

to the 19th day. The results demonstrated that EI₅ is apparently less toxic than streptothricin as judged by the findings of Molitor,¹³ and more nearly equal to the toxicity of actinorubin as determined by Morton.¹⁴ Both actinorubin and EI₅ in large dosage cause focal necrosis of the liver and renal epithelial damage.

Summary. Search for antibiotics active against *Mycobacterium tuberculosis* var. *hominis* has resulted in the isolation of a streptothricin-like antibiotic, termed EI₅, from an unidentified *Streptomyces* sp. It is bacteriostatic for *Mycobacterium tuberculosis* H37Rv in dilution of 1:100,000 and closely resembles

¹³ Molitor, H., *Ann. N. Y. Acad. Sci.*, 1946, **48**, 101.

¹⁴ Morton, H. E., *Proc. Soc. Exp. Biol. and Med.*, 1947, **64**, 327.

actinorubin in its antibacterial spectrum, general properties, and toxicity.

The organisms that produce the two antibiotics, *Streptomyces* sp. A-105 and *Streptomyces* sp. EI₅ are morphologically distinct.

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Multiplicity of Antigens in Ragweed Pollen Extracts as Demonstrated by the Technic of Oudin. (17409)

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The heterogeneity of ragweed pollen extracts has been demonstrated by chemical fractionation¹ and by electrophoretic analysis.² Precipitins to crude extracts of ragweed pollen have been produced by numerous workers,^{3,4} but because of the heterogeneity of the extracts used, it would be expected that the precipitins so obtained were directed to a number of different antigenic substances. It is the purpose of this paper to show, by a new technic, the presence of a multiplicity of antibodies in the antisera produced against crude extracts of ragweed pollen, and consequently, the presence of a multiplicity of antigens in

the extracts used for immunization.

The new technic used to demonstrate this antigenic complexity was that recently described by Oudin⁵⁻⁹ which consists in overlaying a solid immune serum-agar mixture contained in a thin-bore tube with the antigen solution. An antigen-antibody precipitate is formed as a sharp band which moves down the tube as more and more antigen diffuses into the agar. Oudin showed that only one band is formed when a single antigen-antibody system is present, while multiple systems give

¹ Stull, A., Sherman, William, and Wilson, W., *J. Allergy*, 1942, **13**, 537.

² Abramson, H. A., *Ann. Allergy*, 1947, **5**, 19.

³ Kukla, A., and Hirsch, D., *J. Immunol.*, 1946, **53**, 391.

⁴ Eagle, H., Arbesman, C. E., and Winkenwerder, W. L., *J. Immunol.*, 1939, **36**, 425.

⁵ Oudin, J., *Compt. Rend. Acad. Sci.*, 1946, **222**, 115.

⁶ Oudin, J., *Bull. Soc. Chim. Biol.*, 1947, **29**, 140.

⁷ Oudin, J., *Annal. de l'Inst. Pasteur*, 1948, **75**, 30.

⁸ Oudin, J., *Annal. de l'Inst. Pasteur*, 1948, **75**, 109.

⁹ Oudin, J., *Compt. Rend. Acad. Sci.*, 1949, **228**, 1890.

TABLE I.
Supernatant Tests on Ragweed Pollen Extract and Its Homologous Rabbit Antiserum.

Tube No.	Ragweed extract, ml	Precipitable N in mg per ml of antiserum	Precipitin test on supernatant (ring method)	
			Presence of:	
			Antigen	Antibody
1	.04	.060	—	+
2	.08	.086	—	+
3	.12	.120	—	+
4	.16	.126	—	+
5	.20	.140	±	+
6	.30	.156	+	+
7	.40	.166	+	+

more than one band. The number of such bands corresponds to the minimum number of antigen and antibody systems present. Before applying this technic to ragweed pollen extracts the findings of Oudin were confirmed using crystalline antigens and antisera of known purity. This latter work will be published elsewhere.

Materials and methods. Defatted short ragweed (*Ambrosia elatior*) pollen was extracted with distilled water or physiological saline in the proportions of 5 g per 100 cc (5% extract) or 10 g per 100 cc (10% extract). The water or saline used in the extraction contained 1:5000 merthiolate (Lilly). The rabbits were immunized by repeated subcutaneous injections of the ragweed pollen extract using Freund's adjuvant technic.^{3,10} Before use in the Oudin test the extracts were brought to pH 6.5-7.5 with 0.1N sodium hydroxide, and any precipitate which formed was filtered off. The procedure followed in the Oudin technic was essentially that described by him⁷ with only minor modifications. One part of 0.6% Bacto agar (Difco) in physiological saline containing 1:5000 merthiolate was used to mix with one part of antiserum. The agar was kept at 48-50°C and the serum was brought to that temperature before mixing thoroughly with the agar. Precipitin tubes of 3 mm inside diameter and approximately 5-6 cm long, which had been previously coated with 1% agar and dried under vacuum, were filled $\frac{1}{2}$ to $\frac{2}{3}$ full with the serum-agar mixture. The tubes containing the serum-agar were then placed in the refrigerator for

5 to 10 minutes to allow the agar to solidify. The antigen solution containing 1:10,000 merthiolate was now layered on top of the solid serum-agar mixture, and the tubes were sealed with plasticine to prevent evaporation and kept for 2 weeks at approximately 25°C.

Results. Quantitative precipitin tests were set up using rabbit antiserum and short ragweed pollen extract in 50% glycerin in the same manner as reported by Bukantz *et al.*¹¹ The results of the supernatant analysis shown in Table I demonstrate that more than one antigen-antibody system exists, since both antigen and antibody were found in the same supernatant. (See Kendall.¹²) The Oudin test was run using a 10% extract of ragweed pollen in physiological saline. The final concentration of antiserum in the serum-agar mixtures was 50%. An antiserum, in a 50% final concentration, when tested with 5% water extract of the same batch of pollen gave 5 bands. The position of each individual band was measured at 6-18 hour intervals with calipers accurate to 0.1 mm. The results of plotting the distance moved by each of the bands against the square root of the time gave, as found by Oudin with other systems, straight lines (Fig. 1). These experiments were repeated using different antisera with similar results. In some experiments there was an indication of the presence of a sixth band, but this was so faint that one could not be certain of its actual existence.

Discussion. The results of these experiments clearly demonstrate that there are at

¹⁰ Freund, J., and Bonanto, M., *J. Immunol.* 1944, **48**, 325.

¹¹ Bukantz, S., Johnson, M., and Hampton, S., *J. Allergy*, 1949, **20**, 1.

¹² Kendall, F. E., *J. Clin. Invest.*, 1937, **16**, 921.

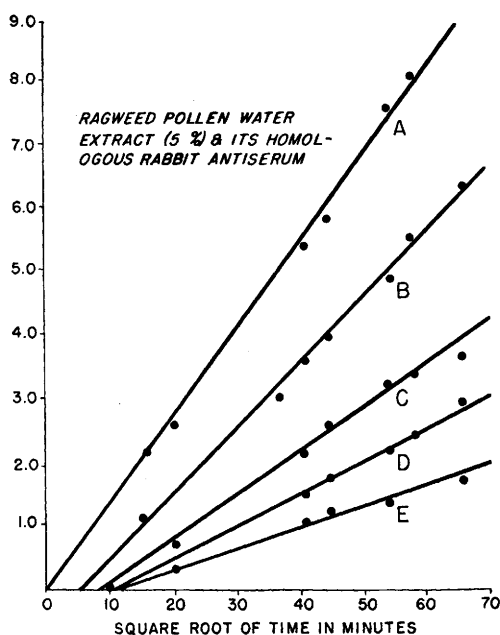


Fig. 1.

Movement of 5 bands of precipitate obtained with ragweed pollen extract and its homologous antiserum plotted against the square root of the time.

least 5 distinct substances in ragweed pollen extracts which are antigenic for rabbits. That the antigenic complexity may be even greater than this is indicated by the observation

that complete absorption of the ragweed extract with rabbit antiserum markedly reduces the direct skin reactivity in specifically sensitive human beings, but does not abolish it.¹³ The relationship of these 5 antigens to the various chemical¹ and electrophoretic² fractions shown to exist in ragweed pollen extracts is not known at present, and more work is needed to elucidate this problem. The technic of Oudin shows great promise in the fractionation of antigenic mixtures. It has been applied by Oudin to the fractionation of horse serum⁶ and by us to demonstrate the presence of at least 3 antigens in cytochrome c of 0.34% iron content.¹⁴ Moreover, work from Oudin's laboratories⁹ and from our own (unpublished experiments) indicates that it may be possible to apply his method as a quantitative tool for the determination of either antigen or antibody concentrations.

Conclusion. Application of the technic of Oudin to ragweed pollen extracts and their antisera demonstrated the presence of at least 5 different antigens in such extracts.

¹³ Becker, E., and Marshall, R., unpublished work.

¹⁴ Becker, E., and Munoz, J., *J. Immunol.*, 1949, **63**, 173.

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Allergic Encephalitis Following Injection of Dialysate of Brain Tissue. (17410)

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The encephalitic process which follows the injection into monkeys^{1,2} and guinea pigs^{3,4} of homologous or heterologous brain tissue has been variously termed meningo-encephalomyelitis,⁷ acute disseminated encephalomyelitis,¹ isoallergic encephalomyelitis³ or allergic encephalitis,⁵ and has been compared with the

post-vaccinal (rabies) paralysis of human beings.¹⁰ The nature of the factor in brain

¹ Kabat, E. A., Wolf, A., and Bezer, A. E., *J. Exp. Med.*, 1947, **85**, 117.

² Morgan, I. M., *J. Exp. Med.*, 1947, **85**, 131.

³ Freund, J., Stern, E. R., and Pisani, T. M., *J. Immunol.*, 1947, **57**, 179.

⁴ Kopeloff, L. M., and Kopeloff, N., *J. Immunol.*, 1947, **57**, 229.

⁵ Bell, J. F., Wright, J. T., and Habel, K., *Proc. Soc. Exp. Biol. and Med.*, 1949, **70**, 457.

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