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Studies With Antigens, VIII. Preparation of Purified Extracts of Ragweed Pollen.

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(Introduced by Isidore Cohn)

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We have described[1, 2, 3] the preparation of purified house-dust and grass-pollen extracts of higher purity, higher concentration, and greater allergenic activity than extracts prepared by previous methods.

The purpose of the present investigation is to apply the technic used in the preparation of purified house-dust and of purified grass-pollen extracts to the preparation of highly purified extracts of ragweed pollens. The pollens of *Ambrosia artemisiaefolia* and *Ambrosia trifida* were studied.

Aqueous extracts of defatted pollens were subjected to fractional precipitation by the addition of water-miscible organic liquids such as dioxane, acetone and isopropyl alcohol. The fractionation technic is briefly described as follows: The precipitate which formed when a small amount of the organic liquid was added to the aqueous extract was removed, then an additional amount of organic liquid was added to the filtrate; this procedure was repeated to give progressively larger proportions of organic liquid with successive separations of insoluble fractions. The fractionations with organic liquids were carried out at low temperatures. Fractionations of aqueous ragweed pollen extracts with dioxane and other organic liquids resulted in the separation of the solutes into 3 fractions, one only slightly and the other 2 completely soluble in water.

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

Dialysis experiments showed that No. 1200 cellophane and Visking Standard Stretch Membrane were semi-permeable and permitted the passage of dialyzable material possessing very little skin reacting potency per unit weight of dissolved material in the dialysates. Dialysis was carried out at low temperatures.

Original aqueous extracts and aqueous solutions of extracts frac-

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*, in press.

3 Boatner, C. H., and Efron, B. G., Proc. Soc. Exp. Biol. and Med., 1940, **45**, 460.

tionated with dioxane were treated with high concentrations of sulphate salts: namely, ammonium, sodium, and zinc sulphate. In the use of ammonium sulphate, 60 g of salt were added to each 100 ml of the extract. The precipitate which formed was removed and treated with water. The resulting solution was cleared and was again treated with ammonium sulphate. The precipitate was removed, was treated with a small volume of water, and the resultant solution was dialyzed against running water until it was freed of sulphate.

Typical results are shown in Tables I and II. The comparative scratch tests[4, 5] were made with equal concentrations of extract on clinically sensitive patients.

The skin reacting potency of original ragweed extracts was found to be significantly greater per unit weight of material than that of the fractions precipitated by low concentrations of dioxane (Table I, 1). The skin reacting potency of the fraction precipitated by the intermediate concentrations of dioxane was significantly greater per unit weight of dissolved material than either that of the original extract (Table I, 2) or that of the fraction precipitated by high concentrations of dioxane (Table I, 3).

The skin reacting potency of the fraction which was precipitated by the intermediate concentrations of dioxane and which was then

TABLE I.
Fractionation with Dioxane.

Extracts	No. equal size reactions	No. smaller reactions	No. larger reactions	Total	P*
1. Original	2	0	18	20	<.01
From low conc. dioxane	2	18	0	20	
2. Original	17	52	7	76	<.01
From intermediate conc. dioxane	17	7	52	76	
3. From high conc. dioxane	16	26	5	47	<.01
From intermediate conc. dioxane	16	5	26	47	
4. Original	32	68	22	122	<.01
Fractionated with dioxane, dialyzed	32	22	68	122	
5. Original, dialyzed	26	30	8	64	<.01
Fractionated with dioxane, dialyzed	26	8	30	64	

*The probability is approximated by the Chi-Square Test.[6]

[4] Efron, B. G., Boatner, C. H., Pabst, M. R., *Int. Corresp. Club of Allergy*, 1940, **3**, 59.

[5] Pabst, M. R., Boatner, C. H., Efron, B. G., *New Orleans Med. and Surg. J.*, 1940, **93**, 142.

[6] Fisher, R. A., *Statistical Methods for Research Workers*, 7th Ed., Oliver and Boyd, London, 1938.

TABLE II.
Fractionation with Ammonium Sulfate.

Extracts	No. equal size reactions	No. smaller reactions	No. larger reactions	Total	P*
1. Fractionated with dioxane, dialyzed	8	21	3	32	<.01
Fractionated with dioxane, $(NH_4)_2SO_4$, dialyzed	8	3	21	32	
2. Original	19	93	12	124	<.01
Fractionated with dioxane, $(NH_4)_2SO_4$, dialyzed	19	12	93	124	
3. Original, dialyzed	11	27	8	46	<.01
Fractionated with dioxane, $(NH_4)_2SO_4$, dialyzed	11	8	27	46	
4. Original, fractionated with $(NH_4)_2SO_4$, dialyzed	18	26	11	55	.02-.01
Fractionated with dioxane, $(NH_4)_2SO_4$, dialyzed	18	11	26	55	

*Footnote, Table I.

dialyzed was significantly greater per unit weight of dissolved material than that of the original extract from which it was processed (Table I, 4). It was also significantly greater than that of the original extract which had been dialyzed (Table I, 5). The dialyzed extracts compared had been subjected to dialysis against running water through similar membranes over the same period of time.

The skin reacting potency of the extract treated by fractionation with dioxane and with ammonium sulphate, and then by dialysis was significantly greater per unit weight of dissolved material than that of the extract purified by fractionation with dioxane and by dialysis (Table II, 1). The skin reacting potency of the former fraction was also significantly greater per unit weight of dissolved material than that of the original extract from which it was processed, than that of the original extract after it had been dialyzed, and than that of the original extract treated with ammonium sulphate and dialyzed (Table II, 2, 3, 4). In each instance in which dialyzed extracts were compared, they were subjected to dialysis against running water through similar membranes over the same period of time.

The increased skin reacting potency of the processed ragweed pollen 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 ragweed extracts. Patients who suffered from allergic disease but who were not clinically sensitive to ragweed pollens failed to show positive reactions to scratch tests with the various fractions. Patients who were clinically sensitive to ragweed pollen showed significantly larger skin reactions with the more highly purified extracts than with the less highly purified extracts or with the untreated extracts.

Summary. 1. Fractionation of aqueous ragweed pollen extracts with water-miscible organic liquids produces purification and concentration of the allergens of these extracts. 2. Fractionation of extracts, purified by the above treatment, with high concentrations of sulphate salts produces further purification and concentration of the allergenic factors.

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Changes in Blood Ketone Acids During Artificial Fever.

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Fever, whether infectious or artificial in origin, increases the metabolic rate and consequently influences carbohydrate metabolism. The initial effect is an increased utilization of carbohydrate in the tissues, resulting in an accelerated withdrawal of glucose from the blood. As a means of compensating for the drain on blood sugar, a rise in the rate of hepatic glycogenolysis takes place, and this in turn leads in the postabsorptive state (or when glucose is absorbed at a lower rate than it is removed from the blood) to a depletion of the glycogen stores of the liver.

Observations on patients and on laboratory animals, undergoing treatment with hyperthermia, clearly show the process in its several phases. In a previous communication[1] we pointed out the fact that the hyperglycemia that occurs during fever is due to excessive hepatic glycogenolysis, and not to blood concentration, as several workers believed. In accord with this view, von Haam[2] and Gunderson and

1 Kirstein, M. B., and Bromberg, L., *J. Lab. and Clin. Med.*, 1939, **25**, 7.

2 Von Haam, E., Changes in the parenchymatous organs and blood vessels produced by artificially induced fever. Presented at conference on fever therapy, under auspices of the Kettering Foundation, St. Louis, Mo., Nov. 11, 1938.