Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 44. JUNE, 1940. No. 2

SECTION MEETINGS

MINNESOTA		
	University of Minnesota	May 15, 1940
NEW YORK		
	College of Physicians & Surgeons	May 22, 1940
SOUTHERN		
	Louisiana State University Medical School	May 3, 1940
SOUTHERN CALIFORNIA		
	University of California, Los Angeles	May 2, 1940

11441

Isolation of an Unpigmented Skin Reactive Constituent from Extracts of Ragweed Pollen by Electrophoresis.*

H. A. ABRAMSON, D. H. MOORE, H. GETTNER, J. GAGARIN AND L. JENNINGS.

From the College of Physicians and Surgeons, Columbia University, the Mount Sinai Hospital, New York City, and the Biological Laboratories, Cold Spring Harbor, Long Island.

In 1938 experiments were undertaken, using a conventional moving boundary technique, to separate the fractions and to determine the electrical charge of the constituents of giant ragweed pollen extracts. At that time it was observed that the pigments in dialyzed ragweed extracts at pH 7.0 were negatively charged. The results during that summer and the succeeding winter, however, were contradictory, because the relation of the pigments to the biologically active constituents was uncertain.

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

Using the Tiselius[1] cell for the study of the moving boundaries of protein solutions and the Philpot-Svensson[2] technic to analyze the quantity and nature of the constituents, further progress may now be reported. Figures 1a and 1 b are illustrations of the type of curves given by the major constituent. This major constituent is negatively charged, slow moving, unpigmented, and highly skin reactive in persons sensitive to ragweed. A minor constituent, about 1/5 that of the unpigmented major constituent, appearing in the pigment moves approximately 10 times as fast as the unpigmented major one in Fig. 1a. The pigments apparently did not all migrate with the minor boundary but also moved towards the positive pole. Fig. 1b, which also illustrates as a major constituent an unpigmented component has, in addition, several minor constituents migrating towards the positive pole. The electric mobility of the unpigmented skin reactive constituent is 0.05 μ/sec at 1.5°C. It is of interest to note that the electrical mobility of quartz particles in similar ragweed solutions studied by Abramson, Sookne and Moyer[3] approximately agrees with this value when temperature corrections for viscosity are made.

In Fig. 2, the section of the electrophoresis cell labelled (a) shows a Schlierung pattern of the boundary between the unpigmented slow moving active constituent and the buffer; section (b) shows a lightly pigmented section; section (c) was so highly pigmented that practically no light came through; and section (d) contained the ad-

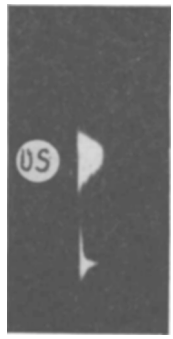

FIG. 1a.
The white area labelled US is the Philpot-Svensson curve of the unpigmented, skin reactive, slow moving constituent. The minor peak is a faster moving constituent in the pigment.

FIG. 1b.
This curve was obtained from another sample of ragweed and illustrates again the US fraction as well as four minor constituents migrating in or with the pigment.

1 Tiselius, A., *Trans. Farad. Soc.*, 1937, **33**, 524.

2 Svensson, H., *Koll. Z.*, 1939, **87**, 190.

3 Abramson, H. A., Sookne, A., and Moyer, L. S., *J. Allergy*, 1939, **10**, 317.

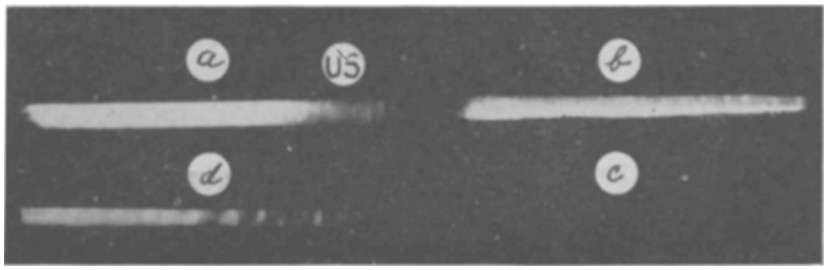

FIG. 2.

To conserve space, these Schlierung or band patterns of the constituents of extracts of ragweed pollen have been rotated 90° with the top of the electrophoresis cell on the left hand side. The US fraction is indicated by the broad band in Fig. 2a. For description of sections b, c, d, see text.

vancing column of pigmented constituents and shows many bands with electrical mobilities closely related to one another but with very low concentrations of each. These bands are essentially similar to those illustrated in Fig. 1b.

It has been previously observed by one of us that the skin reactive constituents of giant ragweed extract may be introduced by an electrical field into the skin by either the negative or the positive pole. Indeed, it was surprising to observe that the positive pole was more efficient than the negative pole with unfractionated dialyzed solutions at pH 7.0. The present studies show that there was no positively charged component observable by the method in the six dialyzed extracts thus far studied. The absence of a positively charged skin reactive constituent is evidence in favor of the point of view of Abramson and Gorin[4] that diffusion forces primarily account for the movement of the skin reactive constituent into the skin during the passage of the current.

Further experimental evidence that the positive pole may transport a negatively charged skin reactive constituent was obtained in the following way. A sample of the unpigmented active fraction was dialyzed for one hour against distilled water to remove phosphate buffer. It was then brought by addition of sodium hydroxide to pH 7.0. The nitrogen content of this solution was 0.25 mg/cm^3. Using this dialyzed negatively charged constituent sufficient ragweed was introduced by electrophoresis from the positive pole for 3 minutes to produce a severe skin reaction in an individual markedly skin-sensitive to ragweed pollen.

Some idea of the skin reactivity of the unpigmented fraction in

[4] Abramson, H. A., and Gorin, M. H., *J. Physical Chem.*, 1939, **43**, 3; Abramson, H. A., and Gorin, M. H., *Chem. Prod.*, 1940, **3**, 37.

relation to its nitrogen content is given by the fact that a 1:1000 dilution of a solution containing 0.3 mg of nitrogen/cm^3 still retained skin reactivity in a ragweed-sensitive case. That is, a solution containing 0.0003 mg of nitrogen/cm^3 scratched into the skin by the usual technic, gave a positive test.

The skin reactivity of the pigmented fractions has not as yet been investigated nor have undialyzed solutions been examined electrophoretically.

11442

Absorption Rates and Biologic Effects of Pellets of α-Estradiol and α-Estradiol Benzoate in Women.

ROBERT I. WALTER, SAMUEL H. GEIST AND UDALL J. SALMON.

From the Gynecological Service of Dr. S. H. Geist, Mt. Sinai Hospital, New York.

In a preliminary communication, we have reported the subcutaneous implantation of crystals of α-estradiol benzoate in a group of 10 menopausal patients who had well-defined morphologic signs and symptoms of estrogen deficiency.[1] It was shown that, by this method of administering estrogens, it was possible to achieve a strikingly more prolonged effect than is obtained with comparable amounts of the hormone, administered parenterally, in solution in oil. It was subsequently demonstrated that a correspondingly prolonged inhibition of the hyperactive hypophysis occurred following the estrogen implantation.[2]

Since Deanesley and Parkes[3, 4] have shown that prolonged estrogenic effects resulted from the subcutaneous implantation of pellets of estrogens in rats, we thought it desirable to study in women the duration of the physiologic and therapeutic effects of pellets as compared with crystals of the same estrogenic substance. Accordingly, 46 patients were implanted with pellets and 55 with crystals of either α-estradiol or α-estradiol benzoate. During a period of observation of approximately one year, it was noted that more prolonged physiologic and therapeutic effects re-

1 Salmon, U. J., Walter, R. I., and Geist, S. H., *Science*, 1939, **90**, 162.

2 Salmon, U. J., Geist, S. H., and Walter, R. I., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 424.

3 Deanesley, R., and Parkes, A. S., *Proc. Roy. Soc. B.*, 1937, **124**, 279.

4 Deanesley, R., and Parkes, A. S., *Lancet*, 1938, **2**, 606.