

INVESTIGATIONS OF SPONTANEOUS HYPERSENSITIVITY OF THE DOG

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SPORADIC reports of spontaneous hypersensitivity in animals have appeared periodically in the veterinary and medical literature.¹⁻⁵ The objectives of the investigations reported here were (1) to study a case of spontaneous ragweed pollen sensitivity in the dog, using some of the clinical and immunologic procedures presently available, (2) to determine the usefulness of such an animal as a model of allergy in investigative studies, and (3) to evaluate the effect of certain pharmacologic materials and other factors on a reproducible quantitative allergic response of the animal.

CASE REPORT

A 2-year-old ovariectomized fox terrier living in southern Michigan was first noted to develop symptoms of lacrimation, conjunctivitis, and severe pruritus in mid-August, 1957, coincident with the pollination of ragweed. Her condition was diagnosed as an allergic reaction, and she was treated with antihistamines with some relief of symptoms. The symptoms subsided at the end of the ragweed season in early October, 1957, and she was next seen in mid-August, 1958, with the return of the same symptoms. J. A. Hergott, D.V.M., then made the dog available to us for investigation.

Physical examination showed the dog to be in good general health. Positive physical findings included reddening of the conjunctivae, profuse lacrimation, and some purulent discharge from the eyes. An erythematous, scaling eruption was present over the back and on the flexural surfaces of the forelegs. The remainder of the physical examination was negative. The symptoms persisted through August and September and began to subside in early October. The ocular symptoms cleared completely by the middle of October. The skin lesions improved but persisted until the middle of December, when they gradually subsided.

Experimental Data.—The total white blood cell count was 13,000, with a differential count of 72 polymorphonuclear leukocytes, 13 lymphocytes, 5 monocytes, and 10 eosinophils. A conjunctival smear stained with eosin and methylene blue showed large clumps of polymorphonuclear leukocytes. Intradermal skin tests with 1:500 concentrations of pollen, mold, and dust extracts were done, using saline as a control. All skin tests were negative, with the exception of those to ragweed, which showed a 3 plus reaction. Serum was obtained from the dog by a sterile technique, and 0.05 ml. of this serum was planted in normal human skin and normal dog skin. In forty-eight hours these skin sites were challenged with 1:500 ragweed antigen, and a 4 plus reaction was observed in both human skin and dog skin with

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negative controls. Conjunctival testing with ragweed pollen was positive, but testing with grass and tree pollen was negative. Similar tests were negative in the normal dog used as a control. Attempts to demonstrate precipitating antibodies with ragweed antigen and dog serum in routine precipitin tests and with the Ouchterlony double-diffusion gel-precipitin technique⁶ were negative. As expected, human antiragweed serum used in identical techniques was negative and rabbit antiragweed serum prepared by hyperimmunization of rabbits with ragweed antigen in Freund's adjuvant was positive. Active cutaneous anaphylaxis by the method of Ovary⁷ was positive in the dog skin. For this test, 0.02 ml. of 1:500 ragweed antigen was injected intradermally and was immediately followed by the intravenous injection of 1 ml. of 0.5 per cent Evans blue dye. A positive reaction was noted, with a dark blue area at the site of the antigen injection, and the saline control was negative. Passive cutaneous anaphylaxis was attempted, using the method of Ovary.⁷ Separate skin sites in

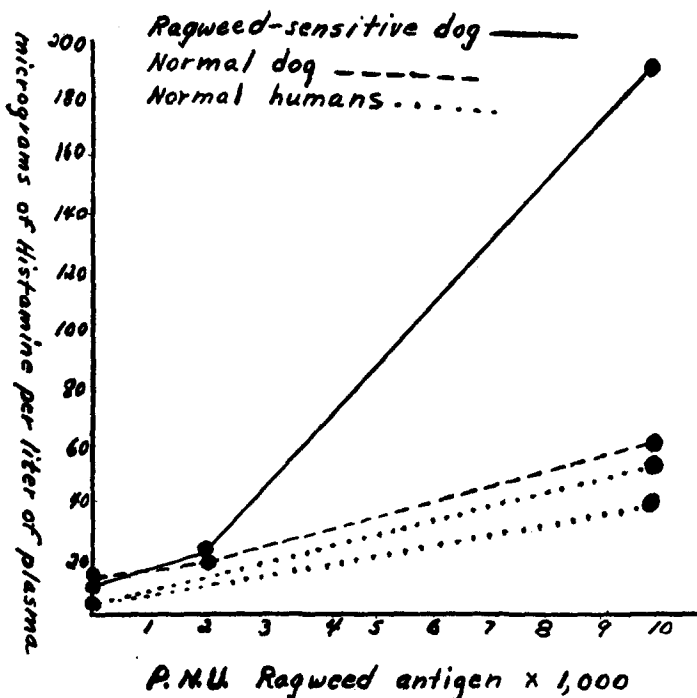


Fig. 1.—Release of histamine from blood of ragweed-sensitive dog and normal controls after addition of ragweed antigen.

the normal white guinea pig were sensitized intradermally with 0.05 ml. of dog antiragweed serum and 0.05 ml. of human antiragweed serum. Twenty-four hours later these skin sites were challenged by intradermal injection of ragweed antigen and intracardiac injection of Evans blue dye. Positive Prausnitz-Küstner reactions were noted, but the passive cutaneous reaction was negative. Similar results using human serum alone have been described by Fisher and associates.¹⁴ A bis-diazotized benzidine hemagglutination reaction, using a modification of the method described by Gordon and associates,¹⁵ was done* and was positive at a titer of 1:1,280.

The release of histamine after the addition of ragweed antigen to blood from the ragweed-sensitive dog and normal controls was measured* as described by Noah and Brand,⁸ and results are shown in Fig. 1.

A summary of the findings in the ragweed-sensitive dog and a comparison with findings in human ragweed sensitivity is shown in Table I.

*Performed by Dr. K. P. Mathews.

TABLE I. COMPARISON OF RESULTS OF CLINICAL AND IMMUNOLOGIC STUDIES DONE ON THE RAGWEED-SENSITIVE DOG AND ON RAGWEED-SENSITIVE HUMAN BEINGS

TEST	RAGWEED-SEN- SITIVE HUMAN BEINGS	RAGWEED-SEN- SITIVE DOG
Skin testing with ragweed antigen	Positive	Positive
Conjunctival testing with ragweed pollen	Positive	Positive
Passive transfer of reaction to normal skin with serum (Prausnitz-Küstner reaction)	Positive	Positive
Precipitin reaction with serum and ragweed antigen (routine precipitin and Ouchterlony plate technique)	Negative	Negative
Active cutaneous anaphylaxis	Not done	Positive
Passive cutaneous anaphylaxis in guinea pig skin using serum from ragweed-sensitive patient	Negative	Negative
Bis-diazotized benzidine hemagglutination titer	Positive (some)	Positive
Symptoms produced by exposure to ragweed antigen	Conjunctivitis, rhinitis, respiratory distress	Conjunctivitis, rhinorrhea, respiratory distress
Symptoms relieved by antihistamines, epinephrine, and adrenal cortical hormones	Yes	Yes
Histamine release from blood after addition of ragweed antigen	Usually	Suggestive

DETERMINATION OF A QUANTITATIVE RESPONSE TO RAGWEED POLLEN

In order to determine the value of the dog as an experimental animal in allergy investigation, it appeared necessary to find which allergic symptom was reproducible and could be measured by either time of onset or severity of response. After exposure to small amounts of ragweed pollen or ragweed antigen in an animal chamber, rhinorrhea, lacrimation, and conjunctivitis developed, but these symptoms were variable in time of onset and degree and eluded any quantitative evaluation. It was then found that exposure to a sufficient quantity of aerosolized pollen or ragweed pollen extract in an animal chamber resulted in a striking symptom complex of severe dyspnea characterized by increased frequency of respirations, thoracic respirations instead of the usual abdominal type, use of accessory muscles of respiration, an audible wheeze, prolonged expiratory phase of respiration, increased expansion of the thorax (an expansion of approximately 2 cm. or 4 per cent of the circumference of the thorax in the normal resting state), and assumption of a sitting or standing position. Auscultation of the chest during an attack demonstrated sonorous musical râles throughout the chest, which were more marked during the prolonged expiratory phase. During the acute attack, the dog refused to eat even her favorite foods, and if the attack was allowed to persist she developed vomiting and diarrhea. The attack of dyspnea would gradually subside over a period of approximately ninety minutes after antigen administration was discontinued or in one-third that time after the subcutaneous administration of 0.3 ml. of 1:1,000 aqueous epinephrine. This attack was then considered to be an asthmatic attack in the ragweed-sensitive dog due to ragweed pollen. It was found that the attack was reproducible, provided that sufficient antigen was delivered to the animal in the chamber, and that no reaction occurred with other pollen antigens, including grass and tree pollen. Normal dogs exposed to ragweed antigen under identical circumstances did not have reactions. This response of

the animal was developed as the method of study of a quantitative allergic response because of its consistent reproducibility and the striking end point. The apparatus designed for the experiments is shown in Fig. 2. The dog is placed in the animal chamber with the pneumograph belt in place and allowed to reach a resting state. The dog is allowed freedom of movement by flexible rubber-tubing attachments. Temperature in the chamber is kept constant at 25° C. by a cooling area in the sub-flooring. Undefined pollen is delivered into the

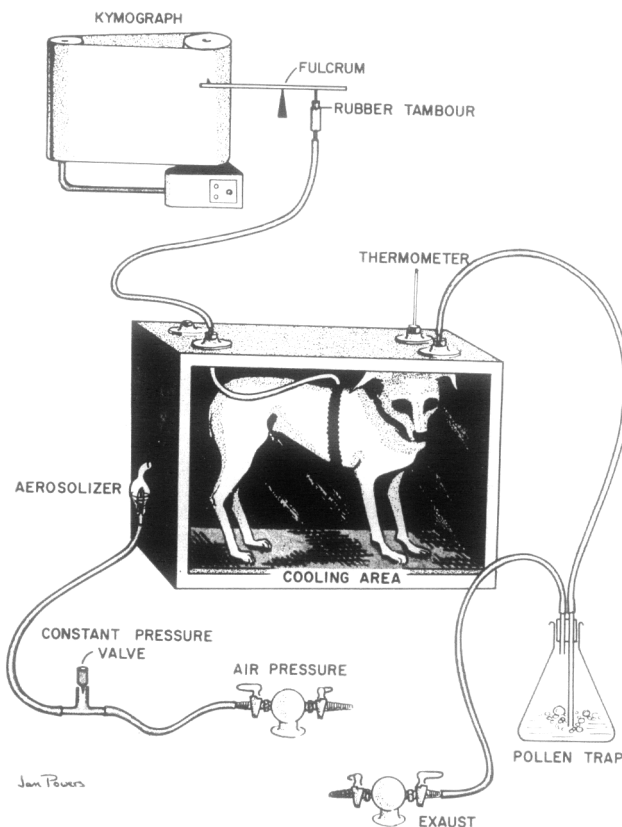


Fig. 2.—Apparatus used for exposure to pollen in animal chamber. Considerable freedom of movement is allowed by the size of the chamber.

chamber through a DeVilbiss No. 40 nebulizer. A constant amount of pollen is delivered per minute by means of a constant pressure valve in the air pressure line leading to the nebulizer. This results in a constant flow of the pollen suspension from the nebulizer. Undefined pollen is suspended in distilled water by thorough mixing. A pollen-in-water suspension is used, since dry pollen alone does not flow through the nebulizer evenly and cannot be counted as accurately as the pollen-in-water suspension. The concentration of pollen is determined by counting in a hemocytometer chamber, using a white blood cell pipette and the technique used for counting white blood cells. Suitable

dilutions are made in order to deliver varying concentrations of ragweed pollen into the chamber. The animal chamber is sealed to prevent laboratory contamination with pollen, and air is evacuated from the chamber at a rate equal to the inflow through the exhaust leading to the pollen trap. Humidity in the chamber is constant during each experiment because of the constant flow of pollen-in-water suspension. In order to record the asthmatic attack graphically, a kymograph record using either smoked paper or an ink-writing apparatus was used.

Development of the typical asthmatic attack is shown by the tracing in Fig. 3 and is characterized by an increase in rate and amplitude of recorded respirations. Inspiration is written as an upward deflection. The increase in amplitude of the recorded respirations is primarily a reflection of the change from abdominal respiration to thoracic respiration and is *not* an indication of the amount of air inspired as compared with the normal resting state. The record of an experiment in which asthma did not develop is shown in Fig. 4. The subsidence of an attack after cessation of pollen administration is illustrated in Fig. 5, and the more rapid improvement after treatment with epinephrine is shown in Fig. 6. The prolonged expiratory phase is illustrated by increasing kymograph drum speed, as shown in Fig. 7. The decrease in the range of thoracic expansion is illustrated in Fig. 8, *B* and *C*, where the amplitude of deflection during the sniffing of food in Fig. 8, *B* is greater than that during the dyspnea in an asthmatic attack in Fig. 8, *C* and is considered to be a reflection of the acute emphysema and decrease in vital capacity during asthma. Respirations of the panting dog recorded in Fig. 8, *A* are not confused with the typical attack because of the irregularity of the panting hyperventilation, the cessation of this hyperventilation with cooling of the chamber, and the lack of other clinical manifestations described above. To avoid confusion, however, the dog was always allowed to reach a resting state prior to administration of antigen. The degree of amplitude of deflection does not reflect severity of attack and varies in different records, depending on positioning of the pneumograph belt on the dog's thorax and technical variations in the recording apparatus.

Following the establishment of an asthmatic response which was reproducible and possible to record, it was next necessary to develop a method by which the minimal amount of antigen necessary to cause an attack could be determined so that observations on alteration of the dose-response relationship could be undertaken. In all subsequent experiments, commercial undefatted *Ambrosia elatior* pollen was used. After a concentration of pollen which would produce asthma had been arbitrarily chosen, smaller but increasing amounts of pollen were administered at daily intervals to find the minimal concentration of pollen which would produce the symptoms. It was then found that the daily administration of pollen to the dog resulted in the development of a state of tolerance to a dose which previously had successfully produced an asthmatic attack. Furthermore, by increasing the pollen concentration delivered during the same experiment after a definite period of time when no asthma occurred, it was found that a certain concentration of pollen was necessary to produce an

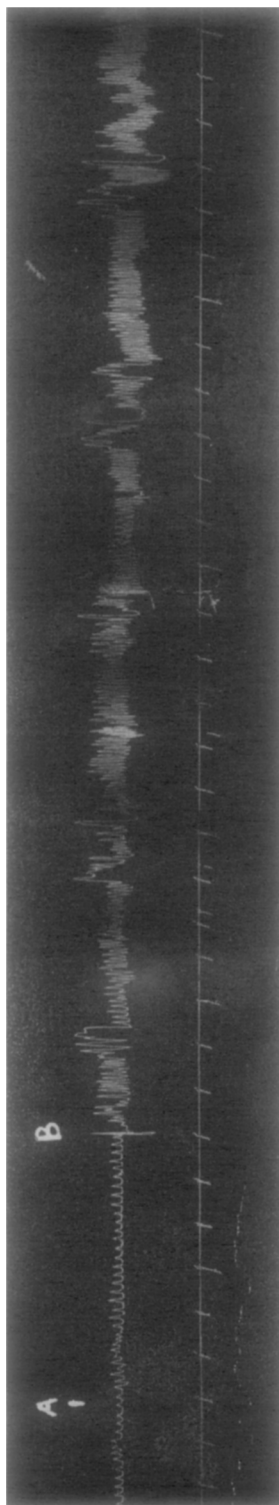


Fig. 3.—Development of asthmatic attack in the dog. *A*, Delivery of ragweed pollen suspension begun. *B*, Recording drum stopped for period of eight minutes. The lower tracing records time intervals of ten seconds.

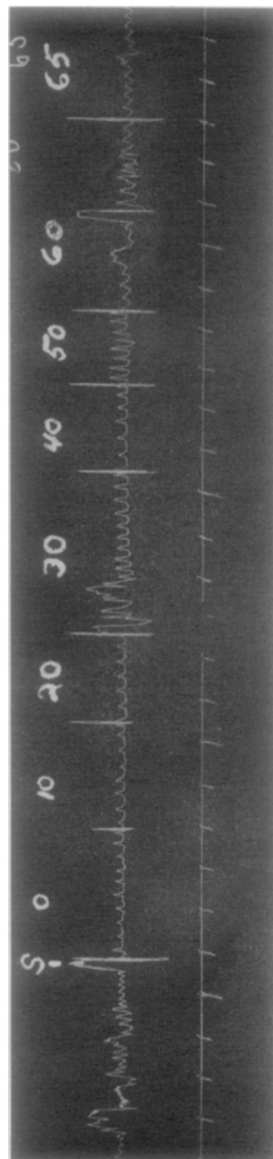


Fig. 4.—Record of respirations during sixty-five minutes of aerosolization of 1.5 per cent serotonin creatinine sulfate. *S*, Initiation of serotonin. Short recordings made every five minutes.

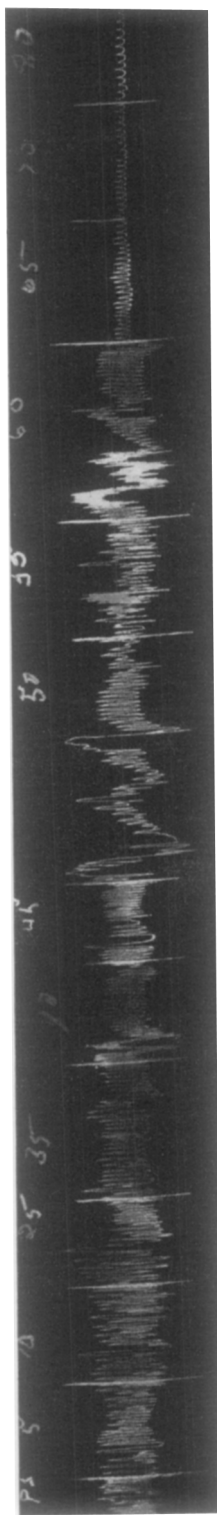


Fig. 5.—Subsidence of asthmatic attack after cessation of ragweed pollen aerosolization. PS, Pollen stopped five minutes for eighty minutes. Kymograph drum speed the same as in Fig. 3.

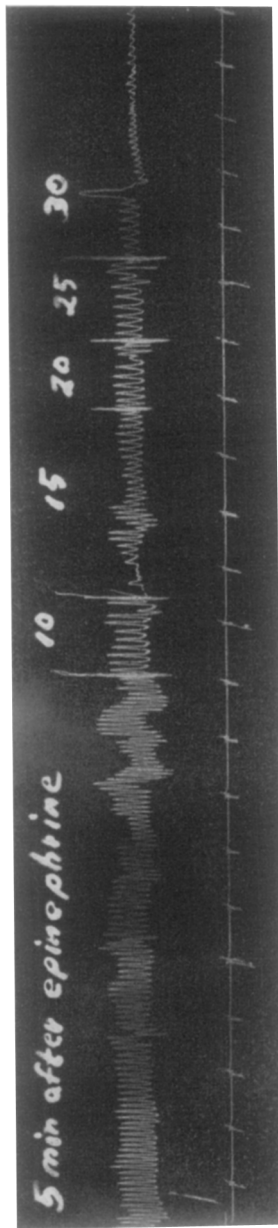


Fig. 6.—Subsidence of asthmatic attack after administration of 0.3 ml. of 1:1,000 aqueous epinephrine subcutaneously. Short recordings at five-minute intervals.

attack of asthma and that below this *threshold* concentration of pollen-in-water suspension no asthmatic attack occurred, even though the pollen was continuously delivered for periods up to three hours. When any concentration of the pollen-in-water suspension above the threshold was delivered, the asthmatic attack occurred within ten minutes. In repeated experiments it was found that this threshold phenomenon was reproducible but that the threshold of concentration varied, depending on the period of time since the previous pollen administration. Daily attacks of asthma or subthreshold concentrations of pollen resulted in an increase of the threshold necessary to produce an attack on the following day.

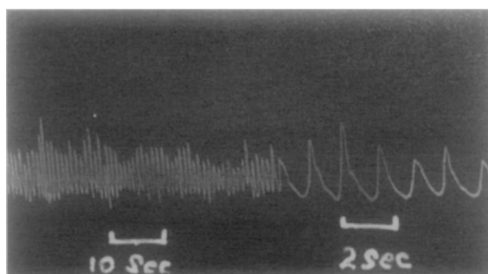


Fig. 7.—Prolonged expiratory phase during asthmatic attack demonstrated by increasing kymograph drum speed.

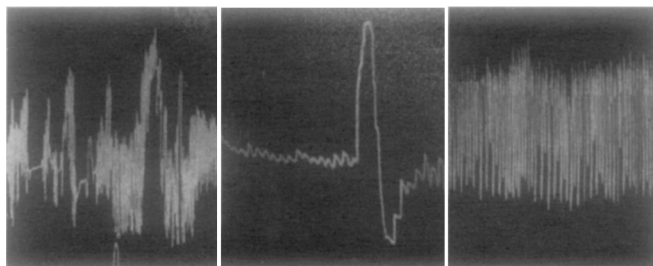


Fig. 8.—Three tracings during one experiment. A, Panting respirations following exercise and before pollen administration. B, Resting state followed by sniffing food. C, Subsequent asthmatic attack which followed pollen administration. Recording speed the same as in Fig. 3.

After a “rest” period of several days without pollen exposure, the threshold dropped to a lower level of concentration necessary to produce an attack. Results of some of these studies are recorded in Fig. 9. It was found that a rest period of seven days resulted in the return of the sensitivity threshold to pollen which existed in the previous experiment. This method was then utilized as the quantitated response to investigate some factors which might affect the threshold.

USE OF THE THRESHOLD PHENOMENON IN SUBSEQUENT EXPERIMENTS

The dog was exposed at weekly intervals to ragweed-pollen-in-water suspension. The pollen was delivered to the chamber for a period of fifteen minutes at the initial concentration; when no reaction occurred at the end of that time, the concentration was doubled and aerosolized for the next fifteen minutes.

This procedure was continued, with the concentration of ragweed pollen doubled every fifteen minutes until definite asthma occurred clinically and as recorded on the kymograph tracing. The results of these studies are shown in Fig. 10. As soon as a definite asthmatic attack occurred, the experiment was stopped and the dog was treated with epinephrine and rewarded with food.

Epinephrine.—Epinephrine shortens the duration of the asthmatic attack of the dog, as described above (Fig. 6). Also, 0.3 ml. of 1:1,000 aqueous epinephrine administered subcutaneously prior to the experiment increased the threshold of concentration of pollen necessary to produce an attack, as shown in Fig. 10.

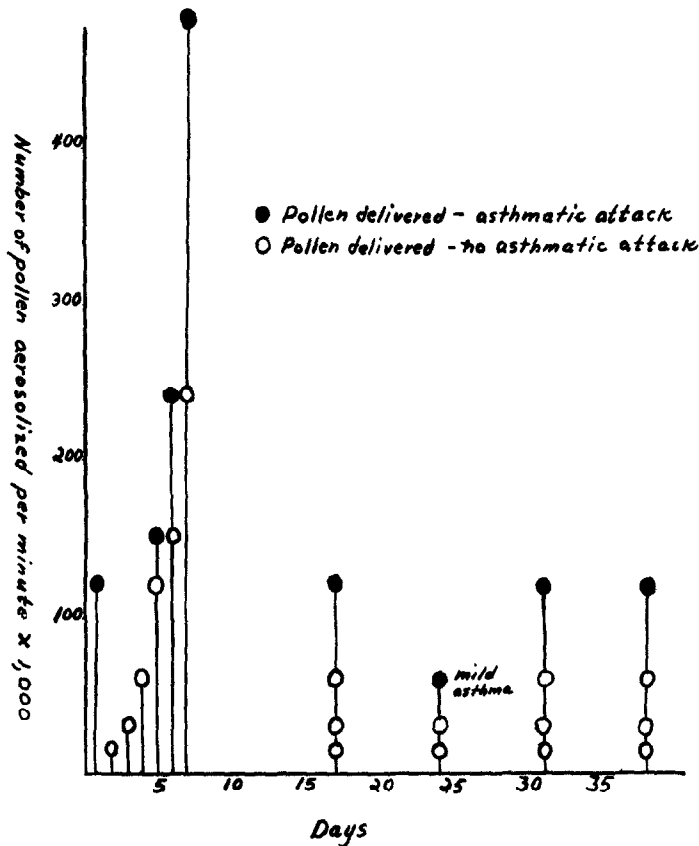


Fig. 9.—Concentrations of pollen required to produce asthma.

Serotonin.—The action of serotonin as an additional humoral mediator in the asthmatic response has been postulated. Serotonin creatinine sulfate, 1.5 per cent, was aerosolized into the animal chamber and no respiratory symptoms were produced in the dog, as illustrated in Fig. 4. This concentration of serotonin was sufficient to produce the transient dyspneic shock syndrome described by many workers in guinea pigs which were placed in the chamber immediately after the dog had been there. A 1.5 per cent solution of serotonin was next used as the suspending medium for ragweed pollen instead of distilled water to

determine whether the addition of serotonin would lower the concentration of pollen necessary to produce an attack. No potentiation of the response with the addition of the aerosolized serotonin was observed.

Lysergic Acid Diethylamide.—The many investigators who have studied its effects have found this agent to be one of the most potent serotonin antagonists. Herxheimer⁹ showed that LSD is capable of inhibiting the serotonin dyspneic shock syndrome in guinea pigs, and Fink¹⁰ reported inhibition of the Schultz-Dale reaction in the sensitized mouse by LSD. It appeared of interest to determine the ability of LSD to modify the threshold of concentration of pollen producing the asthmatic response. The dog was given 0.15 meg. of lysergic

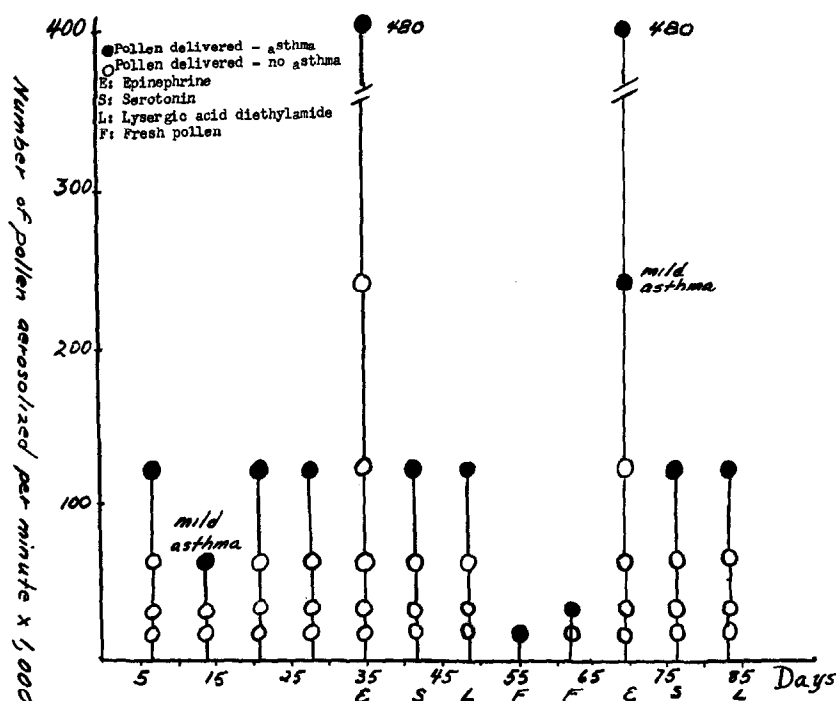


Fig. 10.—Effect of various factors on the pollen concentration required to produce an asthmatic attack in the dog.

acid diethylamide subcutaneously just prior to the beginning of the experiment. This was sufficient to produce definite motor restlessness in the dog but did not modify the asthmatic attack or increase the concentration of pollen necessary to produce the attack.

Fresh Versus Old Pollen.—All previous experiments were conducted with the same supply of commercial pollen obtained two years previously, and this was compared with fresh pollen. Fresh pollen was obtained* out of the ragweed season through a system of forced preseasonal growth and pollination. This pollen was used within two hours of its collection from the plants at 8 A.M., and it was used in the same way as old pollen to produce an attack. The fresh pollen

*From Dr. W. H. Wagner, University of Michigan Botany Department.

showed an increase in antigenic activity over old pollen, as evidenced by the decreased concentrations necessary to produce an attack.

DISCUSSION

It appears evident that the dog described here has ragweed allergy with manifestations suggestive of the response of ragweed-sensitive human beings. It is well recognized that all results and observations depend on the responses of one animal. Confirmation must depend on studies on similar animals, and it is possible that markedly different results may be obtained in other ragweed-sensitive animals when they become available for investigation. We have been told by the veterinary physician supplying the dog that ragweed sensitivity in dogs is not rare in southern Michigan and that these animals may be available for investigation from time to time, depending on their owners. It appears that such animals may prove valuable for certain allergic investigations.

All experiments using the asthmatic response were of short duration, with high doses of pollen being used. The effect of low doses of pollen administered for periods of days must be investigated in the future.

The possibility that the asthmatic response might be conditioned in the dog merely by putting it into the animal chamber without pollen administration after it had had several previous attacks was raised early in the investigation. During the process of working with the recording equipment and evaluating the dog's response, a succession of twenty-five asthmatic attacks were produced in the chamber after exposure to ragweed pollen. Prior to administration of antigen there has been no clinical or kymographic record evidence of any asthmatic symptoms. Environmental and experimental conditions were the same in these experiments. Indeed, since the asthmatic response did not occur below the threshold required in each experiment, it seems evident that the odor of ragweed antigen also did not condition the asthmatic response.

The phenomenon of increasing concentration of ragweed pollen required to produce an asthmatic response when daily experiments were conducted is interesting but not remarkable when such reports as those of Herxheimer¹¹ and of Swineford and colleagues,¹² who used aerosolized antigen as a means of desensitization in the human allergic patient, are considered.

As described in the clinical history, during the ragweed season the dog had no asthmatic symptoms from atmospheric pollen. It is thought that this is because the threshold of concentration necessary to produce an asthmatic attack is never reached during the ragweed season.

Since it has been reported that symptomatic relief occurs in patients suffering from pollinosis who are exposed to artificial negative ionization of air,¹³ experiments are in progress to evaluate this claim, using commercial devices capable of producing negative air ions. Initial results, using the ragweed-sensitive dog in the method described above, indicate that the asthmatic response in the dog to ragweed pollen is not decreased, less severe, or of shorter duration and that the threshold of pollen concentration necessary to produce an attack is not increased by the use of the air-ionizing apparatus. Further results will be presented in a separate communication.

SUMMARY AND CONCLUSIONS

1. A case of spontaneous ragweed hypersensitivity in the dog is described, and clinical and immunologic investigations are reported.

2. The dog with spontaneous hypersensitivity is a suitable model of allergy for experimental work.

3. An asthmatic response in the dog which could be studied in the laboratory was produced by sufficient antigen. Since this response was reproducible and depended on sufficient antigen to produce a response, it may be used as a quantitated response in a rough biologic system.

4. A threshold of pollen concentration was necessary to produce an asthmatic attack in the dog.

5. The threshold of concentration of pollen required to produce an asthmatic attack was increased by frequent asthmatic attacks or pollen exposure and decreased by longer periods of freedom from exposure.

6. Asthma was not conditioned in this dog, in spite of recurrent attacks in the same environment.

7. Serotonin creatinine sulfate, 1.5 per cent, did not produce respiratory symptoms in the dog during aerosolization under the conditions of the experiments described.

8. Aerosolized 1.5 per cent serotonin creatinine sulfate did not potentiate pollen in causing an asthmatic response.

9. Lysergic acid diethylamide did not modify the asthmatic attack or increase the amount of pollen required to produce the attack.

10. A higher concentration of old ragweed pollen than of very fresh pollen was necessary to produce an asthmatic attack.

11. Epinephrine increased the threshold of concentration necessary to produce an asthmatic response.

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