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STABILITY OF HORSE DANDER EXTRACTS

By

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The potency of tuberculin solutions and pollen extracts has been shown to be greatly influenced by the degree of filling of the vials (*Guld et al.* (2), *Magnus et al.* (5), *Hjorth* (3, 4), *Derrick & Trinca* (1)). In pollen extracts the activity of the first milliliter drawn from a 10 ml vial as judged by wheal size was shown to be ten times as great as the activity of the last milliliter in the same vial (3, 4). This reduction in activity—termed the volume effect—is probably due to adsorption to the wall of the vial. The volume effect—increasing with decreasing concentration of the allergen—may be abolished by the addition of “stabilizing agents”, such as the non-ionic detergent Tween 80 (4).

In extracts of purified house dust the original degree of filling of the vial is decisive (6). No difference in potency was found between the first and last ml drawn from originally completely filled vials, whereas a slight, but significant difference in potency was found between extracts from completely filled vials and from vials only one-tenth filled from the beginning.

An analogous investigation using extracts of horse dander has now been performed.

MATERIAL AND METHOD

The experimental subjects were 32 out-patients with hypersensitivity to horse dander, confirmed by intracutaneous tests with approx. 0.02 ml 1:1000 of the

antigen solution. For fear of provoking an allergic systemic reaction, we did not include patients responding by a wheal larger than approx. 12 mm diameter to this test dose. The material comprises 25 males and 7 females, average age 33 years (range 16-62). Twenty-four had bronchial asthma and 8 vasomotor rhinitis. None had urticaria.

Solutions of horse dander were made by Allergologisk Laboratorium, Copenhagen. For the investigation a 1:100 stock solution was diluted with Coca's solution or Coca's solution + Tween 80 0.01 per cent, respectively, and concentrations of 1:10,000 and 1:100,000 were used.

The extracts were supplied in 13 ml, rubber-capped, non-siliconated vials, some of which contained 10 ml and others 1 ml. From some of the vials containing 10 ml concentration 1:100,000 without Tween 80, 9 ml were removed a week or two before use. As a control, we used Coca's solution with Tween 80, 0.01 per cent.

The 10 solutions, coded by the makers, were injected into each patient on the volar aspect of the forearm, according to a prearranged randomization schedule. After 10-15 minutes the reactions were traced on transparent plastic foil by both of us. The study was carried out as a double-blind experiment.

The procedure and method of calculation was exactly the same as that employed in our previous study (6).

RESULTS AND COMMENTS

Extracts without addition of Tween from originally one-tenth filled vials showed a lower potency than extracts of the same concentration from completely filled vials (Fig. 1). This difference (the volume effect) was noted with both concentrations of allergen included in the study, and although very slight (max. 0.3 mm) it was statistically significant (analyses of variance, 99.75 per cent limit).

However, we found almost the same potency of extracts (conc. 10^{-5}) from completely filled vials and from completely filled vials from which 9 ml had been removed shortly before use (7.3 and 7.4 mm respectively).

Thus, the original degree of filling seems decisive, and successive emptying of a vial (the situation met with in allergological practice) does not seem to cause a reduction in activity. This is in contrast to the findings when using extracts of grass pollen.

Addition of Tween 80 did not prevent the volume effect,

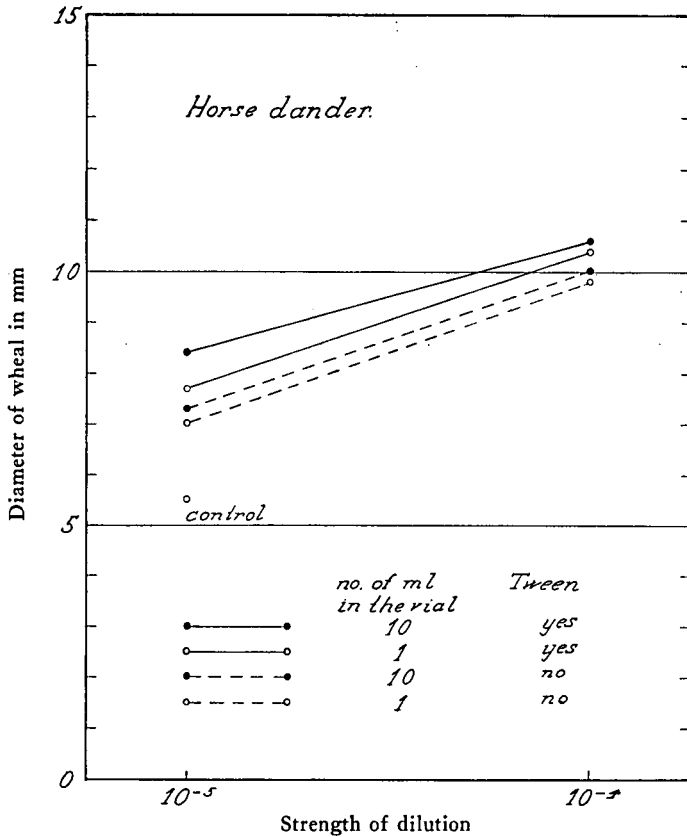


Figure 1.

Dependence of mean wheal size upon concentration, degree of filling of the vial,
and addition of Tween 80.

but increased all reactions (Fig. 1). This effect of Tween was found to be of the same statistical significance (99.75 per cent limit).

The results are in agreement with our previous findings with extracts of purified house dust.

It is concluded that the volume effect in extracts of horse dander is of no importance in clinical work.

SUMMARY

By intracutaneous testing in a double-blind experiment the authors studied the volume effect when using extracts of horse dander. There was a slight, but statistically significant difference (99.75 per cent limit) in potency between extracts from completely filled and from one-tenth filled vials. This difference was of the same magnitude at both concentrations (10^{-5} and 10^{-4}). There was no difference in potency between the first and the last ml drawn from the completely filled vials.

Addition of Tween 80 increased all reactions, but did not prevent the volume effect.

It is concluded that the volume effect in extracts of horse dander is of no importance in clinical work.

REFERENCES

1. *Derrick, E. & Trinca, J. C.*: Alteration in Activity of Grass Pollen Extract. *Acta Allergol.*, 19, 121-141, 1964.
2. *Guld, J., Magnus, K. & Magnusson, M.*: Instability of Potency of Tuberculin Dilutions. *Am. Rev. Tuberc.* 72, 126-128, 1955.
3. *Hjorth, N.*: Instability of Pollen Antigen Solutions. *Acta Allergol.*, 11, 249-260, 1957.
4. *Hjorth, N.*: Instability of Grass Pollen Extracts. *Acta Allergol.*, 12, 316-335, 1958.
5. *Magnus, K., Guld, J., Waaler, H. & Magnusson, M.*: Instability of the Potency of Tuberculin Dilutions. Sec. Report. *Am. Rev. Tub.*, 74: 297-303, 1956.
6. *Osmundsen, P. E. & Aastrup, B.*: House Dust Extract Activity and Volume Effect. *Acta Allergol.*, 22, 61, 1967.

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