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PATCH TESTS WITH NEOMYCIN

Time of Reaction
Patch Test Sensitizations

By

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About ten per cent of all patients with dermatitis or eczema seen during 1964 at the Finsen Institute, Copenhagen, had an allergic contact sensitivity to neomycin. Among patients with varicose eczema the incidence amounted to the unprecedented figure of 26 per cent of all cases referred (*Stoltze* 1966). This alarming increase in the frequency of sensitivity to neomycin appears from Table 1.

Patch tests with neomycin in proprietary creams and ointments are often negative in patients with clinical sensitivity to them. This is probably the single major reason why the frequency of sensitivity to neomycin was not generally realized until it had reached the present epidemic stage.

The false negative reactions to patch tests with remedies containing 0.5 per cent neomycin can be largely obviated by increasing the concentration of neomycin used for testing (*Calnan & Sarkany* 1958). This indicates that the observed false negative reactions may be ascribed to insufficient percutaneous absorption.

S. Epstein 1956 maintains that some patients, sensitive to neomycin, have an exclusively dermal sensitivity, demonstrable only by intradermal tests. In such cases, patch tests remain negative. However, some cases with an apparent dermal sensitivity have been found to give positive reactions to occlusive patch tests, and false negative reactions to patch tests per-

TABLE 1
Frequency of Sensitivity to Patch Tests with Neomycin at the Finsen Institute, Copenhagen, 1961-1964.

		1961		1962		1963		1964	
		Jan. -Jun.	Jul. -Dec.	Jan. -Jun.	Jul. -Dec.	Jan. -Jun.	Jul. -Dec.	Jan. -Jun.	Jul. -Dec.
Neomycin 10 % vaseline	No. %	15 1.9	12 2.8	19 2.2	25 3.1	30 3.2			
Neomycin 20 % vaseline	No. %						54 7.5	75 8.7	64 9.1
Neomycin 10 % anhydrous ointment	No. %						73 10.1	86 10.3	69 9.8
Total number of patients examined		787	423	880	796	918	723	836	702

formed with less occlusive types of test material (*E. Epstein* 1965).

We here wish to emphasize another source of false negative reactions, namely the unusually long latency period between application of patch tests and the development of positive reactions. If patch test reactions are read at the usual time, 48 hours after application, positive neomycin reactions may escape notice. Quantitative estimates of this source of error have not been published, although those who have studied the problem have noted it, (*Boonen & van Waveren Hogervorst* 1964, *Calnan & Sarkany* 1958, *E. Epstein* 1965, *S. Epstein* 1958, *Kirton & Munro-Ashman* 1965).

MATERIAL

The study comprises 1538 consecutive patients with dermatitis and eczema treated at the Department of Dermatology, Finsen Institute, Copenhagen, from 1st January-31st December, 1964. 59 per cent were women, median age 44; 41 per cent were men, median age, 38. Among the women tested 42.2 per cent had positive patch test reaction to one or more substances; among the men 37.6 per cent.

METHODS

Patch tests: Each patient was examined by a standard series of 36 patch tests. The series included two tests with neomycin, placed symmetrically, one containing 20 per cent neomycin in vaseline, the other 10 per cent neomycin in an ointment base containing cetostearyl alcohol 30 per cent, 2-octyl-dodecanol 30 per cent, liquid paraffin 40 per cent.

Lysaplast Special (R) tape was used for the patch tests, which were usually applied on the front of the thighs. The patches were removed 48 hours after application and read 20 minutes later. Repeat readings at 96 hours were made on 84 per cent of the patients. 48 per cent were re-examined on the seventh day, 25 per cent 11 days or more after application of the patches. One of us (K. T.) read the positive reactions.

Intradermal tests: Each patient was tested with an intradermal injection of neomycin. Sterile neomycin was supplied in rubber-capped vials¹. Addition of one ml saline to each vial gave concentrations of 1 per cent, 0.1 per cent, 0.01 per cent, or 0.001 per cent neomycin respectively. Saline was added immediately before injection, and the solutions were discarded after use. All-glass syringes and platinum needles were employed.

Intradermal tests were made on the outer aspect of the upper arm over the distal insertion of the deltoid muscle.

The injections were given by nurses, specially trained in the technique. Readings were made by one of us (K. T.). The minimum requirement for a positive reaction was a seven millimeter papule.

RESULTS

Patch tests: Among the 1538 patients examined, 139 (9 per cent) showed positive reactions to patch tests with neomycin, 20 per cent in vaseline; 155 (10.1 per cent) positive reactions to neomycin, 10 per cent in the anhydrous ointment base (Table 1). This discrepancy will be discussed elsewhere (Thomsen & Hjorth).

The following results are based on patch tests obtained with neomycin, 20 per cent in vaseline.

Time of reaction to patch tests: 19 (15.9 per cent) of 119 patients with positive patch tests reaction to neomycin developed a response within 48 hours, the usual time of first reading. At 96 hours 60 patients (50.4 per cent), and at 144 hours

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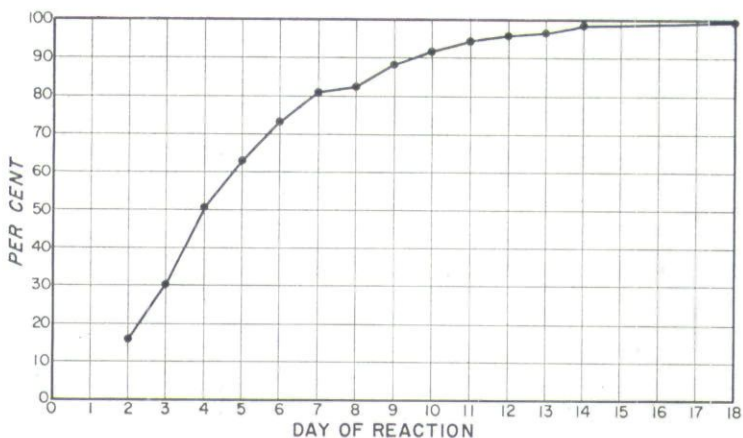


Fig. 1.

Time of Reaction to Patch Tests with Neomycin 20 per cent in Vaseline.
Study comprising 119 patients.

87 patients (73.1 per cent) showed positive reactions (Fig. 1).

In the remaining cases a positive reaction was observed seven days or later after the application. Initially negative responses, which flare up seven days or more after the application of patch tests, are generally taken to indicate sensitization caused by the test. On this assumption, the patients who reacted within six days must be considered as a separate group. Among these, 78.2 per cent were negative at 48 hours, and 31 per cent were negative at 96 hours.

In 27 patients (1.8 per cent of all cases tested, or 22.7 per cent of those with a positive reaction to neomycin) a positive reaction was first observed seven days or more after the application of the test. Among the 27 with the flare-up reaction observed after the seventh day, 20 had been seen on day 2-5, but had not re-appeared in the interval between day 5-9. This precludes an exact determination of the time of reaction. However, among the remaining seven patients (0.5 per cent of all tested) whose test sites were observed at close intervals the reaction developed in three patients on day 9, and in the



Fig. 2.

Time of Reaction to Intradermal Tests with Neomycin. Study comprising 141 patients.

remaining four patients on day 10, 11, 13, and 30 respectively.

Among the 27 patients with the reaction observed after the seventh day, 16 had initially epidermal as intradermal tests. In the remaining 11, a flare-up developed solely as a consequence of a patch test. These patients were then given an intradermal injection of neomycin to determine the level of sensitivity.

Time of reaction to intradermal tests: Reactions to intradermal neomycin develop more rapidly than the patch test reactions (Fig. 2), but even with this method 85 out of 141 patients (60.3 per cent of those with positive intradermal test) were negative at 48 hours.

COMMENTS

Nobody engaged in clinical dermatology will be able to control the test sites in more than a fraction of his patients for seven days after application of patch tests. Inconvenience, distance

or loss of earnings set a limit. The median observation period of six days at the Finsen Institute is probably unusually long. Patients with positive reactions tend to re-appear for longer periods than the average case, and some previously negative may feel inclined to re-appear in the even of a flare-up reaction. Even so, some reactions must necessarily be missed. This source of error cannot be reliably corrected by letting the patient himself read the reaction.

The concentration and the vehicles of most allergens commonly used in clinical practice are adjusted so that an exposure of 24 to 48 hours is sufficient for provoking a positive reaction in sensitive individuals. Patch tests are accordingly read at 48 or 72 hours after application. Such results will be grossly misleading in patch tests with neomycin where 69.8 per cent of positive reactions are missed if the last reading is done at 72 hours.

Comparison of reaction time to patch tests and to intradermal tests shows that the latency period is much shorter with intradermal tests. Accordingly, the slow development of positive reactions may be ascribed partially to a slow absorption of quantities sufficient to provoke a positive reaction.

With some allergens such as turpentine (*Rokstad* 1946) and poison ivy (*Kligman* 1958) a long period of latency may indicate a low degree of sensitivity. Even with neomycin there is some correlation between reaction time to patch tests and sensitivity level, although here a long latency period is the rule. Nearly all patients with an exceptionally high sensitivity (threshold 0.001 per cent neomycin intradermally) had positive patch tests at the first reading 48 hours after the application (Fig. 3). But even those with a sensitivity threshold of 0.01 per cent neomycin intradermally must be considered highly sensitive, and 67.9 per cent of these showed negative reactions at the 48 hour reading. On the other hand, other patients with a low degree of sensitivity had positive patch test reactions at 48 hours. Thus, for practical purpose, the time of reaction to neomycin patch tests is a most unreliable guide to the sensitivity level in a particular patient, since

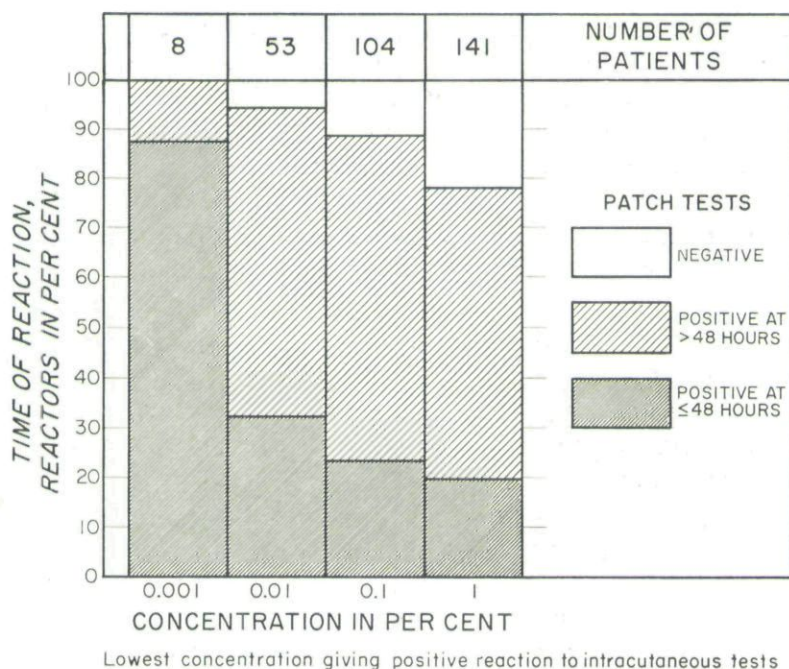


Fig. 3.

Time of Reaction to Patch Tests with Neomycin Related to Degree of Sensitivity as Measured by Intracutaneous Tests. Study comprising 141 patients.

it is dependent on the individual capacity for percutaneous absorption.

The latency period in intradermal tests was largely independent on the level of sensitivity, if estimated by the reactions read at 48 hours. Earlier readings have not been systematically made, since these were prone to biased results from non-specific trauma. The fact that so many reactions to intradermal tests developed later than 48 hours indicates that the long reaction time cannot be due entirely to a slow absorption, but also to immunological phenomena.

The most striking finding in the present study was the high frequency of reactions developing after the seventh day. Such flare-ups are usually seen in testing with strong allergens, such as primula and poison ivy, and are ascribed to 1) sensi-

zation by patch tests, with 2) subsequent reactions to traces of allergen remaining in the upper layer of epidermis. That such deposits of neomycin actually remain, appears from *Munro-Ashman's* finding (*Kirton & Munro-Ashman* 1965) that a positive patch test reaction to neomycin, once disappeared, can be recalled by airtight occlusion of the test site.

Did we sensitize our patients or were the flare-ups an expression of low grade sensitivity and reactions to traces of allergen remaining in the upper parts of the epidermis? Flare-up reactions were also seen after intracutaneous tests. On the other hand a low grade sensitivity cannot be excluded since the patients had in fact been exposed to neomycin, four out of seven patients with flare-up admitting previous use of neomycin. All may have used such preparations since information on previous treatment with neomycin was obtained in only 75 per cent of those sensitive to it. At present it can be stated that flare-ups occur after neomycin patch tests, with a period of latency usually indicative of patch test sensitizations.

It is well established that previous sensitization to one compound involves a tendency to later sensitization by contact with potential secondary allergens (*Hjorth* 1961). Within such limits patch test sensitizations merely represent a widening of the allergic base.

However, "there is a certain amount of evidence to suggest that the skin's previous meeting on one or more occasions with a particular chemical substance does, as it were, oil the wheels of the body's sensitization apparatus in readiness for a future occasion" (*Calnan* 1962). This assumption has been corroborated by a study of patch tests sensitizations to primula, which suggests that the patients sensitized have been so predisposed by previous contact (*Hjorth*). On this assumption, the patients showing flare-up reactions were not sensitized anew, but either they were previously sensitized and had an unusual long period of reaction, or they were predisposed to sensitization by previous exposure to neomycin.

The individual immunological capacity for sensitization to certain chemicals might thus be conditioned by ecological fac-

tors. On this conjecture, the incidence of sensitizations from patch tests would be correlated by the prevalence of sensitivity to a particular substance. The striking incidence of flare-up reactions after patch tests with neomycin may reflect a similarly high incidence of sensitivity to neomycin in Denmark. Fewer late reactions may result in other countries with a low prevalence of neomycin sensitivity, and this may be the reason why such late flare-ups have not previously been reported.

SUMMARY

For several years neomycin has been the most frequent source of allergic contact dermatitis in Copenhagen.

Patch tests with pharmaceutical containing 0.5 per cent neomycin are often negative in patients with contact dermatitis from neomycin. This source of error can be largely obviated by use of neomycin 20 per cent in vaseline for testing. Another important source of error in testing with neomycin, derives from the unusually long time of reaction. This has been subjected to quantitative study in the present paper.

Among 1538 consecutive patients with dermatitis tested at the Finsen Institute, 139 patients, 9 per cent, showed positive reactions to 48 hours' patch tests with neomycin 20 per cent in vaseline.

Among all patients with a positive reaction, 15.9 per cent had developed a reaction within 48 hours after application of the test. At 96 hours, 50.4 per cent had developed a reaction.

Reactions to intradermal tests developed more rapidly, yet only 39.7 per cent were positive at 48 hours after injection.

In 27 patients the positive patch test reaction was first observed seven days or later after application of the test. In seven patients observed at close intervals, reactions developed in three on day 9, and in others on day 10, 11, 13, and 30 respectively. These patients may have been sensitized by the patch tests. Some may have had an unusually long time of reaction.

Patch test sensitizations to neomycin have not previously

been reported. It is suggested that the incidence of such patch test sensitizations might be correlated with the incidence of sensitivity to a particular substance.

REFERENCES

- Boonen, W. & van Waveren Hogerworst, J. W.*: Hypersensitivity to Antibiotics. *Dermatologica* 128, 394, 1964.
- Calnan, C. D. & Sarkany, I.*: Contact Dermatitis from Neomycin. *Brit. J. Derm.* 70, 435, 1958.
- Calnan, C. D.*: The Mechanisms of Allergic Contact Sensitization: Middleton, A. W.: *Cosmetic Science* 1962. Pergamon Press, Oxford, London, 1963.
- Epstein, E.*: Detection of Neomycin Sensitivity. *Arch. Derm.* 91, 50, 1965.
- Epstein, S.*: Contact Dermatitis from Neomycin due to Delayed (Tuberculin-Type) Sensitivity. *Dermatologica* 113, 191, 1956.
- Epstein, S.*: Dermal Contact Dermatitis from Neomycin. *Annals Allergy* 16, 268, 1958.
- Hjorth, N.*: Primula Dermatitis. Transactions of the St. John's Hospital, in press.
- Hjorth, N.*: Eczematous Allergy to Balsams. Munksgaard, Copenhagen, 1961.
- Kirton, V. & Munro-Ashman, D.*: Contact Dermatitis from Neomycin and Framycetin. *Lancet* I, 138, 1965.
- Kligman, A. M.*: Poison Ivy (Rhus) Dermatitis. *Arch. Derm.* 77, 149, 1958.
- Rokstad, I.*: Skin Reactions Caused by Fractions of Oil of Turpentine and Hexadinitrodiphenylamine. *Acta Derm.-Venereol.* 26, suppl. 15, 1946.
- Stoltze, R.*: Dermatitis Medicamentosa in Eczema of the Leg. *Acta Derm.-Venereol.* 1966, in press.
- Thomsen, K. & Hjorth, N.*: to be published.

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