

sectomized frog and the adrenal ascorbic acid depleting test in the hypophysectomized rat were employed. The results demonstrate: 1. Lack of correlation between the 2 activities in various ACTH preparations. 2. Partial separation of intermedin and ACTH activities using a discontinuous pH gradient on cellulose. 3. Differential migration of activities during zone electrophoresis on filter paper. 4. Potentiation of intermedin and inactivation of ACTH by alkali-heat treatment. 5. Differential concentration of ACTH and intermedin activities in the anterior and intermediate lobes of the frog pituitary.

1. Johnsson, S., and Högberg, B., *Nature*, 1952, v169, 286.
2. Sulman, F. G., *Refuah Veter.* (Israel), 1952, v9, 31.
3. ———, *Nature*, 1952, v169, 588.
4. Waring, H., and Landgrebe, F. W., *The Hor-*

mones, VII, ed, Pincus and Thimann, New York, Academic Press, Inc., 1950, p. 508.

5. Sayers, M. A., Sayers, G., and Woodbury, L. A., *Endocrinology*, 1948, v42, 379.
6. Li, C. H., *J. Am. Chem. Soc.*, 1952, v74, 2124.
7. Porath, J. O., and Li, C. H., unpublished.
8. Geschwind, I. I., Porath, J. O., and Li, C. H., *J. Am. Chem. Soc.*, 1952, v74, 2121.
9. Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.*, 1951, v35, 118.
10. Stehle, R. L., *J. Pharmacol. Exptl. Therap.*, 1936, v57, 1.
11. Raben, M. S., Rosenberg, I. N., and Astwood, E. B., *Fed. Proc.*, 1952, v11, 126.
12. Li, C. H., and Pedersen, K. O., *Ark. Kemi.*, 1950, v1, 333.
13. Irving, G. W., Jr., and du Vigneaud, V., *Ann. N. Y. Acad. Sci.*, 1943, v43, 273.
14. Reinhardt, W. O., Geschwind, I. I., and Li, C. H., *Acta Endocrin.*, 1951, v8, 393.

Received June 6, 1952. P.S.E.B.M., 1952, v80.

Effect of Various Allergens and Antibiotics on Experimental Poliomyelitis.* (19651)

ALBERT MILZER AND FREDDIE NICHOLSON.

From the Medical Research Institute, Michael Reese Hospital, Chicago.

Last year we reported that the inoculation of pertussis vaccine, diphtheria toxoid or *Salmonella typhimurium* vaccine would significantly decrease the incubation period before onset of paralysis in mice infected with the Lansing strain of poliomyelitis virus(1). Since then we and others(2,3) have been able to confirm these results. Dean *et al.*(3) reported that mice injected in a foreleg with pertussis vaccine alone or combined with diphtheria-tetanus toxoids were more frequently paralyzed in the injected limb following infection with mouse encephalomyelitis virus than the unvaccinated controls. The present studies were carried out to determine the effect of various allergens and antibiotics on the incubation period of mice infected with the

Lansing strain of poliomyelitis virus. The allergens tested included house dust, mixed tree pollen, mixed grass pollen, ragweed pollen, mixed mold spores, and horse serum. The antibiotics tested were dihydrostreptomycin, terramycin, and penicillin.

Materials and methods. Groups of Swiss mice (Plymouth strain) weighing 13 to 15 g were inoculated intracerebrally with approximately 10 LD₅₀ units of the Lansing strain of poliomyelitis virus. The identity of the Lansing strain used in this laboratory was verified periodically by neutralization tests with hyperimmune Lansing antiserum. Control mice inoculated with nutrient broth were also included in every experiment. The various allergens tested were obtained through the courtesy of the Allergy Laboratory of Michael Reese Hospital and were used in the following concentrations: 1. House dust (Endo) 1:400, 2. Mixed tree pollen 1:500, 3. Mixed grass

* Aided by a grant from the Merton Davis Memorial Fund. Supported in part by the Michael Reese Research Foundation. Read before Soc. Amer. Bacteriologists, April 28, '52, Boston, Mass.

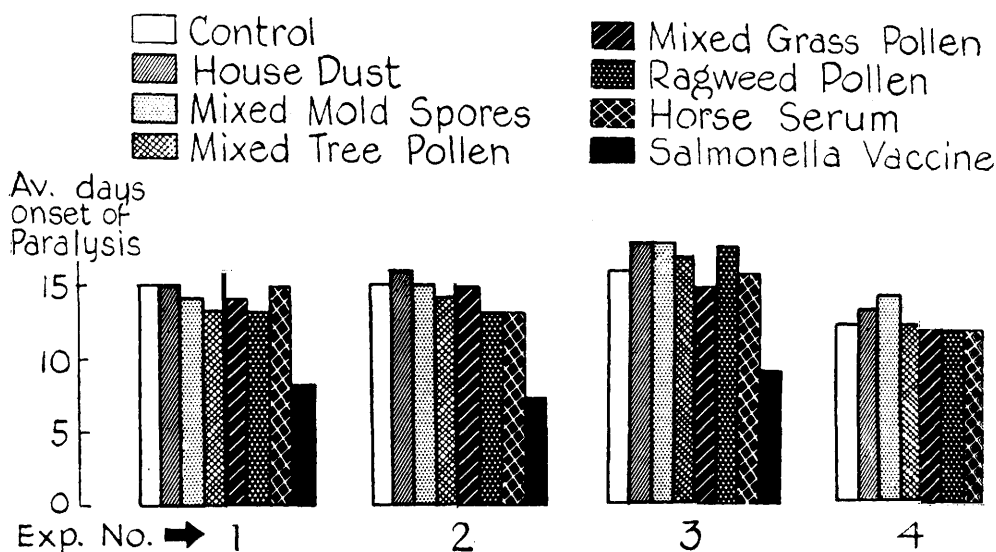


FIG. 1. Effect of various allergens on poliomyelitis.

pollen 1:500, 4. Ragwood pollen 1:33, 5. Mixed mold spores 1:200. The mixed mold spores contained species of the following genera: *Penicillium*, *Alternaria*, *Hormodendron*, *Aspergillus*, *Rhizopus*, *Mucor*, *Fusarium*, *Monilia*, and *Thoma*). The above dilutions of the various allergens studied represent the maximum concentrations that are usually employed for desensitization of patients. It was found that 0.1 cc of these dilutions inoculated intravenously were not toxic for the strain of mice used, although 10 times this concentration was often a lethal dose. The horse serum was used undiluted and was obtained from the Difco Laboratories. The *Salmonella typhimurium* vaccine was prepared as described previously(1). The antibiotics studied were dihydrostreptomycin, terramycin and penicillin. Streptomycin and terramycin were inoculated in 0.5 mg amounts dissolved in 0.1 cc of saline and given intravenously. This dosage was nontoxic and was approximately equivalent to the daily human dose on a weight basis. Thirty thousand units of procaine penicillin G in peanut oil was given intraperitoneally; in other experiments the same dosage of the crystalline form of penicillin G was given intravenously. The amount of penicillin given was approximately 1/10 the human daily dose.

The general plan followed in each experi-

ment was as follows: The mice were divided into test and control groups. All animals were inoculated with approximately 10 LD₅₀ units of the Lansing strain of the poliomyelitis virus. The same pool of Lansing virus was used in all experiments. As previously(1) on the first, third and fifth days after virus inoculation the various allergens and antibiotics in the above dosages were inoculated in 0.1 cc amounts intravenously or intraperitoneally. Control mice were inoculated with nutrient broth intravenously (0.1 cc) after the same intervals. Other control mice were simultaneously inoculated intravenously with 0.1 cc of *S. typhimurium* vaccine (approximately 100 million organisms) and showed little or no reaction.

Results. Allergens. Results obtained in a series of 4 experiments with over 300 mice are given in Fig. 1. The figure shows the average incubation period in days after virus inoculation for onset of paralysis in mice inoculated with the various allergens as well as control mice inoculated with nutrient broth or *Salmonella* vaccine. At least 20 mice were inoculated with each allergen, nutrient broth or *Salmonella* vaccine in every experiment. No mice were inoculated with *Salmonella* vaccine in Exp. 4, however, because of the difficulty in obtaining mice at the time that this experiment was carried out. The range of incu-

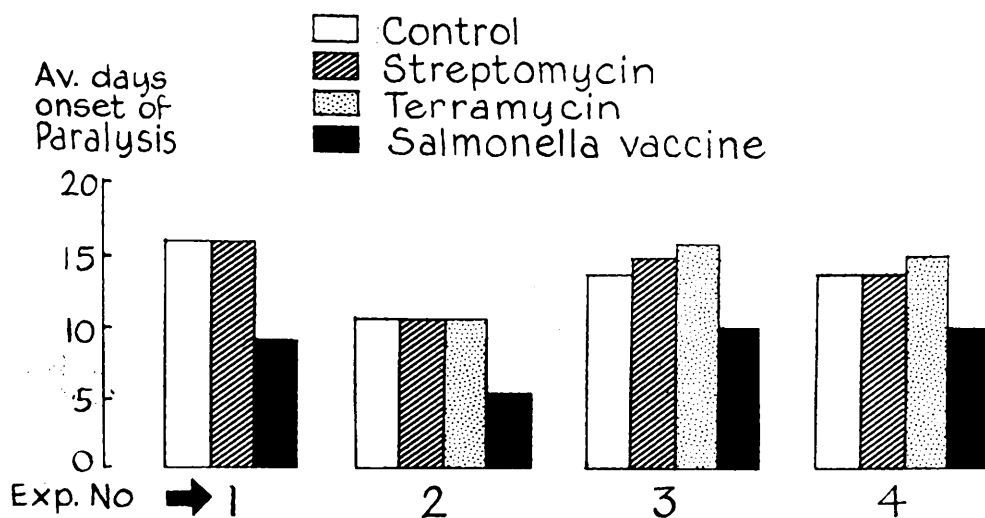
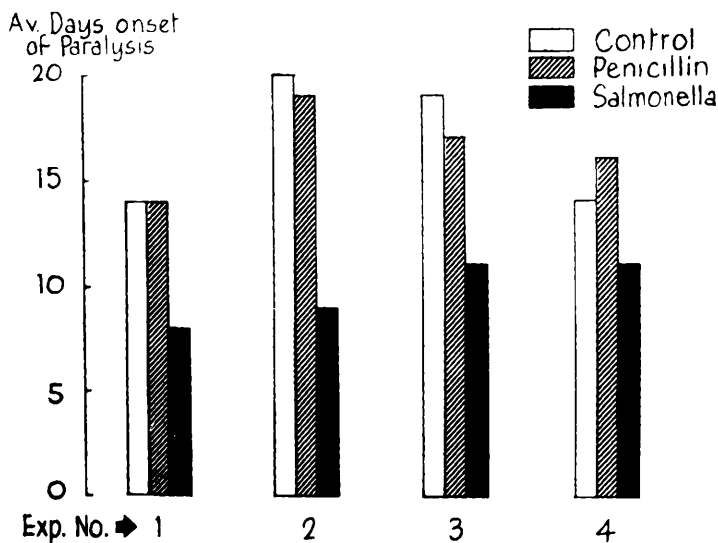


FIG. 2. Effect of various antibiotics on poliomyelitis.

FIG. 3. Effect of penicillin and *Salmonella* vaccination on poliomyelitis.

bation period in both the test and control groups varied from 3 to 30 days. The graphs in Fig. 1 show that there was no significant reduction in the average incubation period before onset of paralysis in all experiments in which mice were inoculated with the various allergens or nutrient broth. In those experiments in which the *Salmonella* vaccine was included there was a consistent reduction in incubation period before onset of paralysis in this group. Statistical analysis of the data using the Student-Fisher *t* test as previously (1) indicated that there was no significant

difference between the groups of mice receiving the various allergens and the controls inoculated with nutrient broth, while the reduction in incubation period following *Salmonella* vaccine inoculation was consistently significant.

Antibiotics. Figs. 2 and 3 summarize the results obtained in the average incubation period before onset of paralysis in infected mice inoculated with streptomycin, terramycin, and penicillin. All of the antibiotics were inoculated intravenously except procaine penicillin G which was given intraperitoneally. In

other experiments the same dosage of crystalline penicillin G was given intravenously with similar results. Again at least 20 mice were inoculated with each antibiotic, nutrient broth or *Salmonella* vaccine. Approximately 500 mice were studied in these experiments. Statistical analysis by the t test showed that there was no significant difference between the groups of mice inoculated with the various antibiotics and controls inoculated with nutrient broth, while the *Salmonella* vaccine consistently produced a significant reduction in incubation period before onset of paralysis.

Discussion. These experiments indicate that inoculation of various allergens and antibiotics do not significantly decrease the incubation period before onset of paralysis in mice infected with the Lansing strain of poliomyelitis. These results are in contrast to those previously reported in which it was shown that inoculation of pertussis vaccine, pertussis-diphtheria toxoid or *Salmonella typhimurium* vaccine show a significantly decreased incubation period before onset of paralysis(1).

It is of interest that clinically there is a more suggestive relationship between inoculation of pertussis vaccine and/or diphtheria toxoid and paralysis in the inoculated limb than in the inoculation of various allergens and antibiotics during an epidemic of poliomyelitis(4-6). This relationship appears to be valid for the most part only if the inoculations are given 7 to 21 days prior to onset which corresponds to the usual incubation period of poliomyelitis. In a personal communication, Abramson(7) states that he conducted a survey of 800 physicians in the United States and collected data concerning

154,000 patients who received pollen injections before and during the hay fever season. Thirty cases of poliomyelitis occurred in the 154,000 patients receiving pollen therapy. "This incidence was considered about the normal rate for the country as a whole. Although only a small number of cases could be followed, there did not seem to be any correlation between injections of pollen and paralysis in the injected limb."

Summary. Mice infected intracerebrally with the Lansing strain of poliomyelitis virus show no significant decrease in incubation period before onset of paralysis following intravenous or intraperitoneal inoculation of certain allergens and antibiotics. The allergens studied include house dust, mixed mold spores, mixed tree pollen, mixed grass pollen, ragweed pollen, and horse serum. The antibiotics studied were dihydrostreptomycin, terramycin, and penicillin. It was found that *Salmonella typhimurium* vaccine significantly decreased the incubation period before onset of paralysis.

1. Milzer, A., Weiss, M. A., and Vanderboom, R., *PROC. SOC. EXP. BIOL. AND MED.*, 1951, v77, 485.
2. Findlay, G. M., and Howard, E. M., *J. Path. and Bact.*, 1950, v62, 371.
3. Dean, D. J., Cohen, S. M., and Dalldorf, G., *PROC. SOC. EXP. BIOL. AND MED.*, 1951, v77, 834.
4. Editorial, *J.A.M.A.*, 1950, v144, 240.
5. Anderson, G. W., and Skaar, A. E., *Pediatrics*, 1951, v7, 741.
6. Greenberg, M., Abramson, H., Cooper, H. M., and Solomon, H. E., *Amer. J. Pub. Health*, 1952, v42, 142.
7. Abramson, H. A., personal communication.

Received June 10, 1952. P.S.E.B.M., 1952, v80.