

Tolerance of dinitrochlorobenzene, a contact sensitizer, in man

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The contact sensitizer, dinitrochlorobenzene (DNCB), was applied according to several schedules of small weekly or biweekly doses to the buccal mucosa of 64 adult men. The responses of these men were then compared with those of 57 control subjects when all were simultaneously given a "sensitizing" series of applications of DNCB to the skin. Buccal administration of DNCB induced detectable contact sensitivity to DNCB in 39 of the 64 subjects. During the subsequent topical exposures, those buccally treated subjects who had been sensitized showed little rise in degree of sensitivity, while many of those who had not been sensitized failed to become sensitized. Control subjects showed a normal response to the same topical exposures, and quantitative tests at the end of the experiment showed that control subjects were significantly more sensitive to DNCB than were those previously exposed to the compound by the buccal route. Thus, application of DNCB to the buccal mucosa partially aborted later attempts to induce contact sensitivity to the same compound by topical application. There is some evidence that this tolerance was more effectively induced by lower doses of DNCB applied to the buccal mucosa at fortnightly intervals than by larger amounts given more frequently.

Sulzberger,¹ Chase,² and many others³⁻¹² have shown that tolerance of contact sensitizers is readily induced in the guinea pig. Such tolerance is most readily noted when the sensitizer is initially given by any one of several non-sensitizing routes—such as orally or intravenously. However, tolerance may also exist concomitantly with sensitivity.⁷⁻⁹ In such a context, tolerance is manifested as a "fixation" of the degree of sensitivity at a low level, regardless of subsequent attempts to raise that level.

We¹⁴ have recently demonstrated a very similar phenomenon in man. A series of applications of dinitrochlorobenzene (DNCB) to the buccal mucosa induced a mild contact sensitivity in some subjects and did not sensitize others; but virtually all subjects then exhibited a refractoriness to subsequent attempts to induce sensitivity (or to raise the level of sensitivity previously induced).

Having demonstrated that tolerance can be induced in man, we have attempted to obtain some practical information concerning the most efficient ways of doing so. Perhaps, as has been demonstrated in the guinea pig,^{8, 13} a single oral administration of the sensitizer would be an effective way to achieve tolerance in man; on the other hand, it may prove necessary to give a series of

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TABLE I. Application of DNCB to buccal mucosa prior to standard topical application

Group	No.	Buccal application of DNCB	Total buccal dose (γ)
1	37	None (controls)	None
2	8	5 mg. in one dose, then 300γ q.o.w.	5,000- 7,400
3	9	750γ weekly for 4-5 weeks	3,000- 3,750
4	16	750γ q.o.w. × 1-4 then weekly for 9-10 weeks	7,500-10,000
5	10	300γ weekly for 20-24 weeks	6,000- 7,200
6	9	300γ weekly for 5-6 weeks	1,500- 1,800
7	12	300γ q.o.w. 8-10 ×	2,400- 3,000

q.o.w. = Every other week; × = times.

small, widely spread doses. Many other schedules are possible. The present study is a confirmation of our earlier work and a preliminary exploration of variables of dosage and schedule.

MATERIALS AND METHODS

Subjects

The subjects were 101 healthy male adults, 21 to 45 years of age, drawn from a prison population.

Sensitizer

2:4-Dinitrochlorobenzene, purchased from Distillation Products Industries, was used. DNCB was preweighed into 12 ml. vials and was freshly diluted to the desired concentration with acetone at the time of application. The probity of sensitization of human subjects to this compound has been discussed elsewhere.^{14, 15}

Paradigmatic design

All experiments consisted of two parts. In the first part, "experimental" subjects were given DNCB by a series of applications to the buccal mucosa (designed to induce tolerance). In the second part, these subjects, plus additional control subjects, were simultaneously subjected to a series of epicutaneous applications of DNCB (designed to sensitize). Any impairment of the rate of sensitization of the "experimental" subjects compared to that of the control subjects during the second part of the experiment was evidence that some degree of tolerance or unresponsiveness was induced by the buccal administration of DNCB.

Buccal application

The 101 subjects were divided into 7 groups as outlined in Table I. In a previous experiment,¹⁴ subjects had been given buccal applications of 750γ of DNCB, first at biweekly and then at weekly intervals. This schedule was also used in the present experiment and compared with doses of 300γ given according to the same schedule. In addition, the schedule of administration was varied in several ways: some subjects received DNCB every other week, and the duration of DNCB administration varied from periods as short as 4 to 5 weeks to prolonged experiments involving 20 to 24 weekly buccal applications. One group of subjects was given a single large dose of DNCB (5,000γ), followed by a series of smaller doses at biweekly intervals.

At the time of buccal application, the subject was requested to tilt his head to the side so that the buccal mucosa was approximately level. Then DNCB in acetonic solution was dropped onto the buccal surface from a 0.1 ml. glass pipette, taking care that none of the solution ran forward over the vermillion border. After each application the subject was requested to take 6 deep breaths with the mouth open and then to swallow about 30 ml. of water. Applications were made to only one buccal surface at a time. The side of application

TABLE II. Sensitization from application of DNCB to the buccal mucosa alone

Group	No.	No. sensitized	Per cent
2	8	6	75
3	9	6	67
4	16	10	62
5	10	7	70
6	9	5	55
7	12	5	42
Groups 2-7	64	39	61

was alternated from left to right so that any local reaction to DNCB on the mucosa would be distinctive by virtue of its unilaterality. When 300 γ of DNCB was applied, it was dissolved in 0.08 ml. of acetone (0.37 per cent), 750 γ was dissolved in 0.075 ml. (1 per cent), and applications of 5,000 γ DNCB were made in a vehicle of a 0.25 ml. (2 per cent).

Topical application

In the second part of the experiment, all of these subjects plus an additional 37 control subjects were given weekly applications of 150 γ of DNCB to each extremity. These applications were made by dropping 150 γ of DNCB dissolved in 0.02 ml. of acetone onto circular sites one inch in diameter and stamped in ink on each upper arm and each knee. These applications were repeated weekly until a visible reaction occurred or for 8 weeks if no reaction was seen. If the reaction which occurred was a mild and relatively asymptomatic one, the topical applications were continued; most subjects in whom this was possible were in the experimental groups.

Quantitation of sensitivity

The intensity of sensitivity was measured by a graded series of epicutaneous tests consisting of application of the following quantities of DNCB in acetic solution to circles 1 cm. in diameter: 50, 25, 25, 2, 1, and 0.4 γ . This series was applied without occlusion to the inner aspect of the arm. All subjects were tested approximately one week after the first reaction was first noted. Subjects in the buccal group who were willing to continue receiving the series of topical applications after having become sensitized were retested at the end of the series.

All tests were read 3 and 7 days after application and were quantitated in two ways:

1. Lowest eliciting dose was determined for each subject on each test. This was the smallest amount of DNCB in the test series which elicited a detectable erythema. Occasionally the weekly application of 150 γ to the lateral arm or anterior leg elicited a visible reaction, indicating sensitization, but the ensuing test series applied to the inner arm (which, in our experience, is a less sensitive area) was completely negative. Such cases are assigned a threshold score of "50 γ +", as more than 50 γ of DNCB would have been required to detect the induced sensitivity in the test area.

2. Total score was obtained by adding scores for each individual test site, which were graded as follows: 0, no visible reaction; 0.5, detectable erythema; 1.0, erythema over 50 per cent of the test circle; 2.0, erythema and elevation of part of circle, or erythema of all of circle; 3, erythema of all of circle with elevation; 3.5, erythema of all of circle with vesiculation. Individual test scores for each test in a test series were added together to derive a total score for each test series.

RESULTS

Sensitization by buccal application of DNCB

As is shown in Table II, slightly more than half of the 64 experimental subjects exhibited signs of contact sensitivity at the end of the series of buccal

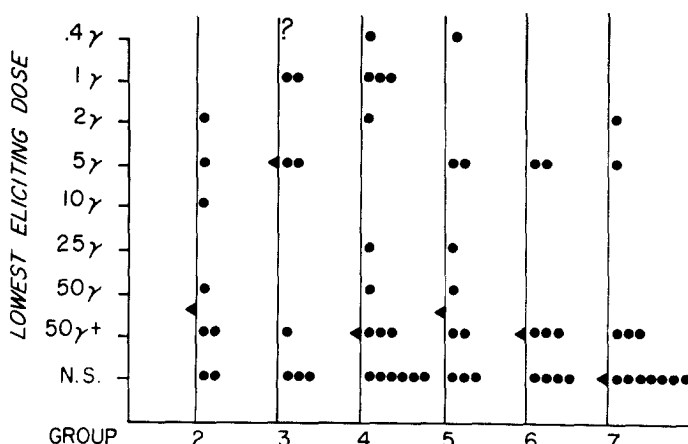


FIG. 1. Levels of sensitivity resulting from buccal administration of DNCB alone. Each dot represents test results for one subject. Tests were read at 3 and 7 days, and the higher sensitivity at either reading represented here. One subject who refused test is represented by question mark and assigned highest possible score. Triangles denote medians. Group numbers are as in Table I.

applications of DNCB. Five subjects developed unilateral stomatitis following buccal applications of 750γ of DNCB; all of these subjects were subsequently tested and found to be quite sensitive (reacting to 2γ or less). No subjects developed stomatitis from application of 300γ of DNCB.

As soon as evidence of sensitization by buccal administration of DNCB was uncovered (most often by the first topical application), the level of sensitivity was determined by a test series. Results of these first tests are presented in Fig. 1. Although there are no differences of true statistical significance, one gains from this figure working hypotheses that, (1) larger doses of DNCB (Groups 2 to 4) tend to sensitize more than the smaller doses and that (2) alternate-week applications tend to be somewhat less sensitizing than weekly ones (compare Groups 4 and 7 with Groups 3, 5, and 6).

Sensitization by topical application of DNCB

The results of all tests conducted after the series of topical applications are presented in Fig. 2.

Most of the 37 control subjects exhibited positive reactions to DNCB after 1 or 2 topical applications, and all but 4 of them had been sensitized after 4 applications. Applications were continued up to 8 weeks but did not sensitize these 4 subjects. Many of the control subjects developed a high level of sensitivity, as is apparent in Fig. 2.

The 25 experimental subjects who had not been sensitized by buccal application were subjected to the same series of topical applications of DNCB. Twelve of these subsequently exhibited contact sensitivity and were then tested, while 13 remained unsensitized after the full series of 8 weekly topical applications. Of the 39 experimental subjects who had been sensitized by buccal applications, 24 exhibited only mild sensitivity and could continue to

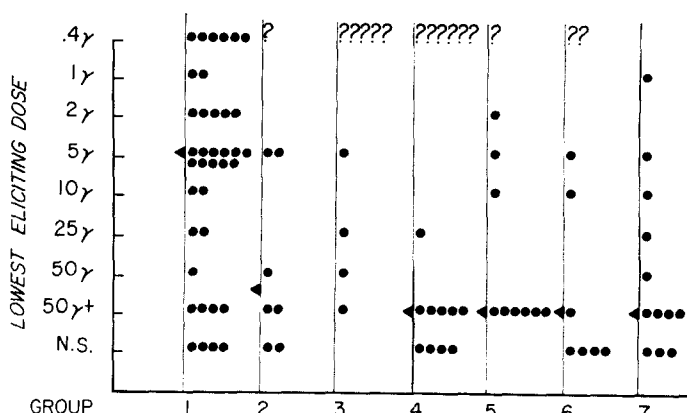


FIG. 2. Levels of sensitivity following series of topical applications of DNCB. Symbols as in Fig. 1. Question marks represent 15 subjects who could not be given 2 or more topical applications and whose response to topical applications is therefore unknown. They were arbitrarily assigned to highest response category for statistical analysis.

receive the series of topical applications. All of these subjects were tested again to determine the level of sensitivity after 1 to 8 additional topical applications. There remained 15 subjects, however, who were too sensitive to continue topical applications, and in them it was not possible to observe the effect of further topical exposure to the compound on sensitivity. These 15 subjects are represented in Fig. 2 by question marks, being arbitrarily assigned the maximal level of sensitivity.

It is obvious from inspection of Fig. 2 that, at the end of the topical series, the final level of sensitivity was considerably less in the experimental subjects (Groups 2 to 7) than in the control subjects (Group 1). This impression is confirmed by simple statistical analysis: 26 of 37 control subjects showed threshold scores above the over-all median, while only 26 of 64 experimental scores fell into this group; this difference is highly reliable ($p < 0.01$ by chi square). An equally high level of statistical significance is observed when the number of subjects who fell into the "50γ+" and "not sensitized" categories are compared for the two groups; that is, more of the experimental subjects than control subjects either were not sensitized or developed a level of sensitivity so low that it was not detected by the test series. The proportion of subjects who were never sensitized was not reliably different in the two groups, however.

The same observations were also recorded as "scores," reflecting the degree of positivity of each test on a numerical scale (see Materials and methods section). Extensive analysis of these scores yielded conclusions identical to those derived from study of the "lowest eliciting dose" data presented above.

The longitudinal trends of both groups are seen grossly in Fig. 3, in which the median sensitivities of both experimental and control subjects are presented at various stages of the experiment. It is obvious that the level of sensitivity of control subjects rose dramatically from an initial unsensitized state during the topical series, while the previously exposed subjects showed little or no rise,

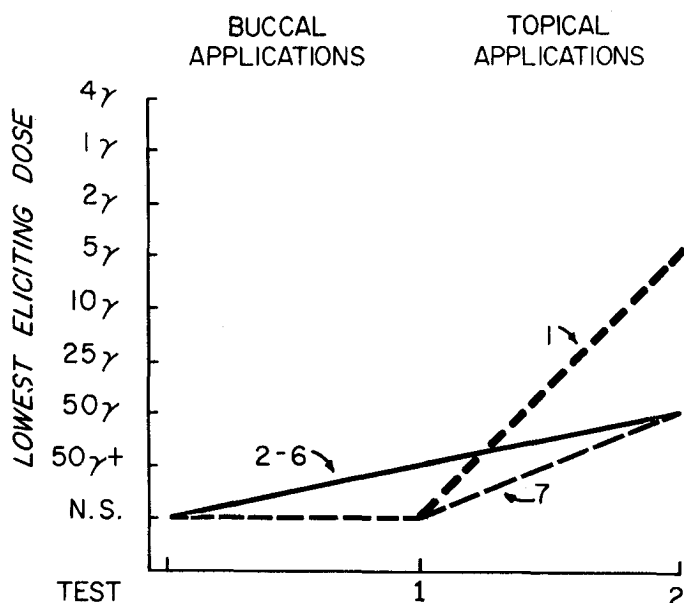


FIG. 3. Changes in median level of sensitivity to DNCB following buccal then topical exposure to DNCB. Groups denoted by number. Group 1 subjects (controls) responded to topical application by rise in sensitivity, while subjects in Groups 2 to 7 showed relatively little rise during this period.

being partially unresponsive during this interval. And at the end of the experiment, control subjects had achieved a higher level of sensitivity than those subjects previously given DNCB by the buccal route.

When the final test results presented in Fig. 2 are reviewed, it appears that, even after the series of topical applications, those subjects who had received the lower buccal dose of DNCB (Groups 5 to 7) and those who had been treated at greater intervals (Groups 4 and 7) tended to be less sensitive than were subjects given buccal applications of larger amounts of DNCB at shorter intervals. From this we may draw a working hypothesis that, in this system, infrequent buccal exposure to low doses of DNCB not only tends to induce lower rates of sensitization but also appears to be more effective in impairing the eventual response to later sensitizing procedures. Perhaps tolerance is more readily induced by such exposures, or perhaps the eventual reduction in response is simply due to fixation at a lower level of sensitivity.

DISCUSSION

The word "tolerance" is used here in a very broad context to refer to any situation in which an immunologic response to an antigenic stimulus is decreased by a previous exposure of the individual to the same stimulus. The response may or may not be reduced to zero, and the previous exposure may have induced some degree of sensitivity, as well as leading to the impairment of the subsequent response. The classical tolerance situation, in which the tolerogen is completely effective and does not in itself sensitize, is just a special case of the broader

concept. Indeed, in these experiments a continuous spectrum of the phenomenon of tolerance was seen, for some subjects exhibited "classical" tolerance, while others were on the fringes, either having been slightly sensitized by the buccal application or responding slightly to the subsequent topical stimulus.

In this frame of reference, examples of "tolerance" are not difficult to find. The simplest example is the response of a group of guinea pigs to a series of identical epicutaneous applications of a sensitizer: the first application sensitizes, but subsequent applications made while that sensitivity is declining fail to do so.⁷ Thus the first application induced unresponsiveness or tolerance to the second. Similar experiments have been reported in man. For instance, Brandriss and Bullock¹⁶ have recently described an experiment in which human volunteers first developed delayed hypersensitivity and then lost much of the sensitivity during a series of identical injections of a dinitrophenyl-protein conjugate.

The present experiments initiate an attempt to find more effective ways of inducing such tolerance. We have previously shown that partial tolerance of DNCB can be induced in man by buccal administration of the compound. We can now state that this is a regularly reproducible phenomenon and that it will occur over a fairly wide range of dosages and schedules of buccal administration.

These data suggest that tolerance may be most readily induced in this situation by application of lower doses of antigen at relatively infrequent intervals. However, this is a hypothesis to stimulate further work and is not a statistically proved fact. Many additional aspects of this area must be explored before formulas describing the most effective ways to abort contact sensitization can be put forth.

There is an obvious theoretic flaw in the design of our experiments, in that the specificity of the unresponsiveness observed was not proved by showing that our unresponsive subjects could be sensitized normally by an unrelated sensitizer. We believe for several reasons that the likelihood that the small doses of DNCB which we gave induced a generalized immunologic deficit is negligible. Manifold larger doses of this antigen have been given to guinea pigs by the intravenous route by de Week and Frey⁶, who observed specific tolerance with normal response to unrelated antigens. Since it has been shown by Feldmann and Maibach¹⁷ that 53 per cent of DNCB applied to human skin can be recovered in the urine within 5 days, it is obvious that topical application of DNCB should also induce any systemic effect which might occur. However, we¹⁵ have shown that larger topical doses of DNCB (e.g., 4,000 γ) tend to induce greater sensitivity, not less, than do smaller doses. The same relationship was seen in the present experiment, in which larger amounts of DNCB, when applied to the buccal mucosa, induced sensitivity more regularly than did small amounts. There is another internal control in the present experiment, for the control subjects given topical application of 600 γ of DNCB weekly (of which 300 γ would be absorbed) exhibited normal sensitization, while experimental subjects given 300 γ by buccal application at weekly or biweekly intervals exhibited unresponsiveness. It would be remarkable indeed if such small doses of DNCB should be found to induce a generalized immunologic impairment in man, while much larger doses have no such effect in the guinea pig; and it also would be surprising for DNCB

to have this effect in man when given orally but not when given topically.

We have no information about the mechanism underlying the tolerance observed in this situation. Nor is there any reason to assume that the mechanisms are the same as those involved in the induction of tolerance in the guinea pig by feeding haptens. A combination of events occurring within various cells, on cell membranes, or in the serum may well be involved. Many of these possibilities have been reviewed by the author¹⁰ elsewhere.

One can expect that more intensive exploration of the circumstances under which tolerance of contact sensitizers is observed will define many of the functional characteristics of the underlying biological events. In addition, correlative studies of serologic and in vitro cellular phenomena associated with this species of human tolerance are obviously needed.

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