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HOUSE DUST EXTRACT ACTIVITY AND VOLUME EFFECT

By

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In high dilutions of protein an apparent instability may be reduced by "stabilizing agents", such as gelatin, gum arabic, or Tween 80, a non-ionic detergent.

According to *Guld et al.* (3) and *Magnus et al.* (6) the activity of tuberculin solutions depends upon the degree of filling of the vials, the activity being higher when the vial is full than when it is only one-tenth full. This effect, termed the volume effect, could be demonstrated regardless of the type of containers (nylon, quartz, silicone-treated glass). It was demonstrated that tuberculin could be adsorbed to glass and that the volume effect was reduced by addition to the diluent of Tween 80. It was suggested that the volume effect is due to adsorption to the surface of the containers.

Hjorth (4, 5) found the volume effect to manifest itself to a marked extent in solutions of mixed grass pollens (*avena elatior*, *phleum pretense*, and *dactylis glomerata*), partial emptying of a vial causing considerable reduction (90 per cent) in the activity of the remaining antigen solution. In these experiments too the volume effect could be abolished by adding Tween 80. Investigations by *Derrick & Trinca* (2) have shown the same phenomenon in solutions of rye grass pollen (*lolium perenne*).

We have investigated whether the volume effect also manifests itself in other allergen extracts. For the present study

purified house dust was chosen, and in a subsequent publication the findings with horse dander will be reported.

MATERIAL AND METHOD

The experimental subjects were 40 out-patients with positive intracutaneous tests with extracts of purified house dust. For fear of provoking an allergic systemic reaction, we did not include patients responding by a wheal larger than approx. 12 mm diameter to 0.02 ml 1:1000. The material comprises 23 males and 17 females, average age 31 years (range 14-64). Twenty-seven had bronchial asthma, 13 vasomotor rhinitis. None had urticaria.

Solutions of purified house dust were made by Allergologisk Laboratorium, Copenhagen. For the investigation we diluted a 1:100 stock solution with Coca's solution¹ or Coca's solution + Tween 80, 0.01 per cent, respectively, and used concentrations of 1:10,000 and 1:100,000.

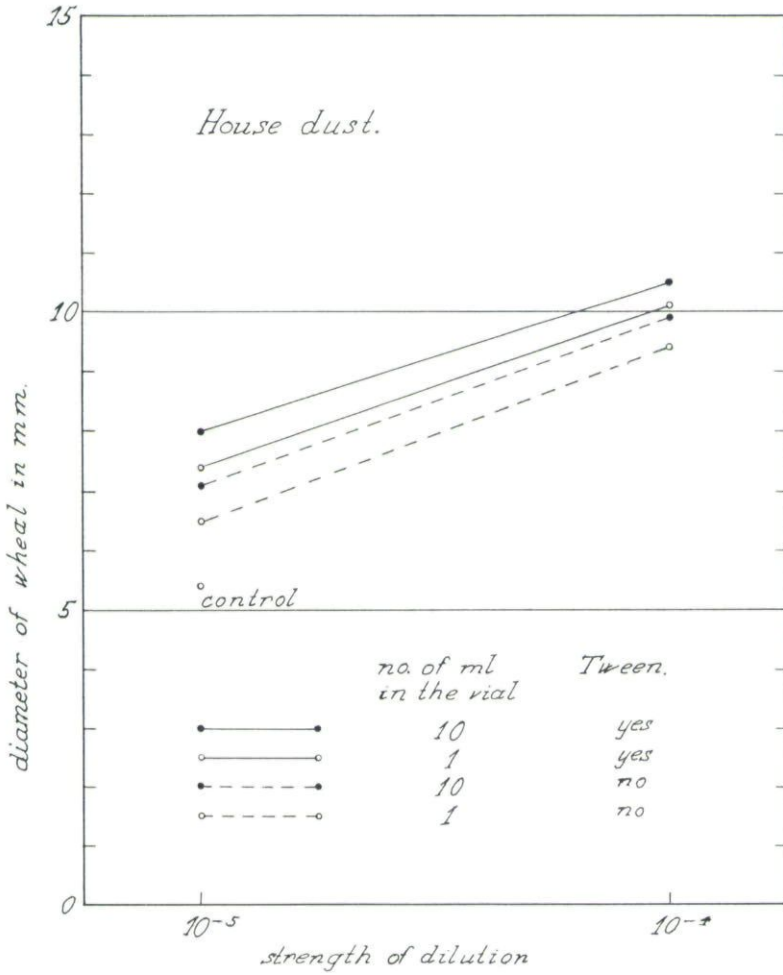
The extracts were supplied in 13 ml, rubber-capped, non-siliconed vials (from Müller & Müller, Holzminden), some of which contained 10 ml and others 1 ml. From some of the vials containing 10 ml conc. 1:100,000 without Tween 80, 9 ml were removed a week or two before use. As a control, we used Coca's solution with Tween 80, 0.01 per cent. All solutions were stored in a refrigerator at 4° C and were used from 2 weeks—4 months after being prepared.

1 ml Yale tuberculin syringes, No. 1YT (supplied by Becton, Dickinson & Co., Rutherford, N.J., U.S.A.) were used after they had been controlled for tightness. The syringes were rinsed after use in tap water and left in 100 per cent nitric acid for 24 hours, then rinsed again and sterilized by dry heat. Dry-sterilized hypodermic needles (chromium nickel steel 18/8) were used. Throughout the experiment the same syringe was used for the same solution.

The 10 solutions were injected into each patient on the proximal two-thirds of the volar aspect of the forearm, into 2 ulnar, 2 radial, and one central site more distally. The solutions were coded by the makers (A, B, C, etc.). The dosage used for all the solutions was 0.02 ml. All the injections were administered by a specially trained nurse according to a pre-arranged randomization schedule (10 × 10 latin square).

At the end of 10 minutes the reactions were traced onto transparent plastic foil by one of us (P.E.O.) and immediately afterwards, by the other (B.Aa.) and measured, the size of the wheal being calculated as the average of the largest diameter and the largest diameter at a right angle to this. As the final measure for each individual wheal we used the mean of the two investigators' results. Not until all the results were available was the code broken, so that the study was carried out as a double-blind experiment.

¹ Sodium Chloride (NaCl) 0.5 per cent, Sodium Bicarbonate (NaHCO₃) 0.275 per cent, Phenol (C₆H₅OH) 0.4 per cent.



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

Fig. 1.

RESULTS

The results are presented in Fig. 1 which gives the dependence of the mean wheal size upon the concentration of allergen, the

filling of the vials, and the addition of Tween 80. Statistical calculation (analysis of variance) showed that the differences at the two concentrations and at the two degrees of filling were highly significant (99.75 per cent limit). The influence of Tween addition was found to be of the same significance.—On the other hand, there was no difference between the wheal size after injection of extract from completely filled vials and from completely filled vials from which 9 ml had been removed a week or two before use. There was also no difference between the results found by the two investigators.

DISCUSSION

The errors inherent in the technique of intracutaneous tests are well-known (1, 7, 8), the size of the wheals depending among other factors upon the injected volume and upon the depth of injection. The present technique reduces this source of error as far as possible. The size of the wheals depends, furthermore, upon the site of injection, the reactivity of the forearm being higher proximally than distally, and higher on the ulnar than on the radial aspect (8). Randomization was such that each solution was injected an equal number of times into each of the 10 injection sites. Thus, solutions to be compared were not injected into symmetrical sites, but it was felt that it was of decisive importance to avoid the bias involved by the knowledge of such "symmetry".

The differences in wheal size following injection of extracts without addition of Tween from completely filled vials and from one-tenth filled vials ought to express the volume effect which, however, is very slight (max. 0.6 mm) compared with the findings when using grass pollen.

In Hjorth's studies on grass pollen (4, 5) and in *Guld, Magnus & Magnusson's* (3) studies on tuberculin the volume effect was independent of the method by which the reduction in volume was brought about. Our results are different, as we found the same potency of extracts from completely filled vials and from completely filled vials from which 9 ml had

been removed shortly before use. Thus the original degree of filling of the vial seems decisive.

The volume effect was equal at both concentrations, and addition of Tween caused the same increase in wheal size at the two degrees of filling. This might indicate that the volume effect in extracts of purified house dust may be fully explained by adsorption onto the walls of the vials and that this adsorption has a certain maximum value. However, it must be emphasized that we used only two different concentrations.

Since the Tween effect in the present experiments proved independent of concentration and degree of filling and since, furthermore, the "volume effect" was the same for extracts with Tween as for extracts without Tween it cannot entirely be ruled out that the observed increase in the reaction indicates an effect by Tween itself, independent of the allergen. This assumption is supported by the not inconsiderable size of the "control wheal". However, in a supplementary investigation we found no difference in wheal size after injecting Coca's solution and Coca's solution + Tween 80, 0.01 per cent, into symmetrical sites of the volar aspect of the forearm in 20 persons.

No difference was found between the first and last ml. in the originally completely filled vials. In allergological practice the vials normally are completely filled from the beginning. Therefore, it is concluded that—unlike the findings when using extracts of grass pollen—the volume effect is of no practical importance in intracutaneous testing and in hyposensitization with extracts of purified house dust.

SUMMARY

By intracutaneous testing in a double-blind experiment the authors studied the volume effect when using extracts of purified house dust. There was a slight, but highly significant difference in potency between extracts from completely filled vials and from vials only one-tenth filled from the beginning. This difference was of the same magnitude at both concen-

trations (10^{-5} and 10^{-4}). However, there was no difference in potency between the first and last ml. of extracts drawn from originally completely filled vials. Thus the original degree of filling of the vial is decisive. Addition of Tween 80 increased the wheal size more than could be attributed to the volume effect. The Tween effect proved to be independent of the concentration and of the degree of filling.

ACKNOWLEDGMENTS

We acknowledge our indebtedness to *O. Horwitz*, M.D. and *P. Nybo*, M.A. (econ.), Dansk Tuberculose Index, for assistance in planning the study and in statistical calculations, to *Niels Hjorth*, M.D. of the Finsen Institute, Department of Dermatology, for interest and critical perusal of the material, and to nurse *Inge Sahl Larsen* for practical assistance.

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