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Studies with Antigens. IV. Purification of Extracts of Grass Pollens.

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We have reported[1, 2] fractionation methods for the purification and concentration of the house-dust allergen. Solutions of purified house-dust produce specific positive scratch reactions in house-dust-sensitive patients in concentrations of 0.50 to 0.87%, and specific intracutaneous reactions in dilutions of 1/50,000 to 1/50,000,000.[3]

The technic has now been extended to the purification and concentration of grass-pollen allergens. (*Phleum pratense, Capriola dactylon, Paspalum dilatatum, Holcus halapensis.*)

Comparative skin reactions which were used for evaluating the effectiveness of all fractionations were made with equal concentrations of extracts. The significance of differences in skin reactions induced by them were estimated by the chi-square statistic.[4]

Aqueous extracts of defatted pollens were subjected to fractional precipitation by the addition of water-miscible organic solvents such as dioxane, acetone, isopropyl alcohol. The fractionation-technic is briefly described as follows: The precipitate which forms when a small amount of organic liquid is added to the aqueous extract is removed, and then an additional amount of organic liquid is added to the filtrate; this procedure is repeated to give progressively larger proportions of organic liquid with successive separations of insoluble fractions. The fractionations with organic liquids are carried out at 5°-7° C.

For purposes of brevity, our description will be limited to the results obtained by the use of dioxane.

The reactivity of the fractions precipitated by concentrations of dioxane less than 25%, or greater than 75% was found to be slight and significantly less than that of the original extracts from which they were precipitated; the fractions precipitated by 25 to 75% concentrations caused marked reactions, which were significantly greater than those induced by the original extracts.

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1 Boatner, C. H., Efron, B. G., and Dorfman, R. I., *Science*, 1940, **91**, 389.

2 Boatner, C. H., Efron, B. G., and Dorfman, R. I., *Allergy*, to be published.

3 Efron, B. G., Boatner, C. H., and Pabst, M. R., *Investigative Dermatology*, to be published.

4 Pabst, M. R., Boatner, C. H., and Efron, B. G., *N. O. Med. and Surg.*, 1940, **93**, 142.

Dialysis experiments showed that No. 1200 cellophane membrane is permeable to much of the inert material in the extracts, but practically impermeable to the allergens. It permitted the passage of dialyzable material of very little potency.

The skin reactivity of extracts fractionated with dioxane followed by dialysis was significantly greater than that of original extracts dialyzed over the same period of time. Therefore, the allergenically inert material removed by fractionation with dioxane is non-dialyzable.

Extracts purified by fractionation with dioxane were treated with high concentrations of salts, such as ammonium, sodium, and zinc sulfate. In the use of ammonium sulfate, 60 g of the salt were added to each 100 ml of the extract. The precipitate was dissolved in water, and again treated with ammonium sulfate. The salt adhering to the precipitate was removed by dialysis.

The skin reacting potency of the extract treated by fractionation with dioxane, with ammonium sulfate, and then by dialysis was significantly greater than that of the extract purified by fractionation with dioxane and by dialysis. The extracts compared had been subjected to dialysis against running water for the same length of time.

Table I compares the results of cutaneous tests of the original

TABLE I.
No. of Reactions.

	Equal-sized	Larger	Smaller	Total
Original timothy-extract	11	1	46	58
Purified timothy-extract	11	46	1	58

$\chi^2 = 90.194$, $n = 2$, $P =$ less than .01.

extract of timothy pollen and of the extract purified by fractionation with dioxane, ammonium sulfate, and dialysis. These data show that the potency of the purified extract is significantly greater than that of the original extract.

Results similar to those shown in Table I were obtained with the other grass-pollens studied.

The increased potency of the purified extracts is due to the purification of the allergens resulting in their increased concentration per unit-weight of dissolved material in the extracts. This conclusion is warranted by the following observations: Scratch tests performed on individuals who had no allergic disease failed to show any evidence of irritation when tests were made with the various fractions of the extracts. Patients who suffered from allergic disease but who were not clinically sensitive to grass-pollen failed to show positive reactions to scratch tests with the various fractions. Patients who were

clinically sensitive to grass-pollens showed significantly larger skin reactions with the more highly purified extracts than with the less highly purified or with the untreated extracts.

Summary. 1. Fractionation of aqueous grass-pollen extracts with water-miscible organic liquids promotes purification of these extracts. 2. Precipitation of extracts purified by the above treatment with high concentrations of sulfates affords further purification.

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Effect of Gramicidin Suspended in Mineral Oil on Streptococci of Bovine Mastitis.

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Gramicidin, an alcohol-soluble, water-insoluble substance extracted from cultures of an aerobic sporulating bacillus, has been found to exert a marked bactericidal effect against Gram positive microörganisms, both *in vitro* and *in vivo*.[1] When injected by way of the cistern into infected quarters of cows suffering from mastitis caused by *Streptococcus agalactiae* (Lancefield serological group B), gramicidin apparently cured a number of the infections.[2]

The method originally used for the administration of gramicidin in the treatment of bovine mastitis consisted in diluting 60-240 mg of the bactericidal substance in 1000 cc of aqueous media and injecting this material into the cistern of the infected quarter. As reported earlier, this method of administration caused an intense swelling of the treated quarter, accompanied by a marked elevation of temperature. Although this reaction lasted only a few hours, it was severe enough to prevent repeated treatments on successive days.

It has now been found that sterile mineral oil is a suitable, non-irritating vehicle for the administration of gramicidin. Of 42 quarters treated with gramicidin-oil mixtures, only 4, which had received 120-160 mg of bactericidal substance, exhibited a severe reaction. On the second and third day following treatment, the fore milk in 3 of these quarters was tinged with blood, whereas the secretion of milk was suppressed for 4 days in a fourth quarter. The reaction in

1 Dubos, R. J., *Ann. Int. Med.*, 1940, **13**, 2025.

2 Little, R. B., Dubos, R. J., and Hotchkiss, R. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 444.