Technical Papers

A Purification of the Active Principle of Short Ragweed Pollen

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Extracts of ragweed pollen as used today for the diagnosis and treatment of ragweed-sensitive patients are generally buffered aqueous solutions, to which has been added glycerine or glucose as a stabilizing agent. These extracts contain the active principle which causes the symptoms of hay fever and asthma in the sensitive individual and when tested on the skin of such a person will cause a reaction of the immediate whealing type. It has been known for some time that the active principle is composed of several antigenic fractions; the presence of 3 to 5 has been observed by several investigators, using electrophoretic (*1*), skin testing (*2*), or modified precipitation techniques (*3*). These solutions are also highly pigmented, containing flavanols (*4*) and possibly other substances, which act as irritants and unnecessary diluents of the active principle. The presence of irritants in food and dust extracts is an important cause of the false positive reactions that are commonly produced when testing for sensitivity to these substances.

We have been successful in removing the pigments and possibly some other irritating substances, by means of a simple adsorptive process, without weakening or denaturing the active material, as determined by skin tests upon ragweed-sensitive persons. The whole short ragweed pollen (*Ambrosia elatior*) was dried to constant weight, and the ether-soluble lipids removed by Soxhlet extraction. Then 18.1050 g of dried, defatted pollen was extracted with 425 ml water at 5° C for 18 hr. The suspension was filtered, and the filtrate made up to 500 ml in a volumetric flask. Aliquots were lyophilized to determine solid content, which amounted to 4.8280 g of golden-brown material in the total extract. Kjeldahl analysis showed 3.26% nitrogen.

An adsorption column of 20 g of acid-washed alumina, slurried in 50 ml of water (pH 5–6), was prepared, and 50 ml of the water extract was chromatographed. The coloring material was strongly adsorbed in a single yellow band at the top of the column. The column was washed with 150 ml of distilled water. Colorless material, amounting to about 60% of the weight of the solids in the chromatographed extract, passed directly through the column. A series of fractions was collected. Each fraction was lyophilized for weight of solids and analyzed for nitrogen content, with the results shown in Table 1.

TABLE 1

CHROMATOGRAPH OF 50 ML OF YELLOW AQUEOUS EXTRACT (PH 5.5) CONTAINING 0.4828 G (9.66 MG/ML)

Fractions	Vol	Wt	Mg/ml	% Nitrogen	pH of solution
1	50 ml	59.0 mg	1.18	.....	6.1
2	15	50.1	3.33	2.28	5.85
3	15	64.8	4.33	2.17	5.91
4	15	76.0	5.06	2.07	5.93
5	15	45.6	3.04	2.21	5.55
6	15	19.4	1.29	3.10	5.40
7	15	11.2	0.75	4.28	5.30
8	15	7.3	.49	2.79	5.30
9	17	6.0	.35	2.78	5.30
10	15	3.4	.23	.....	5.15
11	16	3.8	0.23	.....	5.16

Final washing of all the liquid off the column yielded a negligible amount of material. This procedure has been repeated on other batches of pollen with almost identical results.

All the fractions were white, hygroscopic solids, completely soluble in water. All reacted positively to the Molisch test for carbohydrate. The Benedict's test was positive only after hydrolysis. The starch-iodine test was negative, and all fractions except the first gave a positive biuret test.

The activity of these colorless fractions in causing skin reactions was observed in 10 ragweed-sensitive subjects by doing serial scratch tests, in which varying dilutions of the fractions in an aqueous phenol-dextrose solution (5.0% dextrose, 0.5% phenol) were used. The resulting skin reactions were compared with those produced by similar nitrogen dilutions of

TABLE 2

Dilutions (mg N/ml)	Original extract	Fractions 2	3	4	5	6	7
Subject B. G.							
0.15	+++	++	++++	+++	–	–	–
.05	++	++	+++	+	–	–	–
.017	+	+	+++	+	–	–	–
.0056	±	+	++	+	–	–	–
0.0019	±	+	+	±	–	–	–
Subject J. B.							
0.15	++++	++++	++++	++++	++++	–	++
.05	+++	+++	++++	+++	+++	–	++
.017	++	++	+++	++	++	–	++
.0056	+	++	++	+	++	–	+
0.0019	±	+	+	±	+	–	0

++++ reaction = wheal larger than 1 cm in diam.
+++ and ++ reactions = reactions intermediate between ++++ and +.
+ reaction = clearly defined wheal, with erythema.
± reaction = questionable, not considered positive.
– = not tested.

the original unchromatographed extract. Not all the subjects were tested with all the fractions. Results of 2 typical cases are given in Table 2.

The nitrogen content of the weakest dilution causing a reaction in each subject was determined for each fraction and the original extract. It was seen that in many instances some fractions gave reactions when the unchromatographed extract containing an equal or larger amount of nitrogen did not. This was especially true of Fractions 2, 3, and 4.

We are at present subjecting this material to more extended chemical investigation, electrophoresis, and other immunological studies. We also intend to place a group of ragweed patients upon treatment with it, observing the clinical results, its absorption, and incidence of reactions, as compared with ordinary ragweed extracts. We are extending this work to food, dust, mold, and other allergens. By decreasing the incidence of false positive reactions and by increasing the potency of our testing materials, it is felt that the reliability of skin testing in the diagnosis of allergy will be tremendously increased.

References

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The Mg Content of Various Nucleic Acid Compounds

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Since they can readily be disaggregated by minimal quantities of salts (*1, 2*), a number of polynucleotide bonds can be assumed to be essentially of electrovalent type. In experiments made to determine whether metals were active in this connection, it was found that Mg was present in a relatively high concentration. On the other hand, closely related metals such as Ca and Zn were not present to any marked extent. Traces of Sn and Pb were found, but these metals appeared to be loosely bound and were presumably impurities.

The Mg content varied within fairly narrow limits, and an order of magnitude, that was to some extent characteristic, was found within each group of substances studied. There appears to be some correlation with the degree of polymerization, although the Mg content seems to be more dependent on the mildness of the preparative procedure used. The Mg content can be decreased by dialysis. This is presumably caused by hydrolysis, and this is facilitated by other salts that expel Mg. On longer treatment—for example, with strong NaCl solutions—the Mg content decreases appreciably, but is only freed altogether after treatment with acid. It is therefore understandable that preparations exposed to stronger reagents do not show the same high concentration of Mg.

TABLE 1

Material	Preparation according to	Mg content Spectroscopically estimated order of magnitude (%)
Sodium thymonucleate from calf thymus	Hammarsten (1924)	0.01–0.1
	Hammarsten (1924)	~ 0.1
	Gulland *et al.* (1947)	~ 0.1
	Gulland *et al.* (1947)	~ 0.1
Nucleohistone from calf thymus	Carter and Hall (1940)	0.1–1
	Carter and Hall (1940)	0.1–1
	Carter and Hall (1940)	~ 1
Thymus from calf	(Lipid-freed by treatment with alcohol-acetone)	0.1–1
Ribose nucleic acid from yeast	Johnson and Harkins (1929)	~ 1
	Levene and La Forge (1910)	> 1
(Commercial preparations)	Merck	~ 1
	May and Baker	~ 0.1
	Lemke	~ 0.1

Table 1 shows a survey of the results of spectroscopical studies (for which the writer wishes to thank S. Landergren, of the Geological Survey of Sweden).

Chemical analysis offers considerable difficulty because of the large amount of disturbing alkaline phosphates after combustion. We have tried wet and dry combustion, followed by precipitation as $Mg(NH_4)PO_4$ or $MgCO_3$, but with poor reproducibility. By spot tests (Titian yellow and others) after mild acid hydrolysis, Mg is easily detectable.

The Mg content of sodium thymonucleate is of interest in view of the Mg activation of desoxyribonuclease, but also for other enzymes with a possibly analogous mechanism. As have similar ions (i.e., Ca^{++}), Mg^{++} has evidenced certain special effects on sodium thymonucleate in the way of gelation, hydrolysis, etc., as found by Hammarsten (*3*). An aggregate weight many times higher than normal is found on precipitation of sodium thymonucleate in the presence of Mg^{++} with ethanol (*4*).

No such distinct effects of divalent ions are found in the case of polyribose nucleotide from yeast in which Mg (*5*), as well as Cu and Ca, (*6*) has been found. Many signs of the importance of Mg in processes possibly connected with the metabolism of nucleic acid have, however, been observed (e.g., works by Fulmer *et al.* [*7*]).