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ATMOSPHERIC POLLUTION BY AEROALLERGENS

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ABSTRACT

The ragweed research program undertaken at The University of Michigan has continued to be conducted during the past year by an interdisciplinary team of allergists, botanists, meteorologists, and public health statisticians working together in close cooperation. This team effort has proven very useful in gaining new knowledge of the nature of the ragweed flowers, special studies with ragweed-sensitive patients, methods of sampling ragweed pollen, and preliminary studies for the test chamber.

Research along botanical lines has been concerned with the races of ragweed and their behavior under uniform conditions in the greenhouse; a study of the hybrid ragweed, Ambrosia artemisiifolia X trifida; the function of the flower and pollen discharge in common ragweed emphasizing the effects of temperature, humidity, light, and availability of water; and the biology of the pollen grain. Plans for the coming year include studies of the evolution and migration of ragweeds, the clumping phenomenon, and the chemistry of the outer wall layer of the pollen grains.

Basic immunological studies have been carried out by the allergists. Enzymatic digestion of skin-sensitizing antibody to ragweed was studied to see if partial digestion of the antibody might yield material promising more usual properties of antibodies. A demonstration of a quantitative relationship between skin-sensitizing antibody and antigen was also studied. Research has been done on the effect of ionization on allergic symptomatology using guinea pigs. A ragweed-sensitive dog is being studied in an effort to improve understanding of the problem in humans and in animals. The 1956 Jackson Prison Experiment was reviewed, giving some new insight into questions which will probably be answered in the future chamber experiments. Several extra-seasonal experiments are described using ragweed-sensitive patients exposed to known concentrations of ragweed pollen. Identification of molds and fungi is also discussed, along with plans for the next year's work.

Meteorological research was largely devoted to sampling techniques and waking with several new types of samplers—the flag sampler, roto-bar sampler, and an electronic pollen counter. Both wind-tunnel and outdoor tests have been conducted to check the accuracy of the new samplers. The preseasonal experiment of 1958, held at the Willow Run Field Station, is described in detail, stating the objectives and the instrumentation used. An analysis of some of the Jackson preseasonal data from the 1957 experiment has been included in this report. The in season experiment of 1958, consisting of the Jackson Prison sampling program, the Ann Arbor sampling program, and the roadside experiments, is also described in detail. Work has begun on a theoretical study of diffusion from a continuous

source utilizing the idea of a ragweed-free area and the effect of such an area on downwind concentration. The final plans for the test chamber and a discussion of the construction details are given. The plans for 1959 include a phenological study of ragweed, a test of the theory on the affect of a ragweed-free area, and continued testing of samplers.

The work of the statistical phase has been incorporated into the other sections; the statisticians have aided in the design of present and future experiments. It is planned to enlarge the statistical phase, especially in association with the human test-chamber experiments and design of these experiments. Data processing for use with the large computers will be done in the phenology study and in some of the botanical studies.

A list of papers published, accepted for publication, or submitted for publication, is included. Trips taken and talks given by members of the team are also listed. Acknowledgments are made to the staff of the State Prison of Southern Michigan at Jackson and to the staffs of the Allergy Research Laboratory, the Department of Botany, and the Meteorological Laboratories of The University of Michigan.

Abbreviated minutes of the regular meetings of the research team are included as an Appendix.

OBJECTIVE

The atmosphere is contaminated by two groups of substances, natural and artificial, depending upon their method of production and introduction into the air. Among natural contaminants are the aeroallergens, air-borne substances such as pollens, spores, rusts, and smuts which induce allergic reactions in sensitive individuals. There is evidence that some of these aeroallergens, notably ragweed pollen, which is one of the worst offenders, are becoming more widespread and more serious public health problems as a result of man's use of land.

A comprehensive program of research on an aeroallergen such as ragweed pollen requires an integrated study of the plant and its pollen, of the means by which the pollen is dispersed in the atmosphere, and of the fundamental nature and cause of the physiological reaction of sensitive individuals to it. The present investigation represents a fundamental attack on the problem in all its phases by specialists in allergy, botany, meteorology, and public health, all working in the closest cooperation.

1. BOTANICAL PHASE

by
W. H. Wagner, Jr.

1.1 INTRODUCTION

Collaboration of the botanists with allergists and meteorologists continued to bear fruit in 1958. Not only have we continued to work side by side on such problems as microtechnique, pollen identification, and field behavior of ragweed plants grown out of season, but the botanical investigations have been strongly slanted in the direction of answering questions posed by our colleagues. The specific results of studies by allergists and by meteorologists to which the botanists contributed in small or large part will be presented elsewhere in this report. The botanists will report specifically on the purely botanical aspects of work carried out in 1958.

The several problems to which the botanists devoted special attention during this year include the following: a preliminary investigation of the races of common ragweed (*Ambrosia artemisiifolia*) in terms particularly of their growth periods when grown under uniform conditions; a study of the hybrid formed under natural conditions between the common ragweed and the giant ragweed (*A. trifida*); a detailed description of the behavior of ragweed flowers as revealed by field and laboratory studies with special attention to the process of discharge of pollen; and, finally, the biology of the pollen grain with emphasis on the problem of germination of the pollen tube. These will be discussed in the following sections. The plans that have been outlined for research in 1959 will also be described.

1.2 RACES OF RAGWEED AND THEIR BEHAVIOR UNDER UNIFORM CONDITIONS

Botanists recognize several kinds or varieties of common ragweed (*Ambrosia artemisiifolia*) as well as a number of minor forms. The most important varieties, according to one recent treatment, are the following: the typical variety, var. *artemisiifolia*, a rather uncommon plant that grows along the eastern coast of the northeastern United States and Canada; the common weed variety, var. *elatior*, which occurs practically everywhere east of the Rocky Mountains; and the southern coastal variety, var. *paniculata*, which grows, at least sporadically, throughout the southeastern United States. There are some interesting questions concerning the nature of these varieties. For example, some authorities have treated them as being distinct species, and there is a question of whether and to what extent they should be interpreted as distinct entities. In their typical form, at least, they certainly appear to be distinct. The different varieties are also suited to very different climatic conditions and, evidently, possess different growth periods, as will be discussed below. Perhaps the most interesting question has to do with their original condition, before the arrival of the European in America: the extraordinary variability and vigor of the pernicious weed variety, var. elatior, which is the most serious hay-fever cause, suggests that it may, in fact, be of hybrid origin. Furthermore, var. elatior is apparently intermediate in most respects between the two others. Has man's upsetting of the natural habitats led to the development of var. elatior? These questions lead us to believe that a thorough botanical study of evolution and migration of ragweeds would be very profitable.

A small, pilot study was made by growing all three varieties together under uniform conditions at The University of Michigan Botanical Gardens in the summer of 1958. On April 8 and 9, seedlings were obtained in the field near New Orleans, Louisiana, of var. paniculata, and transported by air to Ann Arbor, where they were immediately planted at the Botanical Gardens. In Ann Arbor, seedlings of the stage of development of those taken near New Orleans (i.e., with two fully developed foliage leaves) are not found until the first week in May under natural conditions. The Ann Arbor variety of ragweed is var. elatior. Accordingly, young plants were started in cultivation in the greenhouse on April 18-20, so that they would correspond to the plants from Louisiana. For var. artemisiifolia we had to depend on Mr. J. S. Erskine of Wolfville, Nova Scotia, who found plants of this variety in the field of the same degree of development in the period June 6-17 and sent them airmail from several nearby localities so that they could be grown with the other varieties in the greenhouses in Ann Arbor.

The results of growing the three different varieties under like conditions were most revealing: The first plants to come into flower were var. artemisiifolia from Nova Scotia; by the middle of July most of the plants were in full anthesis. They were followed by var. elatior, which was in nearly full flower in the middle of August. The plants of var. paniculata, however, continued to grow and far overtopped the plants from Nova Scotia (much the smallest) and from Ann Arbor (medium size); they reached from 50 to 60 inches in height by the time they flowered, and the flowering time was the middle of September. These results indicate definitely that it is not the natural environment alone which determines the differences in growth and flowering of the varieties of common ragweed. On the contrary, there appears to be a "built-in" growth cycle quite independent of environment. These results, based, for each variety, on well over 50 plants, suggest that there are, in addition to the obvious morphological differences, profound physiological differences between the varieties, and indicate that further research on the periodicity of ragweeds will be of fundamental value. We suggest on the basis of our preliminary observations that perhaps it is not light intervals or photoperiod which determines the flowering of ragweed in any given site, but rather a genetically controlled growth period, characteristic for the variety. If this is so, the only result shortening day-lengths would produce would be to modify the activities of seedlings which developed late for a given locality; such late seedlings would be "hurried" to flowering at the normal time by the photoperiodic reaction to short days in the last half of summer. That "tardy" seedlings actually do exist in ragweed is confirmed by our field observations in the spring of 1958, which indicated that, while the bulk of germination of seeds begins in the third week in April in Ann Arbor, germination still takes place in a few individuals as late as the second and third weeks of May.

Morphologically, the plants from Nova Scotia were the smallest, the plants at flowering time ranging from 10 to 15 inches in height. Those from Louisiana grew four or five times as tall, while those from Ann Arbor were intermediate. The most striking difference between the extreme types was in the leaf shape: the leaves of the Nova Scotia plants were small and very coarsely lobed, while those from Louisiana were as much as six times as long, and very finely dissected and lobed, almost lace-like in some individuals.

1.3 THE HYBRID RAGWEED, *AMBROSIA ARTEMISIIFOLIA* X *TRIFIDA*

The hybrid between the common and giant ragweeds is one of the most striking of all weed hybrids because the parents differ strongly in habit, stature, leaf-form, and fruits. During the course of studying the parental species, the botanists found occasional examples of this hybrid and were able to make some observations on it that have not previously been recorded. The first record of this hybrid was in 1915 in a paper by Wylie[1] which has been overlooked by many botanists. Wylie found a single plant of the hybrid on a roadside north of Iowa City, Iowa. Although it produced flowers in abundance, no seeds were formed, even though he observed the plant closely until autumn. Since this original description, various observations on *Ambrosia artemisiifolia* X *trifida* have been published. For example, K. L. Jones[2] recorded a natural hybrid found in 1936 along the banks of the Huron River near Ann Arbor, Michigan. Jones also carried out genetic experiments involving this hybrid under artificial conditions. Among the significant results of his experiments was the fact that the 12 chromosomes in the haploid set of *A. trifida* are recognizably larger than the 18 from *A. artemisiifolia*. Although the pairing between chromosomes that occurred in the hybrid was irregular, such pairing as occurred involved almost entirely chromosomes of similar size. Therefore it was assumed to have been contributed by the same parent. This is considered by Stebbins,[3] in a review of species hybrid in 1945, to be a striking example of pairing between chromosomes contributed by the same parent in species hybrids involving polyploidy.

The hybrid was redescribed by Rouleau[4] in 1944 under the binomial *Ambrosia* X *helenae*. In 1939 the cross was found at Cauderan (Giroude), France, where the parent species have been introduced. In 1950 the intermediate was taken at Urbana, Champaign County, Illinois. Those hybrids which have been recorded have been found in disturbed habitats. Those which we have collected were found along the sides of newly built roads and on construction sites in and around Ann Arbor, in 1956, 1957, and 1958.

The hybrid may pass unnoticed, and may even be confused with other species, despite its peculiar appearance. There are probably unrecognized specimens in various herbaria. To illustrate, a specimen of the hybrid from Pawnee, Oklahoma, was found in the herbarium of Tulane University identified as A. psilostachya, the "perennial" or "western ragweed." Another specimen, heretofore confused with another species, was found in the much-used herbarium of The University of Michigan Biological Station; it bore the identification A. artemisiifolia, probably because the botanists who found it did not know the characteristics of the hybrid, and confused it with a very coarse form of the common ragweed. We now have records that the hybrid has been found in Iowa, Illinois, Oklahoma, Michigan, Quebec, and France, but the hybrid will probably be found in many other places.

The difficulty of recognizing the hybrid may result in part from the extraordinary variation in successive leaves produced from spring through fall. The early leaves are roughly like over-sized leaves of A. artemisiifolia, but the later leaves formed on the main and lateral axes are not directly comparable to those of either parent, having rather large but narrowly three-lobed or simple blades. By late summer and fall, all the spring leaves have usually fallen or completely dried up, so that only the more simple leaf types of the distal parts of the plant remain. At this stage the plant looks something like a narrow-leaved form of A. trifida.

To attempt to show all the normal foliar variations of this hybrid, we studied a single plant, as nearly average as possible—from cotyledons to highest bract. A natural hybrid was found growing with the parents on May 18, 1958, on a construction site at the University Hospital, Ann Arbor. With as little injury to the plant as possible, it was extracted from a difficult position in a crack along the sidewalk and turned over for cultivation to Mr. Walter F. Kleinschmidt of The University of Michigan Botanical Gardens. A sample leaf was removed from each node just before it dried and fell off naturally. The results of this "pruning" of the old leaves of successive nodes are shown in Fig. 1.

The cotyledons and the first foliage leaves are readily distinguishable from the corresponding leaves of A. trifida. On the fourth to eighth nodes the leaves look somewhat like extremely gross leaves of A. artemisiifolia and their margins are lobed and toothed (Fig. 1, especially the second row). By the time the sixth pair of foliage leaves has appeared, there are already lateral branches growing from all except the lowermost nodes, a feature especially characteristic of A. artemisiifolia. At approximately the middle level of the plant, the opposite leaf arrangement gives way to alternate, in this respect also as in A. artemisiifolia rather than in A. trifida, which has opposite branching throughout. The leaves in the upper half of the plant tend to become simpler in structure and finally, in the top five nodes, to become narrowly three-lobed to nearly simple, the margins now practically smooth.

Fruits have apparently not been found previously in Ambrosia artemisiifolia X trifida and this constitutes the first report of them. A natural hybrid, cultured in 1957 under particularly luxuriant conditions in the greenhouse and permitted to develop into an unusually large specimen, was grown side by side with numerous pollinating plants of A. artemisiifolia. By the latter half of September,

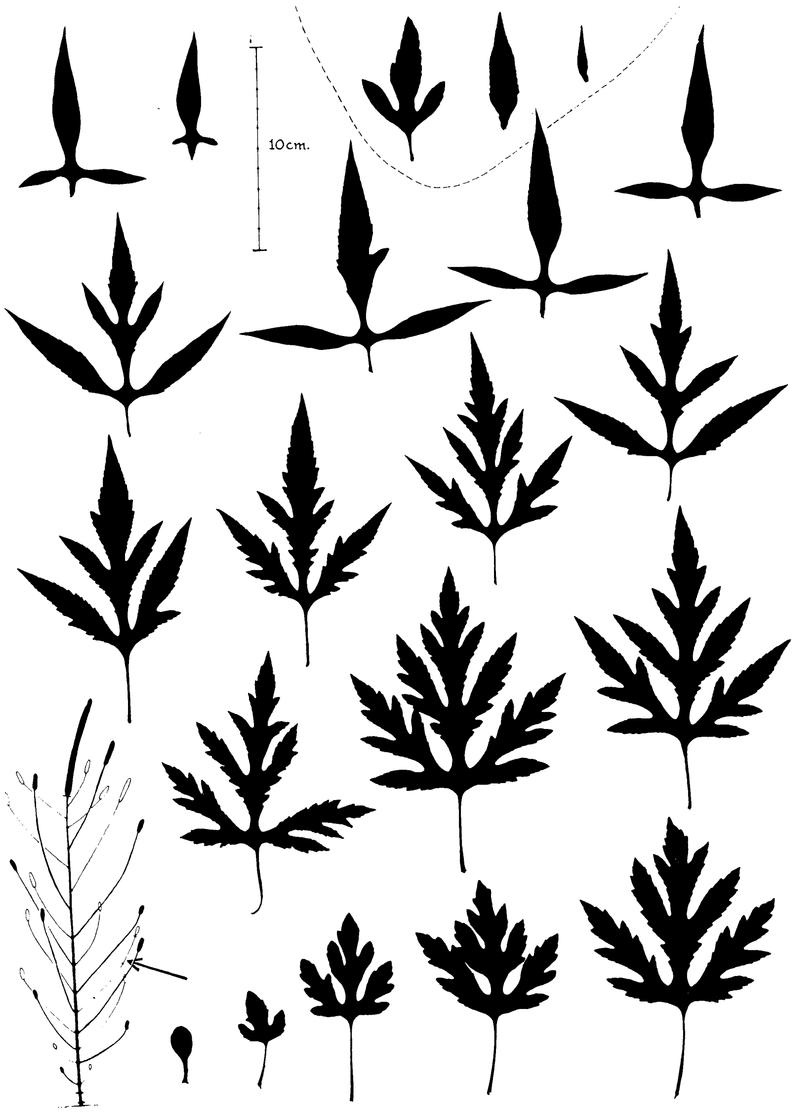


Fig. 1. Leaf variations of Ambrosia artemisiifolia X trifida. Lower left: major branches of "average" plant 50 inches tall, showing successive nodes where leaves were removed. Silhouettes of successive leaves arranged left to right—bottom row, nodes 1-5; second row, nodes 6-8; third row, nodes 9-12; fourth row, nodes 13-16; top row, nodes 17-18 (18 at base of terminal spike). Inset: successive leaves from lateral branch shown by arrow in habit diagram.

the hybrid produced approximately 20 fruits, perhaps stimulated to form by the pollen from the parent species. The fruits, the outer walls of which are involucral in origin and appeared superficially to be normal, represent good intermediates between the rather different fruits of the parents, as shown in the scale drawings in Fig. 2. The fruits of A. artemisiifolia average usually 4-5 mm in total length, with a narrowly constricted beak that makes up one-third to one-half the length. Those of A. trifida are much larger, approximately double in overall length, 7-10 mm, with a more broadly based beak that comprises one-fifth to one-fourth of the total length. The fruits of the hybrid are variable but all those

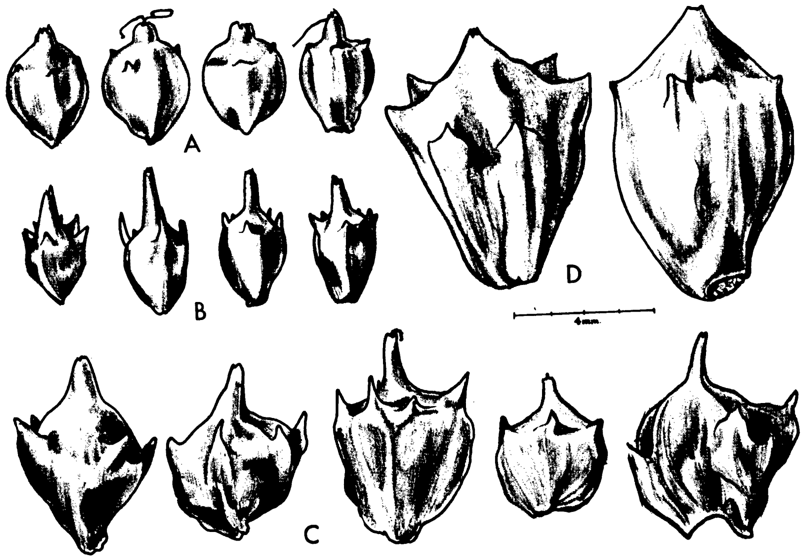


Fig. 2. Fruits of ragweeds. A, Ambrosia coronopifolia; B, A. artemisiifolia; C, A. artemisiifolia X trifida; D, A. trifida.

which did develop on the plant were intermediate both in size and shape between those of the parents. The only major deviation is that the hybrid fruits tend to be more deeply channeled, a feature no doubt related to the collapse or failure of development of the achene within. An attempt was made to germinate some of the hybrid fruits following after-ripening during the winter of 1957-58, but without success. Both the pollen and the fruits, when formed, of this hybrid are probably ineffective in its reproduction.

Because of the great rarity and sporadic occurrence of this hybrid ragweed and because of its probable inability to reproduce or even survive more than one summer by any means, we are inclined to designate it by formula only, i.e., as Ambrosia artemisiifolia X trifida, rather than by formal binomial nomenclature, as A. X helenae Rouleau. There is considerably more justification, perhaps, for designating another ragweed hybrid, A. artemisiifolia X coronopifolia with a taxonomic binomial as A. X intergradiens[5] because the latter hybrid is found frequently in many counties of Michigan (and probably elsewhere) and has the ability to form very large, perennial populations through the years by way of root proliferations.

1.4 FLORAL FUNCTION AND POLLEN DISCHARGE IN COMMON RAGWEED

Ragweeds illustrate strikingly the reduction of flowers in connection with wind pollination. The ancestors of ragweeds were probably typical Compositae like the sunflowers (Helianthus), plants pollinated strictly by insects, the flowers bisexual, pollen in relatively small amounts, and with mechanisms adapted to pollen transfer by insect visitors. The ragweeds in contrast have evolved much reduced, unisexual flowers. The staminate flowers produce great amounts of pollen to be dispersed in the atmosphere by the wind, and the floral structures, homologous to those in other Compositae, now function primarily to present the pollen to the air mass. Our purpose in making this study was twofold—to determine what the stages are in pollen release by the staminate flower, and to determine whether these stages show any correlations with certain environmental factors. The information outlined below represents a considerable advance in our knowledge over that presented in the last report.

We were able from our observations to delimit six more or less independent stages of the staminate flower: (1) maturation of the flower; (2) extension of the pollen sacs; (3) opening of pollen sacs and pollen release; (4) flotation of the pollen; (5) extension of the central structure of the flower—the pistillodium; and (6) withdrawal of pollen sacs and pistillodium and closure of the flower. Our study of environmental factors was focused especially upon the stages of extension and of opening of the pollen sacs. It was hoped that in emphasizing these two stages we could develop preliminary ideas to explain why the amount of airborne pollen seems to fluctuate with varying meteorological conditions, as is commonly reported by allergists and ragweed-sensitive individuals. The external factors which we considered were temperature, humidity, light, and water supply. The physiological phases of this study were carried out by D. E. Bianchi and D. J. Schwemmin.

The common or low ragweed was chosen for study. The extra-seasonal field plot was the source of data for out-of-door conditions. Our methods for obtaining plants under these conditions have been described in previous reports. The plants used in the laboratory studies were treated like those for the extra-seasonal field plot with the exception that the planting was planned so that new lots of mature plants would be available at three-week intervals throughout the summer. A given lot of plants was found to produce pollen for two or three weeks.

Observations on the various stages of flower development and pollen release were made using a hand lens and a stereoscopic dissecting microscope in the field and in the laboratory. It was discovered that the processes of anther extension and pollen release could be observed by dissecting out a single flower at the proper stage and placing it on a slightly moistened paper on the stage of the microscope.

The opening of pollen sacs in the field under different meteorological conditions was quantified by making counts of flowers at definite intervals. Ten of the plants were selected and the number of "dehisced" flowers in the main spike counted at one-half hour intervals. The counting was ordinarily carried out from 4:30 a.m. to noon, except for one day in which the full 24-hour cycle was followed. The changes in temperature, humidity, and solar radiation were measured by the meteorologists at the same time.

Two methods were adopted for treating the flowers in the laboratory. One was to remove individual heads with their involucres from the spike and float them in a small volume of White's mineral solution. In this way the flowers

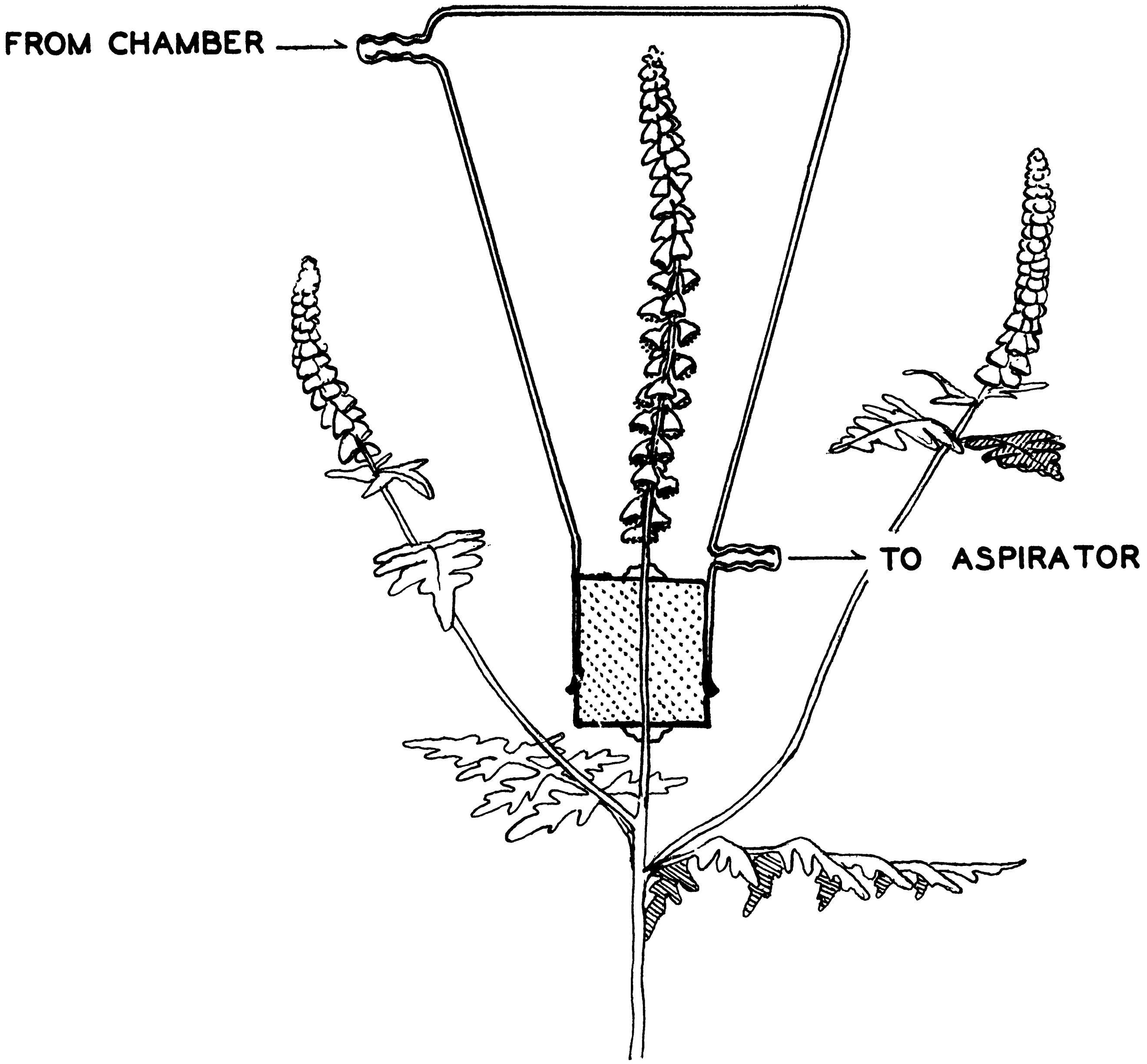


Fig. 3. Method for controlling relative humidity in experiments on pollen discharge.

could be examined in Petri dishes or in deep-welled glass slides which permitted easy control of the temperature and humidity. The second method was to introduce the main flower spike into a flask of water placed upside down over it, as shown in Fig. 3. The flask was sealed by means of a cork which had been cut so as to allow the stem to pass down through a small hole. Lanolin was used to make a watertight seal. The flask was equipped with two side arms which facilitated the removal of the water at the beginning of an experiment, and allowed a continuous circulation of air at a desired temperature and relative humidity.

A glass-enclosed chamber, 3 by 2 by 4 feet, was equipped with a forced air inlet, so that the conditions surrounding the spike could be made uniform. The air was passed through a Y tube, one arm entering a column of silica gel desiccant, the other into a gas-sparging bottle containing water. By regulating the rate of air flow through the desiccant and the water, a given relative humidity could be produced. Air was then continuously drawn from the chamber, through the flask containing the flower spike, by means of an attached water-pump aspirator. The temperature and relative humidity were measured at the outlet of the glass chamber, by means of a constant recording hydrothermograph. In the experiments performed on detached involucres at various relative humidities, the heads were placed on a glass slide in a Petri dish. The slides were suspended above saturated salt solutions with which different relative humidities could be created. The temperature was controlled by placing the Petri dishes in incubators.

The staminate involucres or cups of the ragweed with their flowers are interpreted as modified and secondarily anemophilous heads of the basically entomophilous type found generally in the family Compositae. The pollen-producing or staminate flowers are between 1.0 and 1.5 mm in length and 0.7 and 1.0 mm in greatest breadth. There is no calyx, the only whorl of the perianth being the tubular corolla, narrowed toward the base and broadened in the upper half as shown in Fig. 4A. The flowers are tightly closed except at the actual time of pollen discharge. The anthers, normally five in number, are partially joined to one another laterally. Each anther is composed of four pollen sacs and terminated by a folded-over apical appendage. Between the stamens there extends through the middle of the flower a columnar structure, the pistillodium, capped by a whorl of clavate hairs. The pistillodium is considered to be a modified vestige of the gynoecium of the flower (the ancestral form of which was bisexual), the ovary having now been practically eliminated in evolution. This interpretation is supported by comparative morphology, as well as by teratological flowers that are more or less intermediate in form between typical staminate and typical pistillate flowers such as those which have been illustrated in the work of K. L. Jones.[6]

The fully formed staminate flowers of one head number from 5 to 25 or more, the oldest ones occurring around the periphery, the youngest in the center. Most of the heads that we have examined have several very small and abortive floral primordia in the middle, concealed by the normally developing flowers and not readily seen without dissection. We were not concerned in the present study with the ontogeny of the flowers but investigated only those flowers which had matured and were ready to undergo anthesis.

Early in the morning, roughly between 4:00 and 6:00 a.m., the flower (or flowers) which will discharge pollen undergoes what appears to be a slight enlargement of the swollen apical portion of the corolla tube. This flower then becomes distinguishable from its neighbors in the head. The corolla lobes are still tightly closed. The anthers at this time have pushed forward against the translucent corolla lobes, making the flower which is ready to discharge appear a distinct yellow. The tightly closed corolla lobes which together form a nearly flat cap on the flower (Fig. 4A) now "crack" or break apart slowly, exposing the folded apical appendages of the joined anthers as a shiny, nearly smooth, chrome yellow surface. At this period the cells of the filaments are apparently under-

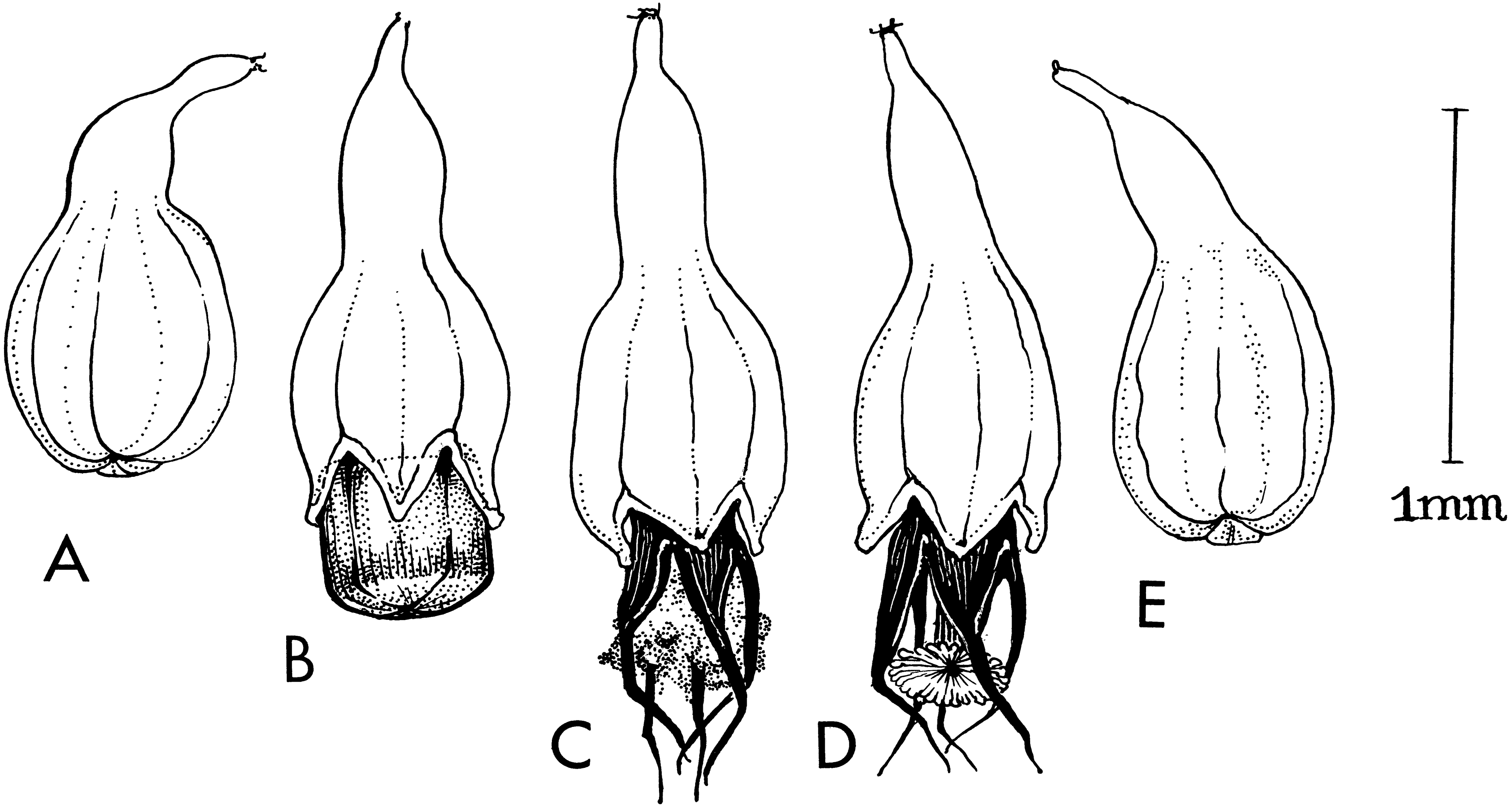


Fig. 4. The stages in the release of pollen. A, the mature flower; B, the extension of the anther sacs; C, the dehiscence of the anthers; D, the extension of the pistillodium; E, the closure of the flower. Closed pollen sacs partly stippled and open sacs in black.

going rapid enlargement, presumably by water uptake, and the whole extension process may take place in only a few minutes. The anther mass is extended forward until it is nearly entirely exposed as a short, barrel-shaped body (Fig. 4B). This completes the stage of extension of the anthers. The laboratory study of the effects of temperature, to be described below, was concerned primarily with this stage.

After full extension of anthers, under normal conditions, the process of discharge of pollen grains is initiated by a longitudinal pleating or channeling of the outer anther walls, the channels at first shallow and then deepening. Soon the apical appendages of the anthers unfold more or less rapidly, and the pleating of the sacs becomes extreme, suggesting that strong internal forces are developed within the tissues of the anther walls. The pollen is freed by this folding process, and the numerous grains are squeezed into a flat-topped or irregular mass, flanked on five sides by the now pleated pollen sacs and their nearly erect appendages (Fig. 4C). This, the stage of dehiscence, was the one in which effects of relative humidity were observed in the laboratory.

The pollen falls in clusters from the flowers. The grains at this stage are strongly adherent and form bright yellow blotches later separated and blown away by the wind on subtending foliage. It should be stressed that there are, on what we take to be normal days, three distinct steps before the actual dispersion of pollen by the air mass: (a) the ejection of pollen in clumps from the flowers; (b) a period, long or short, of adherence of the pollen clusters to adjacent vegetation; and (c) the flotation of the pollen by the wind.

During the next one or more hours of the morning, the pistillodium, which previously extended only up to the base of the anthers and was invisible externally, protrudes and ultimately extends well beyond the morphological limits of the pollen sacs, but not so far as the tips of the appendages (Fig. 4D). The behavior of the pistillodium is variable in time of appearance and orientation with respect to the pollen sacs. Commonly the pollen sacs may withdraw completely, leaving the pistillodium projecting free from the flower. Our knowledge of the activities of the pistillodium is still far from conclusive, but it may be hypothesized that it may well play a role in "sweeping out" such pollen grains as still remain in the flower after the initial opening of the anthers. A similar role of the style has been described for other Compositae by previous writers.

The end of the process of anthesis is accomplished the same day. This involves a full withdrawal of the anthers and the pistillodium back into the corolla, and the final closure of the corolla lobes (Fig. 4E). At this final stage, except for an entirely pale-gray or whitish color and some tendency toward corrugation of the sides of the perianth, the flower has much the same appearance it had prior to anthesis. The flower, so far as we have been able to observe, never opens again; and the tip of the corolla will therefore remain below the level of the flowers of the same head that open on subsequent days.

Our study of the plants in the experimental field plot revealed that there is a definite and well-marked diurnal periodicity in the dehiscence of the ragweed flowers. This period of activity always took place in the morning. The onset of the discharge was characterized by the actual "cracking" or partial separation of the corolla lobes described above, which was detected beteen 6:30 and 8:00 a.m. each day. Extension of the anthers, dehiscence of the pollen sacs, and release of the pollen followed in rapid succession. The time required for the number of opened flowers to reach its maximum for the day varied from one-half to two hours. The actual counts of opened flowers throughout three different mornings are given in Fig. 5. As shown there, the whole process can be initiated and completed quite suddenly, as on June 19, or can be more gradual, as on June 20 and 22.

The release of pollen from the ragweed flowers takes place at a time of day when there is a regular alteration in a number of environmental factors. Light, temperature, and relative humidity were given specific consideration in the field studies. In Fig. 6, the changes in temperature and relative humidity are compared with floral activity. A rise in temperature and reduction of relative humidity characterizes the time intervals during which active dehiscence occurs. There was also a sudden rise in the amount of solar radiation reaching the plants during this period, which may have been responsible for the sudden alteration in the other two factors. On the remaining days, when the flowers opened at a reduced rate, there was also a more gradual change in light, temperature, and relative humidity during the critical period.

Effects of Temperature.—Temperature effects were determined in the laboratory by the use of excised flowers. It had been observed that, if a spike of flowers was removed from the ragweed plant and placed in a small volume of White's mineral solution, the flowers would continue their ordinary diurnal release of pollen through several cycles. This was true also of excised heads, floated on the mineral solution in depression slides. If the depressions containing the heads were covered with a glass microscope slide and sealed with vaseline, the flowers proceeded to a stage where the anthers were presented but not dehisced. The relative humidity of the environment was near saturation. When the cover slide was removed, the pollen sacs of the fully extended flowers would begin to dehisce within a few minutes. Since the behavior of the flower in vitro seemed to approximate that on the intact plant, we were in a position to perform controlled experiments under more convenient conditions than in the field.

If the excised, floating involucres were incubated in the dark for 24 hours at 4, 15, or 20°C, the presentation process would not take place. This was in contrast to the heads that were maintained at temperatures in the greenhouse and which bore fully extended flowers at the usual time the next morning. When flowers incubated at 15 or 20°C for 24 hours were placed at elevated temperatures, the extension of the anthers would take place normally. However, the flowers which had been incubated overnight at 4°C could not be induced to extend by exposure to high temperatures, and the inhibition was, therefore, apparently irreversible. The rate at which the flowers extended depended both upon the temperature of the 24-hour incubation period and upon the temperature of second exposure.

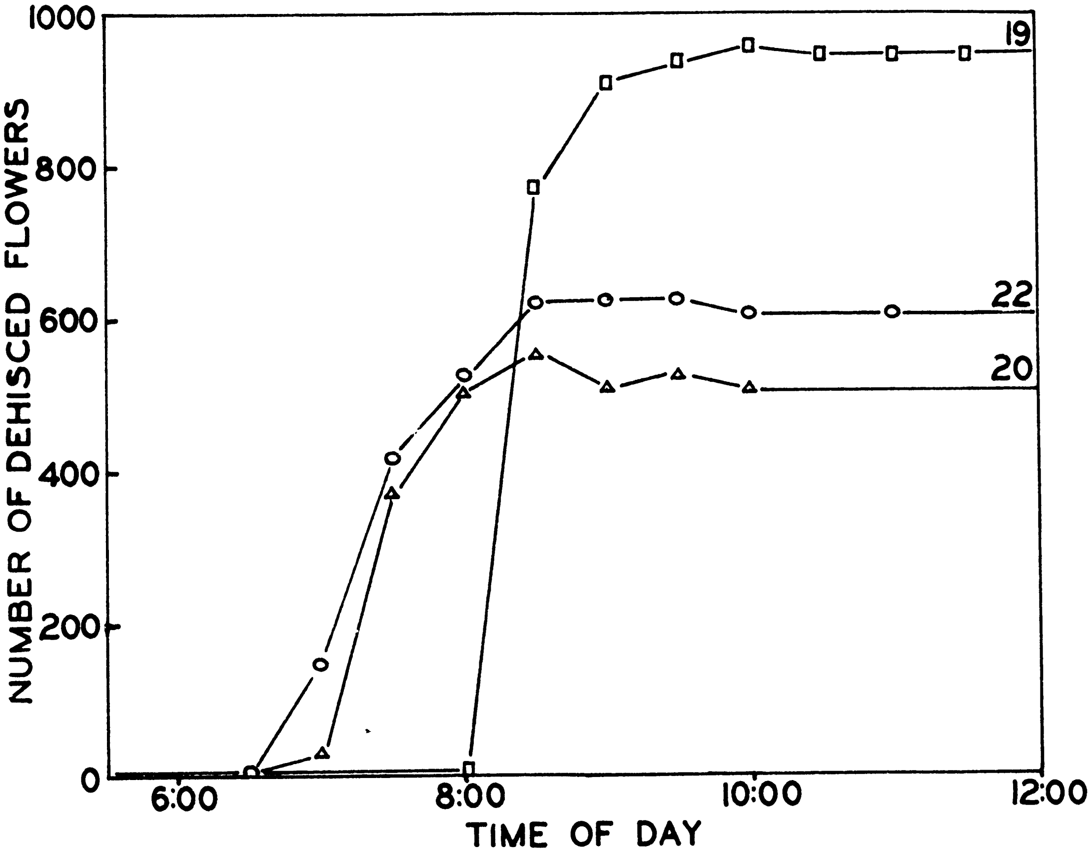


Fig. 5. Time of pollen release in the field. Squares indicate June 19, 1958; triangles, June 20; circles, June 22.

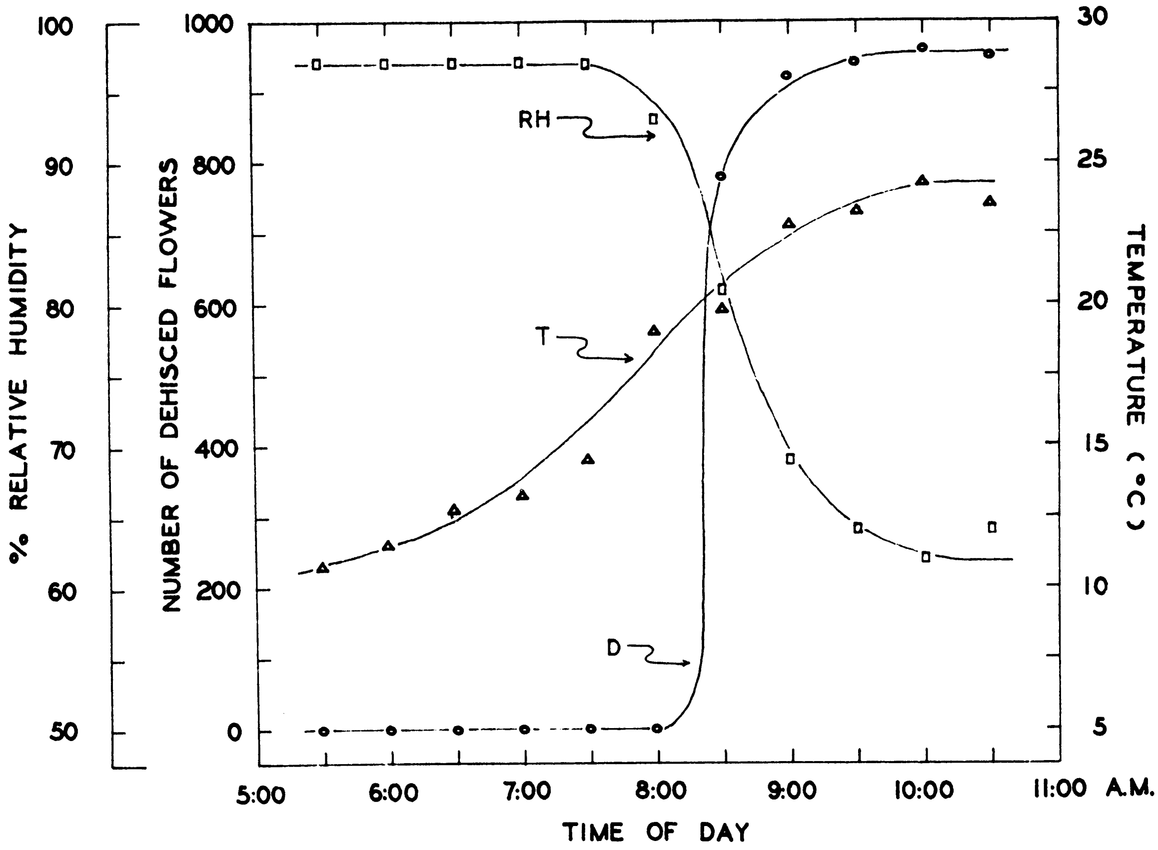


Fig. 6. Dehiscence in relation to temperature and relative humidity in the field. Triangles represent temperature in Centigrade degrees; squares, relative humidity; and circles, number of dehisced flowers.

Flowers incubated at 20°C for 24 hours would extend readily when moved to either 30, 35, or 40°C. The number of fully extended flowers reached its maximum after 100 minutes, as can be seen in Fig. 7. If the 24-hour incubation was carried out at 15°C, the results were slightly modified. In most cases, the flowers failed to extend when transferred from 15 to 30°C. This is illustrated by the data in Fig. 8. At 35 and 40°C, however, extension readily occurred, but it showed a lag in reaction compared with those flowers that were incubated at 20° for 24 hours.

Effects of Humidity.—Studies were made of both intact plants and isolated heads to examine the effects of humidity. Whole plants were prepared the day prior to experimentation by submerging the main inflorescence of the plant in a flask of water as described above. This treatment caused no noticeable change in the performenace of the plant except that it stopped the actual dehiscence of the pollen sacs. The plants with their inflorescences submerged were allowed to remain in the greenhouse overnight, and by morning the anthers would be fully extended but not open.

The flasks were then attached to the exhaust line from the humidity chamber, the water removed from the flask, and the air of a desired relative humidity was drawn through the flask. During the ensuing period the relative humidity, temperature, and number of dehisced flowers were recorded. As the percentage of moisture in the air was decreased, the rate of dehiscence of anther sacs was increased, as shown in Fig. 9. At 20% relative humidity, half of the flowers releasing pollen had done so in 53 minutes, whereas at 60% relative humidity, half of the flowers released their pollen only after 123 minutes, well over twice as long. At 80% relative humidity, under the conditions of these experiments, none of the flowers had released pollen after three hours; however, the pollen was released in only 30 minutes when the humidity was then dropped from 80 to 15%.

Since the temperature of the humidity chamber could not be controlled, further experiments were carried out using detached heads in Petri dishes. The inflorescences to be used were submerged in water the day before experimentation as in the previous experiments. The next morning the inflorescences were removed from water and the heads quickly removed from the spike and dropped in a beaker of water to prevent dehiscence. When the experiment was to be run, the heads were removed from the water and placed on a dry glass slide which was suspended in a Petri dish over saturated salt solutions of different concentrations. These salt solutions were so composed as to offer a range of various relative humidities within the Petri dish. The dishes were then placed in various incubators ranging from 4 to 30°C, and the number of dehisced flowers was recorded over the following time period. As shown in Fig. 10, the rate of dehiscence is affected both by temperature and relative humidity. The rate of dehiscence is thus inversely proportional to an increase in relative humidity and directly proportional to an increase in temperature.

Effects of Light.—All attempts to alter the diurnal periodicity of dehiscence in the laboratory by changing the light environment were without success. Plants

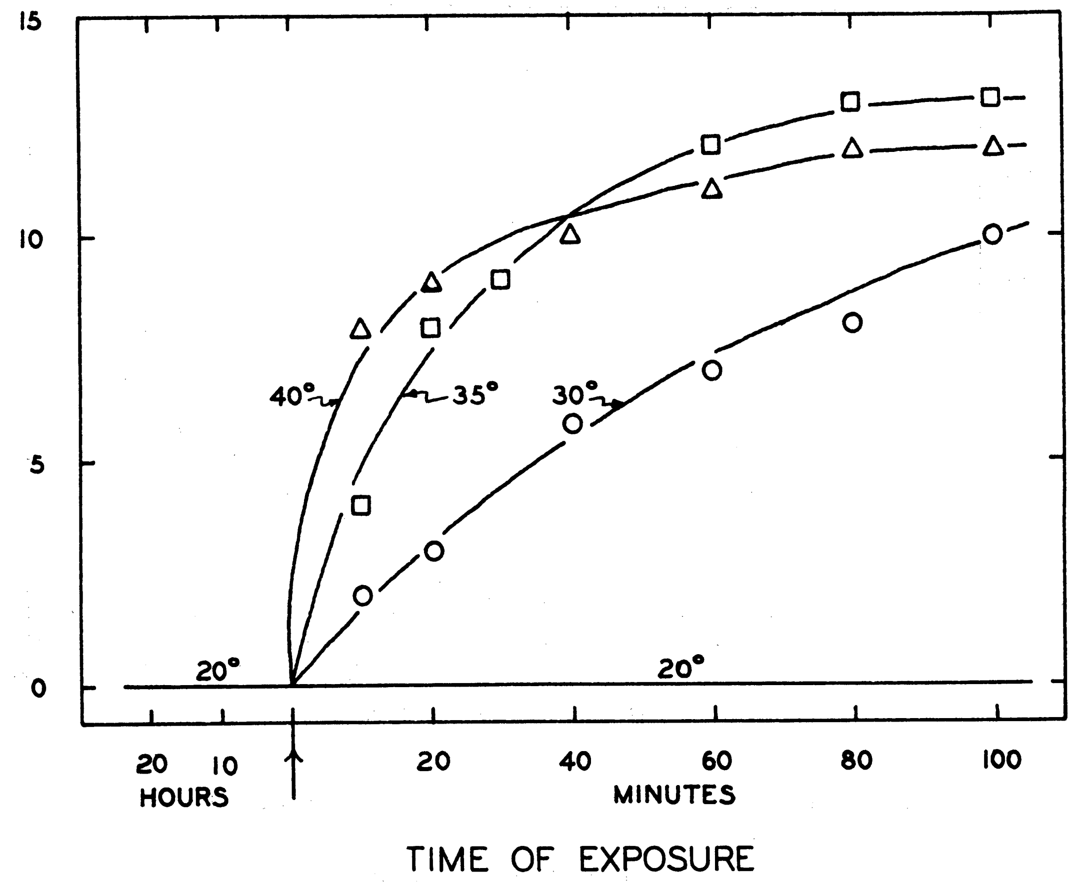


Fig. 7. Experimental effects of temperature on pollen sac extension (excised heads). Flowers were kept at 20°C for 24 hours prior to exposure at the temperatures indicated.

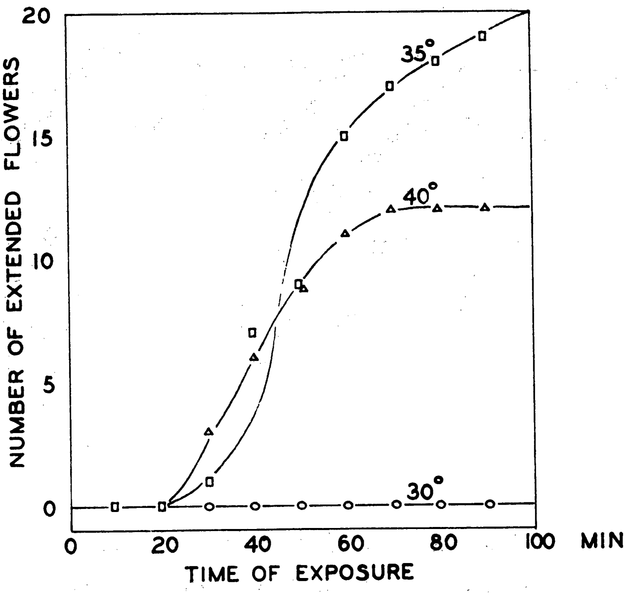


Fig. 8. Effects of prior low temperature on pollen sac extension (excised heads). Flowers were kept at 15°C for 24 hours prior to exposure at the temperatures indicated.

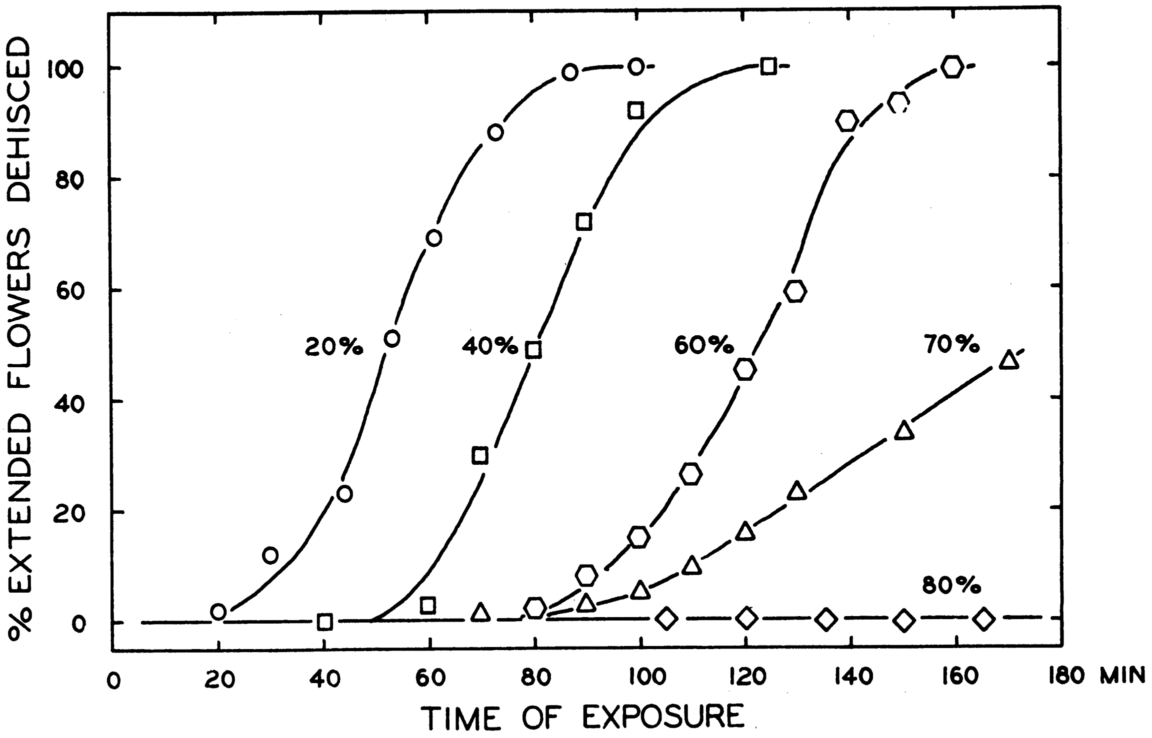


Fig. 9. Effects of humidity on the time and rate of anther dehiscence. Percentages indicate the relative humidities to which the flowers were exposed.

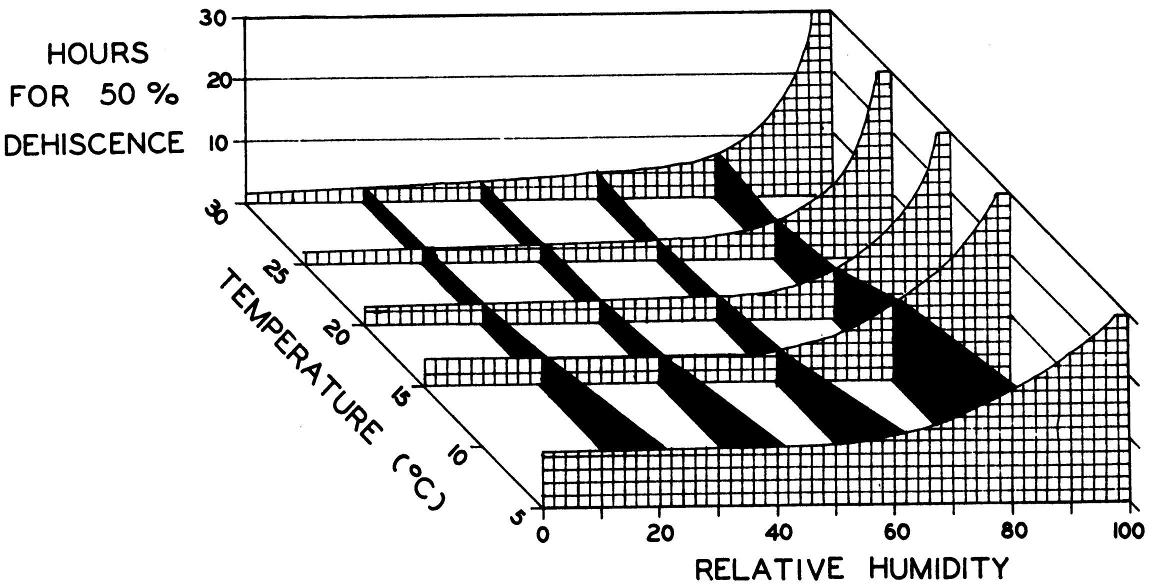


Fig. 10. The combined effect of temperature and relative humidity on excised anther dehiscence.

were kept under constant illumination for approximately two months without any visible alteration in the pattern of pollen release. Flowers of the plants under constant illumination extended and dehisced at the same time as the control flowers on plants under a natural day-night regime. The number of flowers releasing pollen also was not influenced. Equally interesting is that plants that were in total darkness maintained their normal diurnal periodicity for one week. Thus light appears, at least in the conditions of our trails, to have no immediate effects on the release of pollen in ragweed.

Effects of Availability of Water.—The final external factor we considered was the availability of water to the plant. During a typical rainless day of the normal ragweed season, the plants often become very wilted due to the high rate of transpiration which exceeds the uptake of water into the root system. It is conceivable that this diurnal wilting could influence the development and release of the pollen. To test this hypothesis, we placed ragweed plants under two different conditions: one set of plants was kept well watered so that wilting did not occur at all; a second set was allowed to wilt, but was watered enough to prevent the plants from dying. Production of pollen was checked daily on these plants over the period of a week. During that time both the turgid plants and the wilted plants continued to release pollen in a normal way. It was concluded, therefore, that wilting per se is not an important limiting factor.

Comments.—The peculiar features of the staminate flowers of ragweed, i.e., those features in which they differ from most other flowering plants in other families with wind-pollinated, unisexual flowers such as Populus, Betula, Quercus, Amaranthus, and Chenopodium, are primarily those which characterize the family Compositae to which the ragweed belongs. The major distinctive features are (a) the grouping of numerous tiny flowers in heads; (b) the fusion of anthers, which function together as a unit; (c) the closed tubular corolla which opens only when the pollen is discharged;(d) the vestigial gynoecium or pistillodium; and (e) the tendency of the pollen grains to stick together and fall as clumps on adjacent vegetation prior to reflotation by the wind. We have been impressed by the rapidity with which the anther mass is extended at the time of anthesis—a process which may take place, under certain conditions, in less than 30 minutes and sometimes as little as 10 minutes. The pistillodium extends more slowly, and its role is still not clearly defined, although the likelihood is that it functions as the "sweeper" of the last remaining pollen grains in the open space between the dehisced anthers. The extension of both anthers and pistillodium is a process that probably depends upon rapid uptake of water and the associated enlargement of the cells of the stalk-like filaments and style upon which these structures are borne. The elongation of the filaments results in the complete emergence of the mass of the anthers from the mouth of the corolla. The effects of temperature on pollen sac extension which we found may be due to its influence on the rate of water uptake. Further study on this question would be desirable.

Our observations on flowers kept at a relative humidity near saturation indicate that temperature is effective in controlling the rate of extension—extension is stopped altogether when the flower is incubated at low temperatures. The effect of relative humidity is primarily to control the opening process. The anthers may be fully extended, but unless the proper conditions of relative humidity prevail, the pollen sacs will simply not dehisce. We were able to keep the pollen sacs closed either by immersing them in water or by keeping them in conditions of high relative humidity. It seems quite likely, therefore, that the mechanism involved in dehiscence is desiccation of the anther sac walls, resulting, as it does in so many sporangia of land-plants, in the deformation of individual cells in the wall tissues by changing water tensions. These tensions result in the complicated pleating and folding that breaks apart the walls and exposes the pollen. Drying would depend upon a decrease in relative humidity, and the amount of evaporation of water from the tissues of the pollen sac walls would increase accordingly. Moreover, temperature may indirectly affect the process by influencing the evaporation rate. As we were able to find no direct effects of light, we conclude that its role is secondary. Both of the significant effects that we found—the prevention of anther extension by incubation at reduced temperatures and the inhibition of anther dehiscence by high humidities—were reversible under most conditions and the normal sequence of changes was observed when the temperature or humidity was returned to more standard values.

It should be emphasized that the experimental observations reported here were carried out, in the main, on excised structures and not on the intact plant. It seems, therefore, that further study with intact plants is necessary before the exact role of the factors of temperature and humidity can be established. Nevertheless, we expect that our conclusions are applicable to field plants in at least a general way because they correspond rather closely to the observations made in the field on intact plants: the diurnal alterations of temperature and humidity correlate with the opening of flowers under field conditions and the field correlations correspond with the general predictions that could be made on the basis of our laboratory results.

1.5 BIOLOGY OF THE POLLEN GRAIN

Earlier cytological studies of Ambrosia pollen[5] suggested that possibly as much as 60 to 80% of the pollen might be viable, i.e., capable of germination and development of the pollen tube. Members of the family Compositae, to which the ragweed belongs, are reputed, however, to be poor objects for laboratory germination, and the amount of germination that has been observed in artificial media has been small and variable. Mr. T. F. Beals has attempted to study the germination of pollen, both on the living flower and on artificial media. The preliminary results of his study are as follows.

Germination on the living plants was studied by artificially dusting pollen upon the flowers, waiting for four hours to permit sufficient time to elapse for germination to occur, then removing the relevant plant parts and staining them in such a way as to demonstrate the pollen grains and their tubes under the microscope. Under natural conditions the pollen grains fall on the stigmas of the female flowers, the latter located in the axils of leaves, usually around 6 (2-12) per axil. The receptive structure comprises a low nonreceptive area, the style, and two terminal ribbon-like structures with numerous hairs on their surfaces, the stigmas. Once attached to the hairs, the pollen grain germinates and a narrow projection, the pollen tube, grows from the grain into the stigmatic hairs and passes into the tissues of the stigmatic axis, the style, and finally into the ovary at the base of the flower, where the tube releases its sperms in the embryonic seed and fertilization takes place. In this study only the germination was observed. It was found that this process would take place readily but that only pollen that had been freshly discharged from the pollen sacs would develop actively. The period of active germination after discharge was estimated as the first five hours, after which the viability of the grains rapidly decreased.

Once this limitation was established, it was possible to secure good germination under culture conditions _in vitro_. A variety of different culture media were tried, but it was found that a 15% sucrose solution made with tap water and solidified with 2% agar provided the most practical and effective medium for the germination of ragweed pollen.

It should be emphasized that, although germination does occur under the culture conditions we have adopted, the growth of the pollen tube is incomplete and typically terminates in the bursting of the tube. In fact, the largest tubes in our cultures have reached only 40 microns, considerably less than occurs normally in the natural state. Nevertheless, certain facts have emerged concerning germination _per se_ which seem significant.

The first of these has to do with the effect of temperature. It was found that germination was maximal at 30°C with marked decreases in germination at both higher and lower temperatures. Germination was completely inhibited at 4°C. Variations in the acidity of the medium seemed to have but little effect over a rather wide range of pH 5.5 to 8.5. Light apparently has no effect, since germination occurred both in the presence and absence of light.

The most significant observation concerning germination has already been suggested above—the age of the pollen grains is very important. Only the reasonably fresh pollen shows any great degree of germinability. The preliminary tests indicate that the time of maximum germinability is four hours after the pollen has been discharged from the anthers. There is a low level of germination at the time that the pollen leaves the anthers and there is a return to a low level of germination after the first six hours. It is most interesting that the low level of germinability of older pollen continues for periods of twelve months or more. Thus there seems to be a peak of germinability during the first several hours after the pollen is released, followed by a rapid decline to a low level which continues for many months. The maximum amount of germination is approximately 60%, and the persistent low level after the peak period is about 5%.

Further and more detailed investigations of the problem of pollen germination are contemplated.

1.6 PLANS FOR 1959

In planning our 1959 research, we have assumed that specialized personnel will be available for the work involved. There is, however, some possibility that appropriate or sufficient aid will not be available.

The study of pollen grains and related subjects will be a special point of emphasis in 1959. First, our studies of pollen samplers and slides have suggested that it would be well to broaden the basis of identification, to include, especially, fungus spores. For this purpose one of the graduate students in the Department of Botany, Mr. Alan Graham, an expert in palynology, will act in the role of advisor and aid to the meteorologists and allergists.

For two reasons—(1) the possible connection of sticky substances on the fresh pollen grains with allergenicity, and (2) the furtherance of our knowledge of the biology of ragweed—we intend to investigate the clumping phenomenon of the pollen in greater detail if the proper personnel are available. It will be recalled that one of the stages in discharge of the pollen which we described involves the falling of the pollen in clumps which attach themselves to adjacent vegetation. We wish to determine what the effects of environmental factors are on clumping and, if possible, what the chemistry is of the outer wall layer of the pollen grain. We also plan to continue our studies of the germination of the pollen grain, emphasizing periodicity and effects of external factors.

In connection with the flower and floral function, we hope to make further studies of periodicity in the giant ragweed compared to that in common ragweed. Also, we need further information on the behavior of the pistillodium. Mr. R. A. White, a plant anatomist, has been invited to join us in a detailed study of the ontogeny and mature structure of the flowers of ragweed based upon microscopic sections of all stages made earlier in this project.

The evolution and migration of ragweeds will be investigated by Mr. W. W. Payne, a plant taxonomist, emphasizing the historical distribution of *Ambrosia*, the species and varieties, and other aspects which may contribute ultimately to a monograph of the group.

1.7 SUMMARY AND CONCLUSIONS

1. In addition to various collaborations with the members of other disciplines associated with this project, the botanists devoted themselves also to specifically botanical problems, as described below.

2. A pilot study of races of ragweeds from three localities—Nova Scotia, Ann Arbor, and New Orleans—showed strong differences in growth and flowering time when the plants were grown under uniform conditions. Several problems, namely, how distinct the different races are, what role man has played in the origin and evolution of the races, and to what extent photoperiod is the prime determiner of flowering time, are discussed.

3. The hybrid ragweed, Ambrosia artemisiifolia X trifida, was studied from the standpoint of its history, occurrence, leaf variation, and fruit structure. Confusions in botanical identification result from the rather extraordinary variation of leaves which is described here on the basis of all the variations from a single "average" plant. The fruits of this ragweed, heretofore undescribed, are intermediate between those of the parents, and are evidently sterile. Because of the rarity of this hybrid, it is preferred to designate it by formula rather than by a regular taxonomic binomial.

4a. The study of staminate flowers of common ragweed (Ambrosia artemisiifolia) revealed six more or less defined stages, which are described: (1) maturation of the flower; (2) extension of pollen sacs; (3) opening of pollen sacs and dropping of pollen clumps; (4) flotation of pollen by wind; (5) extension of the pistillodium; and (6) closure of the flower. Stages (2) and (3) were examined in relation to environmental factors—temperature, humidity, light, and water availability.

b. The actual pollen release is accomplished mainly by enlargement of the filaments of the stamens which extends the pollen sacs and pushes open the corolla lobes, and by dehiscence of the sacs by pleating and separation of their walls and extension of the anther appendages. The pistillodium may function as a "sweeper" of pollen grains still remaining in the anthers.

c. Field studies showed the occurrence of a definite diurnal periodicity. The extension and opening of the anthers occurs between 6:30 and 8:00 a.m. This is correlated with rise in temperature and reduction of relative humidity.

d. Experimental studies on excised flowers and whole plants confirmed that the opening of flowers is controllable by regulating the temperature and relative humidity. The extension of anthers was slowed or prevented by holding the flowers at reduced temperatures. The opening of pollen sacs was retarded or stopped by increasing humidity. Both types of inhibition were reversible under the conditions of the experiments. Alterations in the amount of light and water failed to induce significant changes.

5. Our studies of the biology of the pollen have focused on the process of germination. Germination on the living plants was studied by artificially dusting pollen upon the flowers and staining them in such a way as to demonstrate the pollen grains under the microscope. It was found that good germination occurred only when fresh pollen was used. Laboratory studies, using culture media, showed that a peak of germinability is reached several hours after the pollen is discharged. After six hours the amount of germination reaches a low level of about 5% which remains for many months. The pollen tubes which grow reach only 40 microns and do not develop normally after this point.

6. Work in 1959 will emphasize the study of pollen grains and related subjects, such as identification, the clumping phenomenon, chemistry of the outer wall layers of the grains, and germination; the flower and floral function in-

cluding a comparison of pollen release in giant ragweed and common ragweed plus further studies of the pistillodium, and the maturation and detailed mature morphology; and the evolution and migration of ragweeds embracing the historical distribution, the species, and varieties.

2. MEDICAL PHASE

by
J. M. Sheldon, J. N. Correa, K. P. Mathews,
J. A. McLean, K. Mikat, and R. Patterson

2.1 INTRODUCTION

During the past year the medical group has again had the privilege of working as a team with the meteorologists, botanists, and statisticians. As explained in other sections of this report, this has included the Willow Run extra-seasonal ragweed studies, the wind-tunnel experiments, the proposed tapetal fluid studies, and plans for the chamber construction and operation.

Medical research has been carried on in several phases including basic immunological studies, animal experimentation, and clinical studies.

From a basic immunological standpoint, research was carried out on the enzymatic digestion of skin-sensitizing antibody to ragweed and also on a demonstration of a quantitative relationship between skin-sensitizing antibody and antigen. Studies were carried out in the Allergy Research Laboratory in an attempt to develop better in vitro methods for demonstrating and measuring antigen-antibody reactions. Since this is currently one of the major objectives of all research in allergy, it was thought that both studies were proper and necessary in a project of this type.

Research is being carried out at the present time on the effect of ionization upon allergic symptomatology. Since changes in ionization are known to occur under certain specific meteorological changes, the possibility has been entertained that changes in ionization may either influence or change allergic symptomatology.

Besides the guinea pig anaphylaxis experimentation and air ionization, the medical group has a dog which is sensitive to ragweed pollen. This animal provides a further investigating tool in the study of ragweed pollinosis.

Clinical studies and analysis have been carried out using the data collected at Jackson Prison in 1956. Although this effort was not as successful as had been hoped, it will serve as a guide in the future for many studies. Several patients were exposed to natural ragweed during the 1958 preseason experiment. The results of these experiments, although not completely analyzed, should aid considerably in understanding the human's reactions to ragweed pollen.

Plans for future research are briefly outlined in Section 2.9.

2.2 ENZYMATIC DIGESTION OF SKIN-SENSITIZING ANTIBODY TO RAGWEED

The purpose of this study was to see if partial digestion of human skin-sensitizing antibody to ragweed might yield material possessing more usual properties of antibodies, e.g., ability to precipitate. It has been suggested that some nonprecipitating antibodies are large, asymmetrical molecules. By steric hindrance, inert portions of the molecule might prevent one of the combining sites from being accessible to antigen. Recent data suggesting that skin-sensitizing antibody is present in material sedimenting rapidly in the ultracentrifuge is compatible with this concept. Katsura[7] actually converted a nonprecipitating, delipidized diphtheria antitoxin into a precipitating serum by partial proteolytic digestion. Presumably, when an inert portion of the molecule is removed, the remainder of the molecule is free to react as a more ordinary antibody.

Sera from five different untreated ragweed-sensitive persons were studied. The globulin fraction was separated by salting out, and then redissolved in saline solution. Pepsin first was used for proteolytic digestion, partly because considerable work had been done in the past on peptic digestion of antibodies to bacterial products. The globulin solutions containing skin-sensitizing antibody were submitted to peptic digestion in acid buffers at 37°C for varying periods of time and with different concentrations of pepsin. The process was terminated by neutralizing the acid reaction mixtures. In all of a large number of tests, there was rapid inactivation of the skin-sensitizing activity. However, it was noted that there was rapid inactivation of skin-sensitizing activity in control tests carried out at an acid pH in the absence of pepsin. This inactivation occurred within a few minutes and was observed even at a pH of 4.7, the highest pH which allows for substantial peptic activity. Such instability of skin-sensitizing antibody in this pH range was unexpected and has apparently not been documented previously. Antibodies to bacterial products generally are not inactivated under these conditions. Further studies in this laboratory are being planned regarding the effect of various pH values on skin-sensitizing activity. Despite the loss of this activity, these digests were tested for precipitating activity in the precipitin ring test, but these were negative.

Tryptic digestion then was studied, employing Worthington 1^x crystallized trypsin. The globulin fraction of human serum again was used, the incubation being carried out at a pH of 7.2 and a temperature of 37°C. Trypsin concentrations were varied from 0.0004 to 10.24 mg per ml and time varied from 30 minutes to 24 hours. The digestion was terminated by freezing. With sufficient time and trypsin concentration, partial to complete loss of skin-sensitizing activity could be demonstrated by passive transfer tests. However, there was no concomitant appearance of precipitating activity, ring tests on all samples being negative. A few digested samples incubated with ragweed antigen at 4°C for up to one week, as in the quantitative precipitin test, also failed to show precipitation. A number of samples also were tested for their ability to agglutinate red cells to which ragweed extract had been conjugated by bisdiazotized benzidine using the technique of Gordon, Rose, and Schon.[8] No enhancement of agglutinating activity was observed following partial or complete destruction of skin-sensitizing activity by trypsin.

2.3 THE DEMONSTRATION OF A QUANTITATIVE RELATIONSHIP BETWEEN SKIN-SENSITIZING ANTIBODY AND ANTIGEN

Studies of the quantitative relationship of atopic antibody and antigen have been limited by the failure of this type of antibody to react with antigen in the usual precipitin reaction. Newer methods, particularly the agglutination reaction using the bisdiazotized benzidine type of coupling as applied by Gordon, Rose, and Schon,[8] appear more promising for in vitro demonstration of skin-sensitizing antibody, but the quantitative relations of antigen and reagin remain obscure. The purpose of this study is to present data demonstrating a quantitative relationship between skin-sensitizing antibody and its corresponding antigen by the passive transfer neutralization titration technique.

The quantitative linear relationship to be described has been demonstrated with different reaginic sera and ragweed antigens and is apparently constant under the conditions of our experiments. This evidence that reagin and antigin react quantitatively as would be expected in accordance with present immunologic theory is therefore proposed as an additional characterization of skin-sensitizing antibody and of antigens capable of stimulating its production.

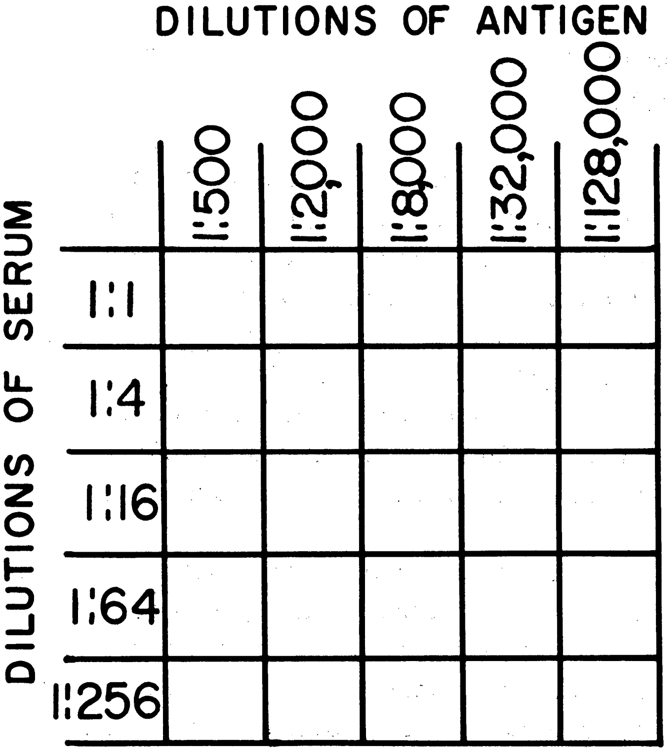
1. Method and Materials

Sera.—Blood samples were obtained from untreated patients who had a history of typical respiratory tract allergic symptoms during the ragweed season and who demonstrated a 4 plus reaction to ragweed antigen with cutaneous skin testing. The serum was separated from the blood, cultured for sterility, and stored in a refrigerator until use.

Ragweed Antigen.—Stock ragweed antigen extracts used in the experiment were: (A) a 1:50 extract of Ambrosia elatior prepared in our laboratory, (B) a 1:50 extract of Ambrosia elatior prepared in The University of Michigan Medical Center Pharmacy, and (C) a 1:50 extract of "Ragweed Family" prepared in The University of Michigan Health Service Pharmacy. These antigen extracts were prepared by extraction with buffered saline in the manner described by Sheldon[9] but were prepared at different times.

Recipients.—Normal students without an atopic history were used in this study. Each recipient had negative cutaneous and intracutaneous skin tests with ragweed antigen.

Passive Transfer Neutralization Studies.—Prior to final determinations, several serial fourfold dilutions of each serum were tested in preliminary passive transfer neutralization titrations with fourfold dilutions of the antigen to find the approximate range of antigen concentration necessary to neutralize the skin-sensitizing antibody in these various dilutions of each serum. The method of preliminary titration is shown in Fig. 11.



DILUTIONS OF ANTIGEN

DILUTIONS OF SERUM

	1:500	1:2000	1:8000	1:32,000	1:128,000
1:1					
1:4					
1:16					
1:64					
1:256					

Fig. 11. The method of preliminary titration used to find the approximate amount of antigen necessary to neutralize various dilutions of serum.

After the approximate strength of the serum was determined in the above manner, serial twofold dilutions of serum and serial twofold dilutions of antigen were prepared. Mixtures of 0.15 ml of each dilution of serum with .15 ml of each dilution of ragweed antigen were prepared in a sterile vial. These mixtures were incubated for one hour at 37°C; 0.05 ml of each mixture was then injected intracutaneously on the back of the normal recipient. In 24 hours, each skin site was challenged with .02 ml of 1:500 Ambrosia elatior extract. The results of the skin tests were read in the usual manner.[9] A plus-minus reaction had significantly more erythema than a negative reaction. Because the preliminary neutralization reactions indicated the approximate amount of antigen which would neutralize each serial twofold dilution of serum, the total number of skin sites which were sensitized with each dilution could be limited and still indicate end points of dilutions. This enabled the use of more sites on each recipient.

Combinations of 4 sera and 3 ragweed extracts were studied on 9 recipients in a total of 11 procedures.

2. Results

The results of these passive **transfer neutralization studies indicated that neutralization of reagin** is dependent on stoichiometric combination with its inciting antigen when both reagin and antigen concentrations are at equivalence.

Figure 12a illustrates the results of testing with serum M and extract "B" on recipient RG. These results show that if a dilution of antisera is neutralized by a dilution of antigen, the next serial dilution of antigen will just neutralize the corresponding dilution of antisera. Figure 13a shows the results with the same serum, M, extract "C" and recipient PV. Figure 14a shows the results with the same serum, M, extract "A" and recipient MJ. Figure 15a illustrates a more extensive titration extending from complete neutralization of undiluted serum M with antigen "B" on recipient WB through 14 serial twofold dilutions of antigen and serum. Thus, the same relationship exists throughout this extended range of serial twofold dilutions at points of equivalence. Figure 16a illustrates a second extensive titration with serum R, antigen "A" and recipient RJ. The results are similar although all three components of the system are varied.

A total of 11 experiments were conducted using the 4 skin-sensitizing sera, the 3 extracts and the 9 recipients. In all experiments, the same quantitative relationship was observed. Figures 12b through 16b indicate the results of plotting the first negative end point in each neutralization titration for the corresponding Figs. 12a through 16a. The general slope of the curve is more important than individual end points because of the crudity of this biological test.

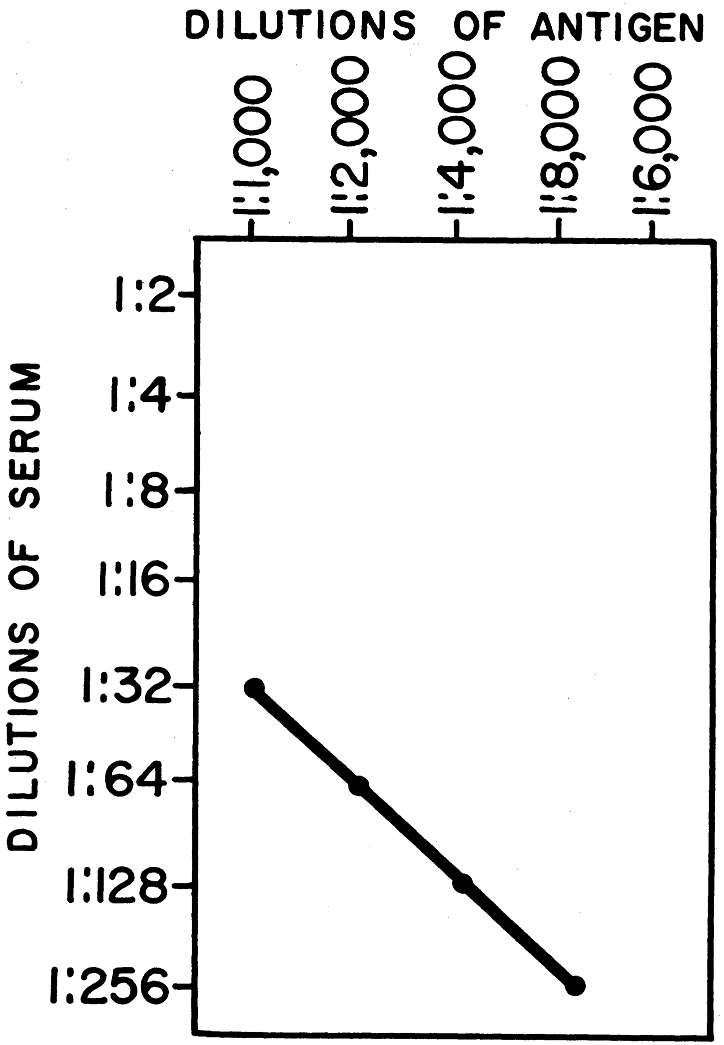
3. Discussion

Examination of the results of these titrations indicates that a quantitative relationship exists between skin-sensitizing antibody and antigen. This relationship is such that if sufficient antigen is present in the initial antigen extract to neutralize undiluted serum, serial dilutions of the antigen will neutralize equivalent dilutions of the reaginic serum. If the serial end point results are plotted graphically as illustrated in Figs. 12b through 16b, the result is approximately a straight-line curve at a 45° angle.

Since these experiments included the use of ragweed antigen prepared at different times and in different laboratories, different sera from untreated patients and different recipients, and all results showed this constant quantitative relationship, it is suggested that this quantitative relationship is a characteristic quality of skin-sensitizing antibody and the antigen which stimulates its production. Although this relationship has been demonstrated using ragweed antigen and skin-sensitizing antibody against ragweed antigen, it seems reasonable to assume that this relationship might also be true for reagin against house dust, mold, and other pollen.

	DILUTIONS OF ANTIGEN				
DILUTIONS OF SERUM	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000
1:2	++	+++			
1:4	+	++	+++		
1:8	+	++	+++	+++	
1:16	±	+	+++	+++	
1:32	−	±	+	+++	+++
1:64	−	−	+	++	+++
1:128		−	−	+	+++
1:256			−	−	+

(a)



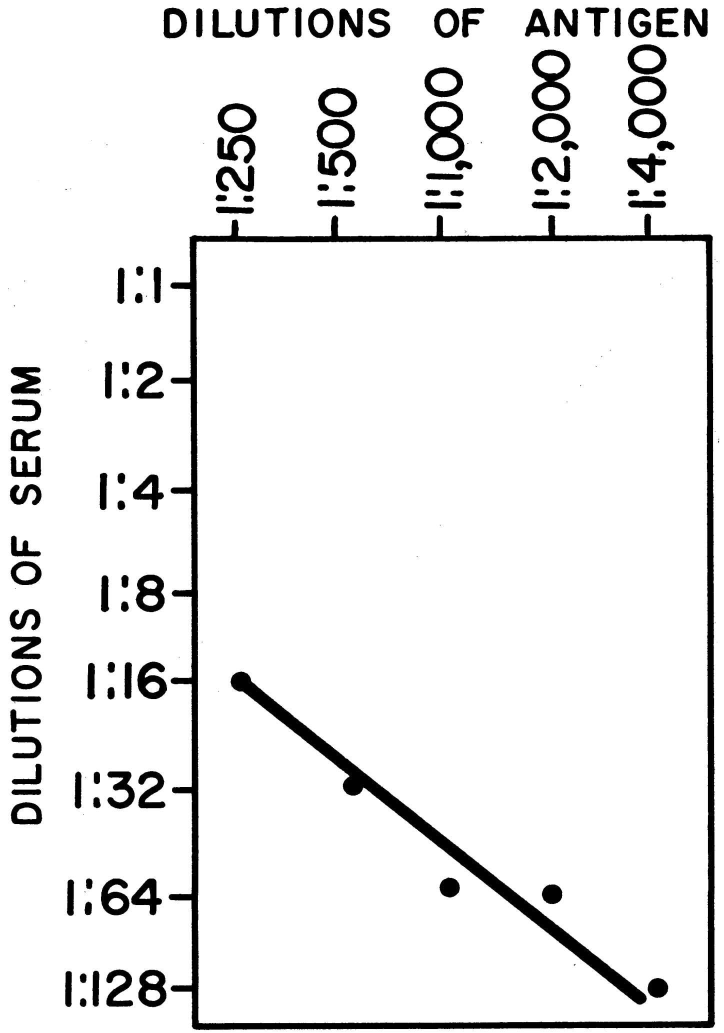
(b)

Fig. 12. (a) Passive transfer neutralization titrations with serial dilutions of serum M and extract "B" on recipient RG; (b) plotting of the first negative end points of Fig. 12a.

DILUTIONS OF ANTIGEN

DILUTIONS OF SERUM	1:250	1:500	1:1,000	1:2,000	1:4,000
1:1	+	+	+++		
1:2	+	+	+	+	
1:4	+	+	+	+	++
1:8	±	+	+	+	+
1:16	−	±	−	±	±
1:32		−	±	+	±
1:64			−	−	±
1:128				−	−

(a)

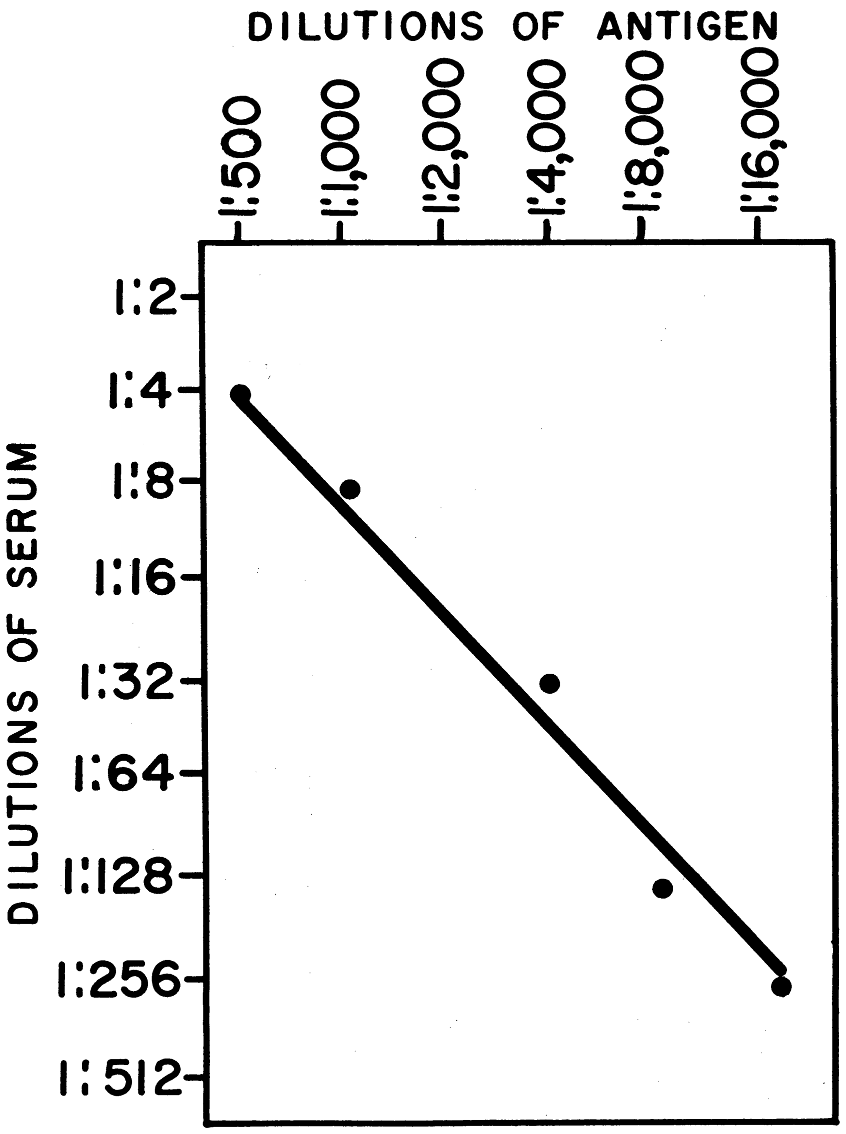


(b)

Fig. 13. (a) Passive transfer neutralization titrations with serial dilutions of serum M and extract "C" on recipient PV; (b) plotting of the first negative end points of Fig. 13a.

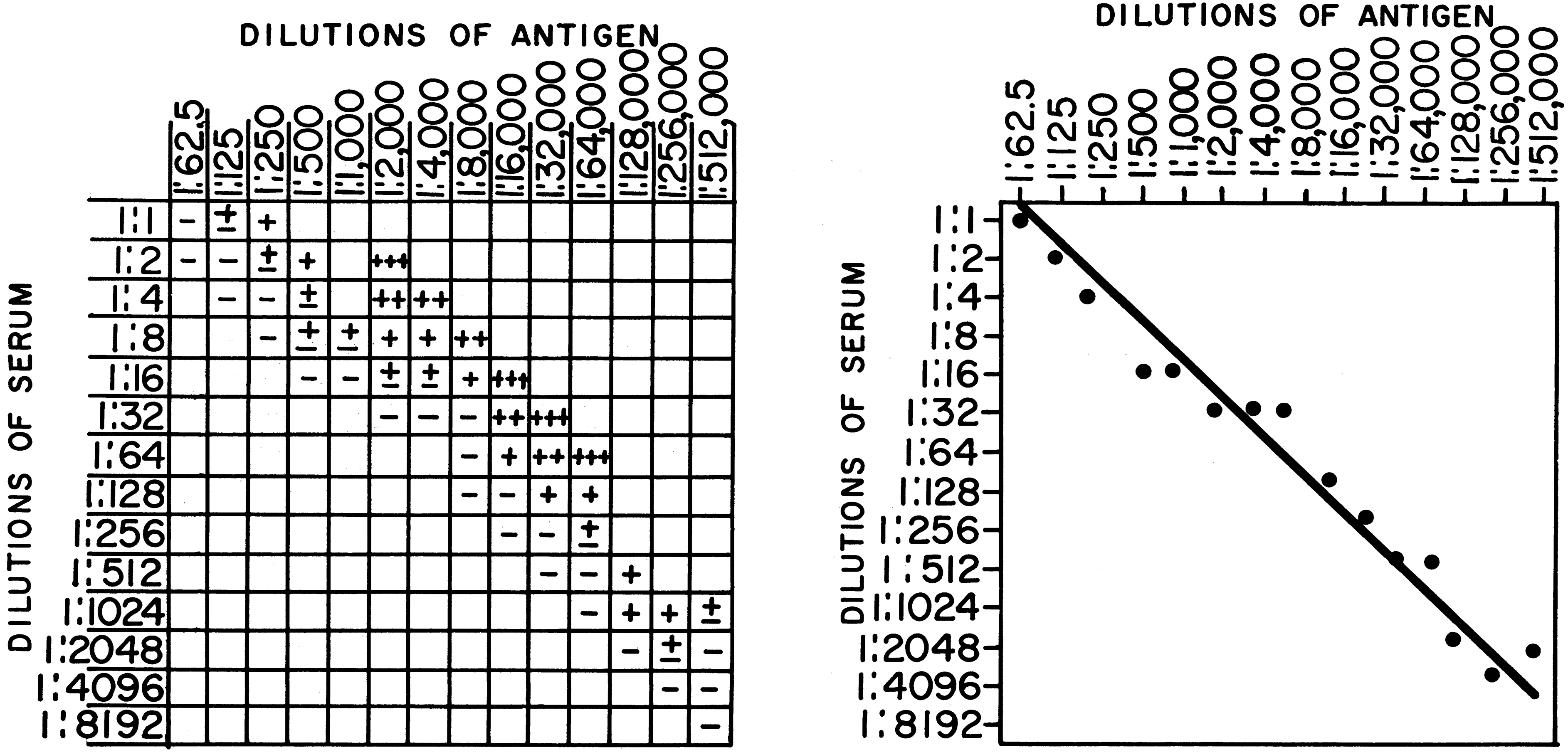
DILUTIONS OF SERUM	DILUTIONS OF ANTIGEN					
	1:500	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000
1:2	+					
1:4	−	+				
1:8	−	−	−			
1:16	−	−	−	+		
1:32	−	−	−	−	+	
1:64	−	−	−	−	+	++
1:128		−	−	−	−	+
1:256				−	−	−
1:512					−	−

(a)



(b)

Fig. 14. (a) Passive transfer neutralization titrations with serial dilutions of serum M and extract "A" on recipient MJ; (b) plotting of the first negative end points of Fig. 14a.

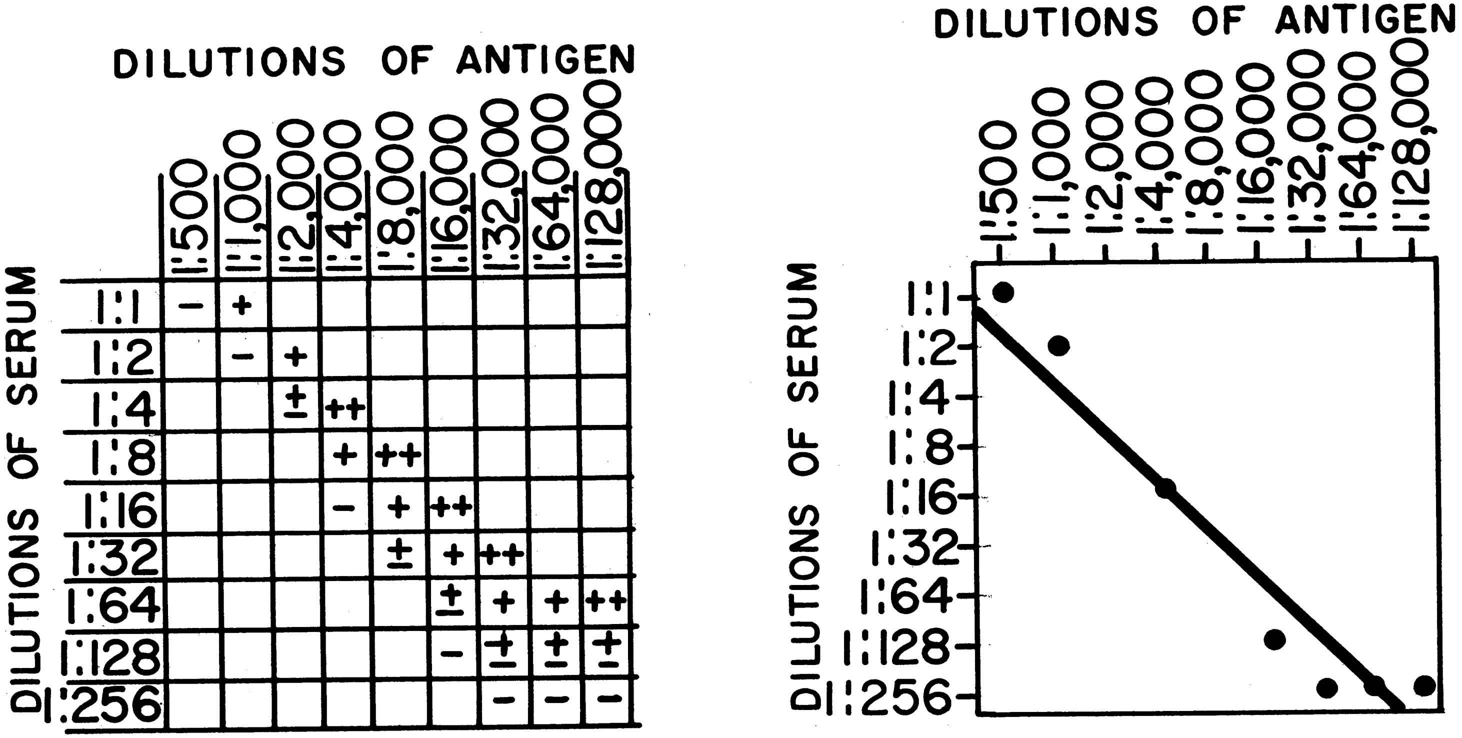


	DILUTIONS OF ANTIGEN													
DILUTIONS OF SERUM	1:62.5	1:125	1:250	1:500	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000	1:32,000	1:64,000	1:128,000	1:256,000	1:512,000
1:1	-	±	+											
1:2	-	-	±	+		+++								
1:4		-	-	±		++	++							
1:8			-	±	±	+	+	++						
1:16				-	-	±	±	+	+++					
1:32						-	-	-	++	+++				
1:64								-	+	++	+++			
1:128								-	-	+	+			
1:256									-	-	±			
1:512										-	-	+		
1:1024											-	+	+	±
1:2048												-	±	-
1:4096													-	-
1:8192														-

(a)

(b)

Fig. 15. (a) Passive transfer neutralization titrations with serial dilutions of serum M and extract "B" on recipient WB; (b) plotting of first negative end points of Fig. 15a.



DILUTIONS OF SERUM \ DILUTIONS OF ANTIGEN	1:500	1:1,000	1:2,000	1:4,000	1:8,000	1:16,000	1:32,000	1:64,000	1:128,000
1:1	−	+							
1:2		−	+						
1:4			±	++					
1:8				+	++				
1:16				−	+	++			
1:32					±	+	++		
1:64						±	+	+	++
1:128						−	±	±	±
1:256							−	−	−

(a)

(b)

Fig. 16. (a) **Passive** transfer neutralization titrations with serial dilutions of serum R and extract "A" on recipient RJ; (b) plotting of first negative end points of Fig. 16a.

The passive transfer neutralization reaction as a method of study of antigen-antibody relationships was devised by Cooke _et al_.[10] and Stull and Sherman[11] and was used as an assay technique by Arbesman and Eagle.[12] Their work indicated that passive transfer neutralization titrations with ragweed extracts gave a measure of comparison of the antigenic strength of one extract with another. Arbesman and Eagle[12] also conducted experiments similar to those reported here with the conclusion that there is no linear relationship between the amount of extract necessary to neutralize a given serum and its reagin content and that passive transfer neutralization techniques are not applicable to the assay of serum reagin content as had been previously described by Lippard and Schmidt.[13] A review of their findings indicates that our observations are compatible with their reported results but that there is a variation in interpretation. The linear relationship presented in these experiments seems also true for the work of Arbesman and Eagle. However, these investigators plotted specific _end points_ of the passive transfer neutralization reactions rather than the general slope of the curve. The plotting of specific end points of passive transfer neutralization reactions does not seem justifiable in view of the experimental error inherent in this rather crude biological assay method. The sources of error are multiple: variations in amount of antigen and antibody added to the mixtures, inadequate mixing of small amounts of material in serum vials, variations in the amounts of the mixtures injected in the recipient, variations in the site of challenge in relation to the original injection sites, and variation in reading of skin tests in addition to other possible experimental errors. Thus, if the curves of Arbesman and Eagle[12] are plotted considering the general slope of the curve rather than the specific end points, it appears that their results are compatible with ours. The unreliability of the individual end points in this system is illustrated in Figs. 15a and 16a where these end points show their individual unreliability but where the general slope of the curve indicates the linear relationship which exists.

All sera used in these studies were from patients who showed a marked positive test to antigen with cutaneous skin tests. Passive transfer neutralization sites with undiluted aliquots of all sera gave strong reactions when tested with antigen. While the immediate reactions resulting from injection of the mixtures of serum and antigen were not studied as part of the experiment, several recipients showed immediate reactions which were more marked with higher concentrations of serum and antigen. This probably accounts for the absence of strong reactions in incompletely neutralized serum passive transfer sites as shown in Fig. 14a. Bowman and Walzer[14] have demonstrated the hyporesponsiveness of skin sites which have undergone previous reactions.

The extensive investigations of several laboratories have provided evidence of several antigens in crude ragweed extract. Therefore, while it is not unlikely that the present study involves the use of mixed antigen-antibody systems, it should be emphasized that these determinations are measurements of biological activity rather than chemical entities.

4. Summary

A constant quantitative relationship existing between skin-sensitizing antibody has been demonstrated by means of equivalent titrations of antigen and reaginic serum using the passive transfer neutralization reaction. This relationship is such that if an extract contains sufficient antigen to neutralize the reagin in serum, any dilution of the extract will neutralize an equivalent dilution of the serum. This quantitative relationship at equivalence indicates that skin-sensitizing antibody and its corresponding antigen react in accordance with present immunologic theory and this relationship is proposed as an additional characterization of skin-sensitizing antibody and antigen.

2.4 EFFECTS OF AIR IONIZATION ON GUINEA PIG ANAPHYLAXIS

Over the last several years, articles have appeared with increasing frequency in the medical and engineering literature relating studies showing apparent relief of pollenosis in human subjects exposed to artificial alterations in air-ion concentration. There are devices already on the market claiming to afford relief by these means.

Since the nineteenth century, when Dessauer[15] first artificially produced air ions using MgO as a source, reports of various beneficial and detrimental effects of air ions on human health and disease have flooded the literature. Unfortunately, much of the work, especially the early work, lacked sound scientific methodology and actual measurements of ion density. Maintenance of accurate, significant levels have been extremely difficult or impossible to obtain. Some have noted marked relief of hay fever and asthma in negative ionized air. But the relief has been temporary, symptoms recurring from a few minutes to two hours after the test. It has been postulated that an increase of negative ions over the level normally present in outdoor air may affect "physically and/or chemically" microscopic airborne contaminants such as dust, spores, bacteria, and pollen. Others have observed increased ciliary beating of animal trachea under negative ionization, with an accompanying increased rate at which mucus moves through the trachea under these conditions.

In our human chamber work we hope to study the effects on pollenosis of alterations in atmospheric variants including air ionization. As a preliminary basic pilot project, we wanted to test possible effects of artificially altered ion concentrations in ambient air on guinea pig anaphylaxis, since this is a well-studied, readily reproducible phenomenon in the laboratory. If air ions influence this immunologic event in the animal world, human pollenosis may likewise be affected to a significant, measurable degree.

To measure and maintain significant altered ion densities within the confines of a small space, a wooden chamber was constructed with a glass front so that events within could be constantly observed. The chamber, approximately 26 by 14 by 12 inches, accommodates three guinea pigs with two standard nebulizers and ion-density metering equipment consisting of a Keithly electrometer and strip-chart recorder. Air ions of either positive or negative polarity are fed in through ports from Wesix ionizers utilizing a tritium source.

Under usual conditions guinea pigs exposed to aerosolized histamine in such an environment will die in typical anaphylactic shock in a few minutes. Likewise, pigs previously passively sensitized with rabbit anti-egg albumin will die quickly in anaphylaxis when challenged with aerosolized egg albumin. We hope to learn if there is a significant effect on this readily reproducible phenomenon under conditions of predominant positive or negative air ionization in the animal chamber.

Antigens may be in the dry state, as well as in solution, when used for challenge. Powdered egg albumin or dried ragweed pollen may also be used. Our ragweed-sensitive dog is a candidate for use in this chamber too.

Numerous problems have confronted us. The major problem has been the extreme difficulty encountered when attempting to measure accurately the density of air ions being released into a medium containing liquid droplets such as are produced by the standard nebulizers. Because of a recombination effect, ion density levels fall precipitously during aerosolization thus making good measurements practical only when the nebulizers are turned off. Though this is a serious difficulty, it is felt that the data obtained will be of importance if only in a negative sense.

To date about 10 animals have been exposed to aerosols in the chamber without ionization primarily to establish base-line values and the LD_{50} for the substance used. A 1% solution of serotonin (creatinine SO_4) was first used. Definite objective symptoms of sneezing and scratching of the nose were noted within 4 minutes, but further symptoms and anaphylaxis were not observed. The seratonin available was felt to be too old for our purposes and a fresh quantity will be obtained.

With a 1% solution of histamine (acid PO_4), the early nasal symptoms are seen in 4-6 minutes and death in typical anaphylaxis ensues within 8-9 minutes.

A 1% solution of aqueous egg albumin failed to produce typical anaphylaxis under the experimental conditions in our chamber, yet death was seen to follow intra-cardiac injection of sensitized pigs. This indicates that the aerosol concentration was insufficient in the chamber. Therefore, animals will be confined in a 6-inch-diameter wire cage directly in front of the two nebulizers. In addition higher concentrations of egg albumin will be used.

At this writing it is too early to predict with certainty significant differences in observed guinea pig anaphylaxis with positive, negative, and normal air-ion concentrations. Normal or usual outdoor air is slightly more positive than negative in polarity. Our next report will clarify this point and the work will be extended as indicated from there.

2.5 RAGWEED ALLERGY IN THE DOG

Sporadic reports of allergic manifestations in animals have appeared in the veterinary medical and medical literature for some time.[16-20] We were given an opportunity to study a dog with a history of symptoms suggestive of ragweed allergy, to determine whether this was indeed an allergic manifestation, and to compare the clinical and immunologic responses of the animal with those of human allergic patients.

Case Report.—A two-year-old spayed fox terrier living in southern Michigan was first noted to develop symptoms of lacrimation, conjunctivitis, and severe pruritus in mid-August of 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 of 1958 with the return of the same symptoms. The dog was then made available to us for investigation through the efforts of J. A. Hergott, D.V.M. Physical examination showed the dog to be in good general health. Positive physical findings included reddening of the conjunctiva, profuse lacrimation and some purulent discharge from the eyes. An erythematous scaling eruption was present over her back and on the flexural surfaces of her forelegs. The remainder of the physical examination was negative. Her symptoms persisted through August and September and began to subside in early October. Her 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 eosinophiles. 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 ragweed which showed a 3 plus reaction. Serum was obtained from the dog with sterile technique and .05 cc of this serum was planted in normal human skin. In 48 hours, this skin site was challenged with 1:500 ragweed antigen and a 4 plus skin reaction was observed with a negative control. Attempts to demonstrate precipitating antibodies using ragweed antigen and serum from the dog with the Ouchterlony double diffusion gel technique[21] were negative. The same result was obtained with human anti-ragweed serum. Active cutaneous anaphylaxis using the method of Ovary[22] was positive in the dog skin. For this test, .02 cc of 1:500 ragweed antigen was injected intradermally and immediately followed by the intravenous injection of 1 cc of .5% Evans Blue dye. A positive reaction was noted with a dark blue area at the site of the antigen injection. The saline control was negative. Passive cutaneous anaphylaxis was attempted using the method of Ovary.[22] Separate skin sites in the guinea pig were sensitized with .05 ml of dog anti-ragweed serum and .05 ml of human anti-ragweed serum intradermally. Twenty-four hours later these skin sites were challenged by intradermal injection of ragweed antigen and intravenous injection of Evans Blue dye. Positive Prausnitz-Kustner reactions were noted but the passive cutaneous anaphylaxis reaction was negative. Similar results using human serum alone have been described by Fisher et al.[23] A hemagglutination reaction using a modification of the method described by Gordon et al.[8] was done by Dr. K. P. Mathews. A high titer was found.

To observe the dog's reaction to ragweed pollen and ragweed antigen after her symptoms had subsided at the end of the ragweed season, the dog was placed in a small chamber and either dried ragweed pollen or water soluble ragweed extract 1:500 was nebulized into the chamber by means of a No. 40 DeVilbiss Nebulizer. After approximately 10 minutes of exposure to high concentrations of ragweed pollen or the water soluble ragweed antigen, the dog developed the symptoms noted during the ragweed season: lacrimation and conjunctivitis. This was followed by striking rhinorrhea and by respiratory distress characterized by a marked change in her respiratory rate, prolonged expiratory phase, and occasionally an audible wheeze. If exposure was sufficient, vomiting and diarrhea occurred. Her symptoms were relieved in approximately one-half hour by subcutaneous epinephrine, .1 cc of 1:1000 aqueous epinephrine. The lacrimation and rhinorrhea were relieved by antihistamines, for example, parabromdylamine maleate, 2.5 mg orally. Control experiments using the same animal and either buffered saline or grass pollen antigen 1:50 did not result in the production of the symptoms. A summary of these studies and the results found in human ragweed sensitive patients are shown in Table I.

Discussion.—From clinical observations and experimental data presented here, it seems evident that spontaneous ragweed sensitivity is present in the dog. This naturally occurring hypersensitivity is similar to that seen in human ragweed-allergic patients. The conjunctivitis, rhinitis and respiratory distress seen in the dog are all seen in the human. The dermatitis occurring in the dog presents some similarity to the atopic dermatitis seen in some allergic humans. Positive skin tests to the suspected antigen with appropriate negative controls are further evidence of ragweed pollen sensitivity. The immunologic investigations conducted to date provide some evidence that the dog has skin-sensitizing antibodies similar to the skin-sensitizing antibodies in humans. These antibodies are passively transferable to normal skin and are nonprecipitating, which are two characteristics of the type found in humans with proven atopic disease. In allergic conjunctivitis, a cytological study of conjunctival secretions would be expected to show eosinophiles rather than polymorphonuclear leukocytes. However, we believe that the dog had a secondary bacterial infection of the conjunctiva which had followed the allergic conjunctivitis. As the allergic conjunctivitis improved at the end of the ragweed season, the bacterial infection also subsided concomitantly without specific treatment.

Summary.—A case of ragweed hypersensitivity in the dog is presented with clinical and experimental evidence indicating that a true spontaneous ragweed hypersensitivity may exist in the dog and that this hypersensitivity is similar to that occurring in human allergic conditions. Further investigations of such animals may well provide information leading to better understanding of this problem in humans and in animals.

TABLE I

COMPARISON OF STUDIES CONDUCTED ON THE RAGWEED-SENSITIVE DOG WITH SIMILAR TESTS ON HUMAN BEINGS

Test	Ragweed-Sensitive Humans	Ragweed-Sensitive 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-Kustner reaction)	Positive	Positive
Precipitin reaction with serum and ragweed antigen (Ouchterlony plate technique)	Negative	Negative
Active cutaneous anaphylaxis	Not done	Positive
Passive cutaneous anaphylaxis in skin using serum from ragweed-sensitive patient	Negative	Negative
Hemagglutination titer	Positive (some)	Positive
Symptoms produced by exposure to ragweed antigen	Conjunctivitis Rhinitis Respiratory distress	Conjuncti Rhinorrhea Respiratory distress
Symptoms relieved by antihistamines and epinephrine	Yes	Yes

2.6 REVIEW OF THE 1956 JACKSON PRISON EXPERIMENTS IN AEROALLERGENS

A comprehensive review of the medical data collected during the 1956 ragweed season at Jackson Prison was carried out by Dr. Robert Lewis and Dr. J. A. McLean. The 1956 experiment at Jackson Prison involved (1) the measurement of the naturally occurring pollen from ragweed, (2) daily observations of the number of prisoners known to respond to ragweed pollen exposure, and (3) a variety of measurement of meteorological variables. The purpose of this experiment was to obtain information about the general nature of the following inter-relationships: (1) pollen concentration and patient reactivity and (2) effect of meteorological variation on pollen concentration. The exact methods used in this experiment have been described in the previous reports.[5,24]

A review of the medical data was made to obtain information about pollen concentration and the patient's reactivity. Meteorological variations were not considered at this time. The purpose was described in terms of the following objectives: (1) to determine whether evidence existed of a simple, direct, and fairly immediate relationship between the level of pollen concentration and the response of the patient in terms of results of objective and/or subjective clinical measurements; and (2) to calibrate the results of tests of patient response for "sensitivity," in other words, to determine which tests were sensitive to the degree of indicating reaction to minimal stimuli, and to determine the effectiveness of the tests in indicating changes in the amounts of pollen stimulation. It was recognized that there were certain variables within the subject, that is, the subject's normal test response and the possibility of accumulative effect of continuous or discontinuous pollen exposure. In addition, there were variables related to the procedure of testing and instrumentation which were recognized. It was also known that certain unrecognized variables would be present in any experiment of this type.

After reviewing the results of the 1956 Jackson experiment, it seemed that there were no strong relationships between the pollen concentration and the test results as observed by this group. There was an indication that the patient subjective report roughly corresponded to fluctuations in the pollen concentration. Since we were not able to determine if an accumulative effect did occur, no conclusion about the sensitivity of the test could be drawn.

It was thought, in addition to the initial impressions gained on a clinical basis, that the necessary information to determine any conclusions about the relationship between exposure and response would require a more controlled environment than that at the Jackson Prison during the 1956 season. The available data for the 1956 Jackson Prison "natural season" certainly contained very relevant information. However, this information will be more readily understandable after the chamber experiments have shown the relationship between pollen concentration, duration of exposure, clinical response, and accumulative effect. In addition, the calibration of any of the tests to show any change in a given degree of exposure requires the knowledge which we hope to gain from the chamber experiments.

Thus the exact relationships between dosage and physiological response and accumulative effect are unknown and it is difficult at this time to interpret more fully the medical work done at Jackson Prison. It is hoped that the chamber experiments will give us some of the keys to this interpretation. Then the Jackson Prison material can be re-analyzed and further relevant information can be obtained.

It is hoped that the forthcoming chamber experiments can answer the following questions: (1) what is the patient's "reactivity time" for a given pollen threshold? (2) is there an accumulative effect which modifies his reactivity time? (3) are there different reactivity times for hay fever and asthma in a given patient? (4) is the response in pollen exposure doses predictable? (5) can laboratory and pulmonary function studies detect quantitative changes in the pollen exposure? and (6) will medication significantly modify the laboratory and pulmonary function test results in the patient's objective and subjective symptomatology?

Another phase of the clinical work along the same lines has been the review of the medical data of the 1957 Jackson Prison experiments. The material is now being coded on IBM cards for a detailed analysis.

2.7 1958 EXTRA-SEASONAL RAGWEED EXPERIMENTS

During the 1958 preseasonal experiment conducted by the botanists and meteorologists at Willow Run, certain pertinent medical observations were also made. Two volunteers known to be sensitive to ragweed were exposed in an area downwind from the ragweed pollen plot to natural airborne ragweed pollen during a non-ragweed season. Any symptoms at that time could be attributed to the ragweed pollen since neither subject was sensitive to the only other aeroallergen present at that time, grass pollen.

As far as specific medical objectives were concerned for the 1958 preseasonal experiment, it was hoped (1) to determine if the patient would develop symptoms during an off-season exposure to ragweed pollen and (2) to determine, if possible, how much pollen concentration was necessary to produce symptoms. The latter objective would then help with determining the specific pollen concentrations necessary for the chamber experiment.

The first subject (C.S.) had severe hay fever of many years duration and had never had specific hyposensitization therapy. During the tests he was seated approximately 40 feet from the center of the ragweed plot. The first day he was exposed for 5 minutes. On the second day within 7 minutes after exposure started, he had mild hay fever symptoms. On the third day the symptoms began within 15 minutes. These mild hay fever symptoms persisted all that day. On the fourth day he began to have symptoms after 15 minutes of exposure but they persisted for only one hour. There was a total exposure of 20 minutes on that day. Symptoms began 10 minutes after exposure on the fifth day. These symptoms persisted all day. On the seventh day he had definite objective findings of hay fever with the symptoms coming on about 10 minutes after exposure and again persisting all day. The same findings occurred on the eighth day of exposure. On the ninth and final day of tests, there was very little pollen due to rainy weather. Although the subject stayed under an umbrella for approximately one hour, objectively, there was no symptomatology.

It should be added that the first subject would develop wheal lesions on his face where abrasions occurred following shaving during the natural ragweed season. During this extra-seasonal experiment, the same type of wheal lesions occurred on his face where the skin had been cut during shaving. This is further substantiating evidence that truly allergic symptomatology was occurring upon exposure to the ragweed pollen. Allergic symptomatology occurred on an inhalant and probably on a skin-absorptive basis at the site of the cuts from shaving.

The second volunteer (P.B.) was a known ragweed-sensitive individual who had been receiving hyposensitization treatment for approximately three years. Since treatment he had only mild symptomatology during the natural ragweed season. This subject was exposed to the same pollen concentrations at the same location as the other subject, except that his initial exposure was for 15 minutes. Each succeeding day his exposure was increased by 15-minute periods. It should be noted that on the third day with a 45-minute exposure, he noted some mild hay fever symptoms within 55 minutes. On the fourth day with a 60-minute exposure he began to notice mild symptoms within 20 minutes after the exposure started. These symptoms persisted for about an hour. During the fifth day with a 75-minute exposure, he had hay fever symptoms beginning within 15 minutes after exposure started. In addition he experienced hay fever all day with some asthma that night. Severe hay fever began within 5 minutes after exposure on the sixth day. After 20 minutes of exposure, the symptomatology was severe enough for him to leave the pollen area voluntarily.

Table II shows pollen concentrations, and the variability between symptomatology and pollen concentration.

The question of an accumulative effect could be raised concerning the second patient since his symptoms occurred with a decreasing time exposure each day. It should be noted, however, that both patients had exposures on successive days and their response patterns may be different when the interval between exposures occurred two or more times a day or continuously.

It was thus shown that ragweed-sensitive patients could have symptomatology from ragweed exposure in a nonragweed season from exposure to fresh airborne natural ragweed, artifically grown. One subject had consistent symptoms 10 to 15 minutes after exposure and with more prolonged exposure, he had a longer duration of these symptoms with somewhat more severe symptomatology. One subject had more severe symptoms upon less time exposure following 5 days of ragweed inhalation.

Later in the year, but again before the regular or "natural" ragweed pollen season occurred in this area, both subjects were exposed to dried, stored ragweed pollen and fresh pollen (3-1/2 hours old) in a wind-tunnel experiment. The first subject noted the same type of wheal reactions on the face where abrasions occurred with shaving and he also noted some mild hay fever symptoms. The symptoms consisted of a running nose, some sneezing, and some itching of both the nose and the eyes on exposure to both types of ragweed pollen. The first subject was ex-

TABLE II

EXPOSURE TIMES, ONSET OF SYMPTOMS, DURATION OF SYMPTOMS, AND POLLEN CONCENTRATIONS DURING THE 1958 WILLOW RUN EXTRA-SEASONAL RAGWEED EXPERIMENT

Patient	Date	Exposure Time, min	Symptom Onset After Pollen Exposure, min	Symptoms	Duration of Symptoms	Severity of Symptoms	Pollen Conc., g/m^3	Pollen Conc., $g/m^3/1$ min
C.S.	25 June	5	0	None			622	124
	26 June	10	7	Hay Fever	15 min	Mild	1032	103
	27 June	15	15	Hay Fever	All day	Mild	1313	88
	28 June	20	15	Hay Fever	1 hour	Mild	6468	3234
	29 June	25	10	Hay Fever	All day	Mild	16	< 1
	30 June	30	10	Hay Fever	4-5 hours	Mild	2491	83
	1 July	35	10	Hay Fever	All day	Mod.	20300	580
	2 July	40	10	Hay Fever	All day	Mod.	4667	117
	3 July	60	0	None			86	1
P.B.	25 June	15	0	None			1867	124
	26 June	30	0	None			3107	104
	27 June	45	55	None			3940	88
	28 June	60	20	Hay Fever	1 hour	Mild	19404	3234
	29 June	75	15	Hay Fever	All day	Mod.	49	< 1
				Asthma	That night	Mild		
	30 June	20*	5	Hay Fever	2-3 hours	Severe	3622**	181
	1 July	0	0	None				
	2 July	0	0	None				
	3 July	60	0	None			86	1

*The patient had to leave the plot because of severe symptomatology.

**Usually both patients were exposed together, but when P.B. had to leave the plot, the pollen concentration changed before C.S. came in from his exposure.

During the past 10 months the application of the BDB and tanned cell antiglobulin techniques to hemagglutination tests with sera of pollen-sensitive patients has been under study. Much technical difficulty has been encountered with the BDB method. Although not mentioned in the literature, it has been found essential not to bleed the rabbits whose cells are used in the BDB test more often than every 4 to 6 weeks; otherwise false positive results are obtained. Even with fresh animals, results obtained with the blood of different rabbits show considerable variability, and cells are not usable for longer than about 7 days. Positive as well as negative controls have been incorporated in our daily experiments to provide as much standardization as possible. It was also found that sera stored at 4°C for longer than a few months consistently give false positive BDB tests, although frozen sera are satisfactory for prolonged use. With the conditions which we have adopted to avoid false positive reactions, only a small portion of untreated sera have given positive BDB tests, although consistently positive results have been obtained in substantial titer (e.g., 1:1280) with sera of treated patients. Both water and saline pollen extracts have been used as antigen. Our percentage of positive results with untreated sera is much lower than reported by some other laboratories, but with the conditions which have been adopted our controls are consistently negative, while in some other laboratories more than 50% of normal serum controls are positive.

The tanned cell antiglobulin test has not presented any technical difficulties, although it is more tedious than the BDB test. All control sera thus far have been negative and sera of treated patients are positive in titers similar to those obtained with the BDB test. Positive results have been obtained in 20 of 27 sera of untreated patients, but the titers are relatively low—1:20 to 1:320, commonly 1:40 or 1:80.

A major question is whether or not either of these tests is actually measuring skin-sensitizing antibody. There does not seem to be any apparent correlation between the hemagglutination titers and passive transfer titers in our limited material, and the reports from elsewhere regarding the BDB cell test are conflicting. In two of two instances, adsorption of serum with sensitized cells to the extent that the BDB test became negative did not affect the passive transfer titers of the serum. Similarly, in two of two instances adsorption of serum with sensitized cells to the extent that the tanned cell antiglobulin test (TCAGT) became negative did not change the passive transfer titers. Although, obviously, further work in a larger number of sera is needed, these data strongly suggest that neither type of hemagglutination test is measuring skin-sensitizing antibody. Results with a few of the more thoroughly studied sera are as follows:

	PK Titer	BDB Test	TCAGT	Serum Adsorbed with			
				BDB Sensitized Cells		Sensitized Tanned Cells	
				BDB Test	PK Titer	TCAGT	PK Titer
ML (untreated)	1:1280	1:320	1:80	Neg	1:640	Neg	1:640
EM (untreated)	1:640	1:80	1:160			Neg	1:640
PB (treated)	1:1280	1:1280	1:1280	Neg	1:1280		

An incidental observation that rather large amounts of viable human skin failed to adsorb out skin-sensitizing antibody *in vitro* will receive further study.

2.10 PLANS FOR 1959

Further work with fungi as described in Section 2.8 is contemplated. Once these have been fully identified, further medical work will be carried out to assay the allergenicity of selected species which have not been well studied medically.

Further work is planned with air ionization studies in animals. It also is hoped to study the effect of definite ionization changes on human allergy in chamber experiments. If significant results are obtained, studies could be carried out to determine the amount of ionization changes which occur with known meteorological variations.

The ragweed-sensitive dog allows us to quantitate this animal's response to ragweed pollen. Thus, she can be used in pilot studies for the human chamber experiments. The effect of medications on her symptoms can be documented. The effect of serotonin and serotonin antagonists will be determined. Study of biopsy specimens will afford a unique opportunity to make pathological and immunological observations in fixed tissues. In addition, the effect of ionization could be determined on this animal as well as human subjects.

From a clinical standpoint, further wind-tunnel experiments are planned with the following objectives in mind: (1) to determine the approximate amount of pollen necessary to produce allergic symptoms in ragweed-sensitive humans; (2) to determine if the same subject has the same pattern of response under similar test conditions; and (3) to determine if an accumulative effect occurs after repeated pollen exposure. In addition, the very important question must be answered concerning the relative effectiveness of "fresh" versus "old" ragweed pollen in producing allergic symptomatology, since it is fundamental to forthcoming test chamber experiments.

Future plans are being made for the all-important test chamber experimentation. Construction of the test chamber has just been completed. It is hoped that the test chamber experiment will help us with the following specific questions. What role do the following factors play in determining the response of allergic subjects to pollen exposure: (a) pollen concentration? (b) duration of exposure in the chamber? (c) temperature and humidity in the chamber atmosphere? and (d) date, duration, and frequency of previous exposure? Also, what are the relevant response variables* which can be used to indicate the subject's reactions? After a single stay in the test chamber, how soon can a subject be used again with the assurance that the values of all the response variables are independent?

It is realized that all these questions may not be specifically answered. In the later stages of experimentation, we will have to determine the strength of variation of these response variables as influenced by each of the relative factors. Statistical design is possible and desirable in this series of investigations. It will also be highly desirable that both the person who measures the response variables and the subject be "blind" to the level of each factor, especially pollen concentration. It should be emphasized that the statisticians are in active, close cooperation with all the medical phases of this experiment, so that any data can be subjected to statistical analysis.

Further work concerning the antigenicity of tapetal fluid of the ragweed plant will be carried out during the next year.

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*Response variables denote a quantity not under experimental control but possibly influenced by selected levels of the factors.

We will further exploit our observation that skin-sensitizing antibody to ragweed is inactivated more readily at an acid pH than other antibodies.

Similarly, our observation that skin-sensitizing antibody is not adsorbed by viable skin *in vitro* as readily as expected will be investigated further.

We will seek to verify Hjurt's report of a major adsorption of pollen antigens on glass surfaces.

Immunochemical studies will be initiated along lines outlined in the research grant proposal.

The current hemagglutination work will be completed by studying a larger number of sera, particularly with adsorption tests.

2.11 CONCLUSIONS

In conclusion it may be stated that the medical phase of this research program has evolved along three major lines. Research is being carried out on the basic immunologic processes of the antigen-antibody reaction. This is being done by *in vitro* studies. Animal experimentation is also being carried out using both guinea pigs and naturally occurring ragweed sensitivity in a dog. It is hoped that these animal experimentations will give us knowledge of the more basic immunologic processes, as well as the possible effect of air ionization on allergic reactions. From a clinical standpoint, we are analyzing further the medical data of the natural ragweed season of 1956 and 1957 at Jackson Prison. We are also attempting to produce allergic symptoms in human volunteers during extra ragweed seasons. These experiments are being carried out with natural ragweed pollen from plants induced to pollinate before the normal season and are being used to gain further knowledge for the coming experimentation in the test chamber.

3. METEOROLOGICAL PHASE

by

E. W. Hewson, J. Akerman, E. W. Bierly, A. N. Dingle, F. C. Elder
G. C. Gill, J. B. Harrington, Jr., B. R. Warr, and C. Yang

3.1 INTRODUCTION

Extensive research has been carried out during the past year in an effort to develop improved techniques for sampling ragweed pollen and to determine pollen concentrations volumetrically. The first section of this part of the report describes briefly the theory of sampling, pointing out the various criteria that determine the efficiency of a sampler. The several known types of samplers are then discussed in detail. Several new techniques developed under this grant are described: the flag samplers, the roto-bar samplers, and the electronic pollen counter. These samplers have been tested in the wind tunnel and out of doors. The tests are reported on and the results of the tests summarized to show the effectiveness of the new samplers and why they have been adopted for our use.

An analysis of wind direction and pollen concentration from the 1957 preseasonal experiment is presented, showing that the pollen distribution along the mean wind direction is quite different from that observed in the 1956 experiment. The preseasonal experiment of 1958 is described in detail. The layout of the experiment, instrumentation used, and questions to be answered are presented, along with a report on the progress of the analysis of the data collected during the field test. The in-season experiment of 1958 is also discussed, along with some results of the analysis of the data.

A theoretical study was made of the effect of a ragweed-free area on diffusion from a continuous area source to see if clearing an area of ragweed plants would be beneficial to the surrounding area. It was found that such was actually the case.

The final details for the human test chamber are given, as is a report on the future plans for the preseasonal and in-season experiments of 1959. The 1959 experiments are designed to help answer some questions relative to forecasting the concentration of air-borne ragweed.

During 1958, the meteorological group has continued to lend aid to the other groups in a cooperative effort to answer some of the fundamental questions that have arisen outside the field of meteorology. Such assistance has been extended to the botanists and allergists during the 1958 preseasonal experiment this year and again to the allergists in wind-tunnel studies. The botanical phase of the 1958 preseasonal experiment involved observing the ragweed plants while in the field. To help the botanists make their observations of pollen emission, the meteorologists furnished them detailed observations of air temperature, relative humidity, wind speed, wind direction, net radiation, and solar radiation. These meteorological data are available to both groups for a complete analysis of the emission of pollen. The allergists availed themselves of meteorological assistance when allergic patients were exposed to ragweed pollen during the 1958 preseasonal experiment at Willow Run, and during wind-tunnel studies when ragweed-sensitive persons were subject to various concentrations of pollen on successive days.

These cooperative efforts have been described in earlier sections of this report and they will not be discussed here.

3.2 SAMPLING THEORY

The efficiency of a sampler depends on the following criteria:

1. The ability to sample air with the same particulate concentrations that exist in the environmental air.
2. The success in retaining the particle once it has touched the collecting surface.

The second criterion above has not been considered in subsequent calculations of concentration or efficiency throughout this section unless specifically mentioned.

It may be worthwhile to review briefly the various techniques used in air sampling and to indicate their limitations in a turbulent atmosphere. Most may be included in one or another of the following classifications:

1. Drawing a known volume of air through a filter.
2. Impinging a known volume of air on a suitable collecting surface.
3. Precipitating particles thermally or electrostatically from a known volume.
4. Allowing particles of a known fall rate to settle on a measured area.

3.3 VOLUMETRIC SAMPLERS

The filters, the precipitators, and several of the impingement type of samplers draw air into the sampler through an aperture. Unless the air enters the aperture isokinetically, i.e., without acceleration or deceleration, the collection will not represent the true concentration. For example, suppose a filter sampler is oriented into the wind. When the wind is stronger than the rate of flow through the filter, particles will cross the streamlines due to their inertia and impinge on the filter producing an unreasonably large catch (see Fig. 17a). Under the same conditions, if the wind is slower than the flow into the filter, the catch will be too low (Fig. 17b). When the sampler is mounted with the aperture downward or facing normal to the flow, the effect of particle inertia is even larger and the catch will be exceptionally low, as illustrated in Figs. 17c and 17d. Experimental confirmation of these very important factors is given later in this report.

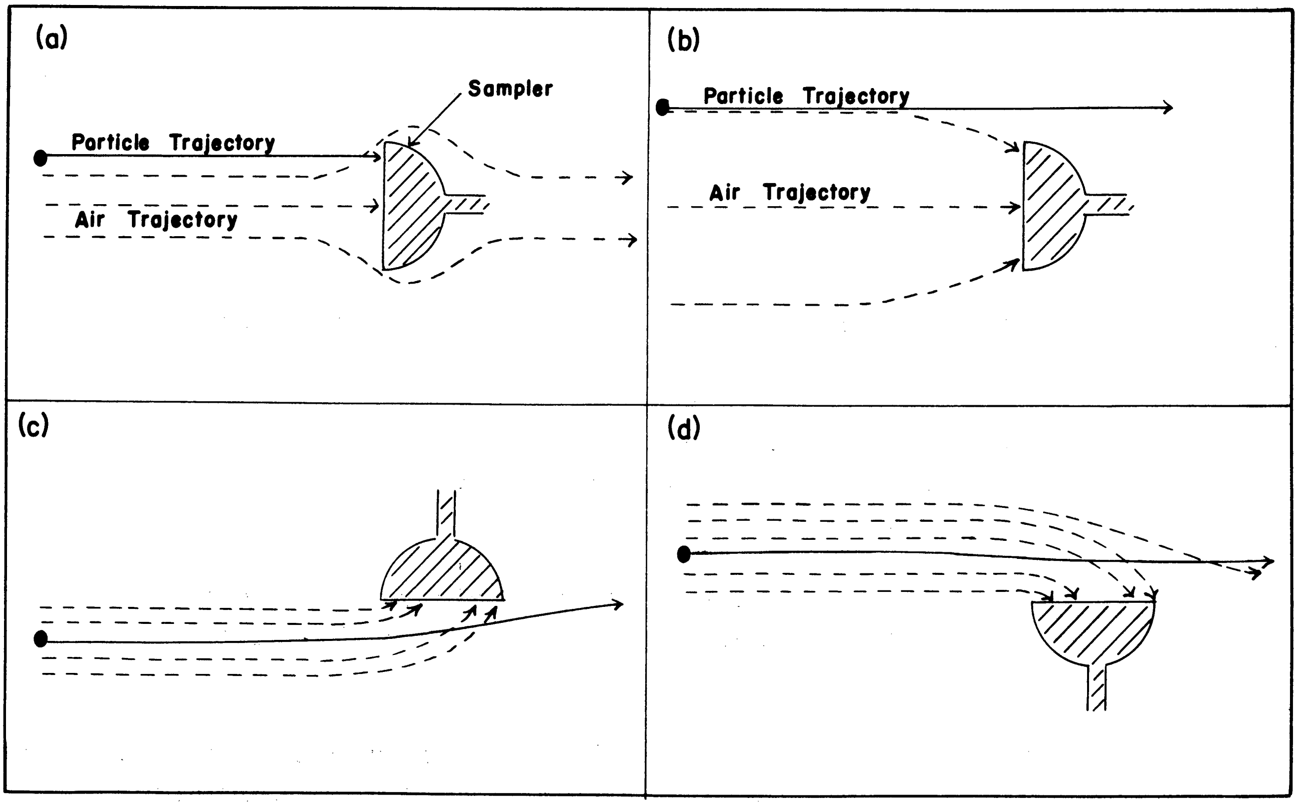

Fig. 17. Schematic diagram showing the effect of particle inertia on the collection efficiency of aperture type samplers in a moving airstream. (a) Sampler facing into a wind of higher speed than the flow into the sampler. (b) Sampler facing into a wind of lower speed than the flow into the sampler. (c) (d) Sampler facing normal to the wind.

It cannot be assumed that the wind flow is as simple as described above. Turbulent eddies create rapid fluctuations in wind direction, making it impossible to keep a sampler oriented into the wind. The same eddies cause rapid fluctuations in wind speed which make it impossible to sample isokinetically. Moreover, vertical gusts carry particulates into upward or downward facing apertures increasing the collection. Unfortunately, there is no way to separate the collection due to the volumetric sample from the collection due directly to turbulent impingement.

In addition to the inertia, the drag coefficient of the particle being sampled has an important bearing on collection efficiency. Very small particles have relatively small inertias and large drag coefficients; hence they tend to follow the streamlines better than large particles and are therefore sampled more efficiency. Unfortunately, most particles of importance as aeroallergens are 13-35μ in diameter and have relatively large inertias.

At very low wind speeds and with small flow rates into the sampler, particle acceleration will be minimized and reasonably high collection efficiency should result. For this reason, the type of sampler in which air is drawn through an aperture should be effective indoors, and also outdoors when the winds are very light.

The design of the impinger has been adequately described in an earlier report.[5] During the 1957 experiments, several impingers were operated at Jackson Prison. A subsequent inspection of the tape roll revealed an almost complete absence of ragweed pollen although smaller particles appeared to have been collected in great numbers.

It was decided to run tests on the impingers to determine the cause of their failure and to see if they could be made operational. The first series of tests were conducted with the tape stationary. The impinger was suspended with the aperture facing down. The collecting surface used was scotch tape. A vacuum was applied and a gas meter was used to measure the rate of air flow. Pollen was introduced by placing some on a sheet of paper and then tapping the paper sharply with a finger. The tape was removed and counted after each test. The results of this series can be summarized as follows:

1. Pollen was not all concentrated in a narrow band but was spread longitudinally along the tape.
2. The degree of spread was least when a high vacuum was applied and greatest when a low vacuum was used.
3. Cigarette smoke which was drawn through the aperture during the experiments concentrated in a narrow band, suggesting that the impingement efficiency for particles the size of pollen grains may be high, but the collecting surface was not able to retain the particles.

A second series of tests was conducted with the tape moving at the rate of 2.54 cm/hr. Pollen was introduced into the impinger at 10-min intervals. The results indicated that most of the pollen from a single, instantaneous puff impinged in a very narrow band. Of each puff, 99% fell in a band 0.1 cm wide which represents a time period of 2-1/2 min. Thus the impinger gives excellent time differentiation.

The third series of experiments was conducted to measure the efficiency of various collecting surfaces. The tape moved while measured numbers of pollen grains on the end of a wire were tapped into the impinger orifice. The number of pollen grains on the wire was first counted under a microscope. The wire was then placed in the impinger opening and tapped vigorously, and then the grains left on the wire were recounted. The ratio of the number of grains collected on the tape to the number released from the wire was considered to be the tape efficiency. The results can be enumerated as follows:

1. The efficiency appeared to be extremely erratic, due probably to deposition of pollen on the entrance of the impinger.
2. Vaseline proved more efficient as a collecting surface than did scotch tape. Gregory's gelatin and dilute rubber cement also were good collecting agents.

Several wind-tunnel tests were run with the impinger using vaseline as a collecting surface. These tests indicated that the impinger efficiency is a function of wind speed, decreasing as the wind increases.

The following conclusions may be drawn regarding the use of the impinger as an automatic pollen sampler:

1. Time differentiation is good.
2. Small particles around 1μ in diameter impinge on the tape in a very narrow band.
3. Air does not enter the orifice isokinetically, so that sampling in wind for particles over 5μ in diameter is not feasible.
4. Spherical particles larger than 10μ in diameter have a tendency to blow off the scotch tape surface.
5. The scotch tape rolls when pressed together have undulations and air bubbles enclosed which make microscopic analysis difficult.

3.4 GRAVITATIONAL SAMPLERS

The gravitational sampler can be used to measure particulate concentration only if it can be assumed that the particle falls at a constant rate V_f through the air. When this is the case, the concentration may be expressed as follows:

$$\chi = \frac{C}{V_f A t} ,$$

where C is the number of particles collected,
A is the area of a horizontal sampling surface,
χ is the volumetric concentration of the particulate, and
t is the time.

Durham[25] and others have pointed out that the fall rate cannot be assumed constant, because of the large vertical motions associated with atmospheric turbulence. Even a 1-mph wind will produce vertical velocities in the lowest 20 ft of the atmosphere of the order of 4.5 cm.sec under normal wind conditions.[26]

These velocities are large compared to the average fall rate for most pollen or spores. For instance, low annual ragweed falls at about 1.6 cm/sec according to Crawford[27]—just 1/3 of the vertical gust speeds that occur with a 1-mph wind. Wind speeds of 10 mph reduce this ratio to 1/30. This means that under all but the calmest conditions more pollen will be brought into contact with the sampler through turbulent eddies than through gravitational settling.

Wind-tunnel tests showed that there is another, even more important objection to the use of the "gravity slide" method of air sampling. When wind moves past a horizontal slide, the stream is split by the edge of the slide, forming a thin laminar boundary layer between the slide and the free stream, as shown in Fig. 18. Most particulates will bypass the slide although eddies in the free stream may carry some particles into the laminar boundary layer where they may settle onto the slide.

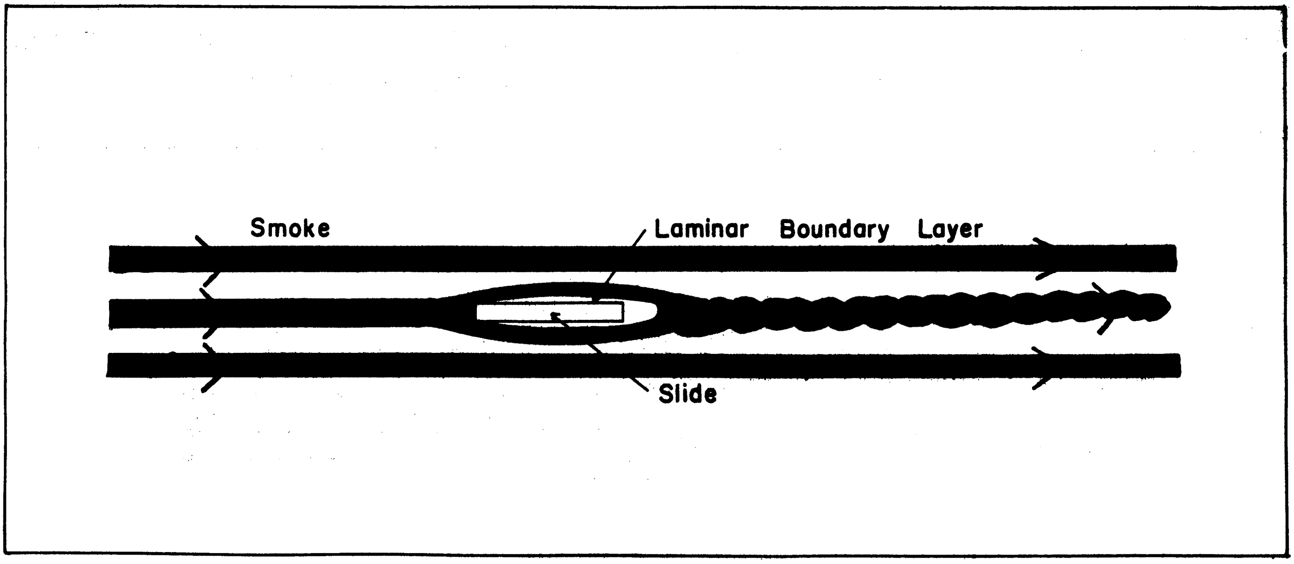

Fig. 18. Schematic diagram of the flow of smoke around a microscope slide under laminar (nonturbulent) flow.

The orientation of the sampler has an important bearing on the catch. Slides whose long axes are parallel to the wind catch 10 to 100 times more pollen than those whose short axis is in the direction of the wind. The reason for this effect is probably that, when the long axis of the slide is parallel to the flow, pollen has three times as much time to fall through the laminar layer onto the slide. Both outdoor and wind-tunnel experiments confirm this result.

The size and shape of the slide holder will affect the flow of air around the sampler. In general, a bulky holder will produce a greater deflection of the airstream, resulting in a poorer catch.

The objections which have been raised to the use of the "gravity slide" in wind do not apply when the slide is place in still air. Here neither orientation of the slide nor its shape can in any way alter the catch.

In the open air the height above ground at which the air begins to move varies from a fraction of a millimeter over a smooth flat surface to several meters in a dense forest. If a gravity slide is placed inside the layer of still air, the collection on the slide should represent the rate of deposition on a level surface. Unfortunately, the deposition may vary markedly from place to place due to minor undulations in the ground or filtering action of the foliage.

3.5 NEW TECHNIQUES

Rempe[28] and later Gregory[29] have shown that vertically oriented cylinders of an appropriate size were effective volumetric samplers under normal outdoor conditions. These samplers utilize the impingement principle. Particles moving in the air stream are carried across streamlines by their inertia to strike the cylinder instead of following the flow around it (see Fig. 19). A suitable adhesive placed on the cylinder retains the particles collected. Gregory demonstrated that the cylinder impingement efficiency* increases with increasing particle inertia and wind speed, and with decreasing cylinder diameter.

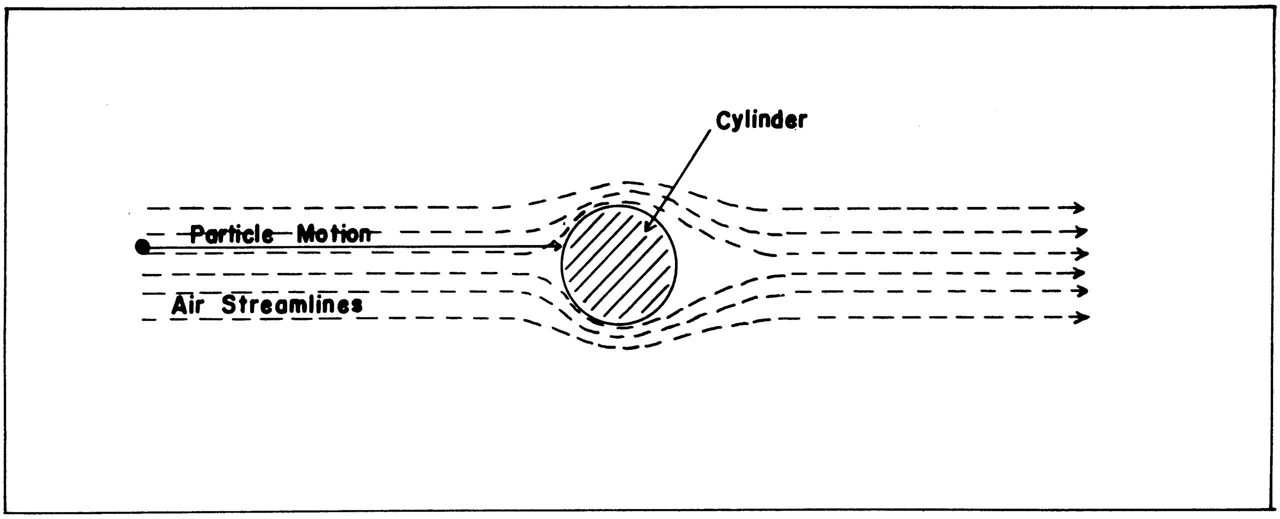

Fig. 19. Schematic diagram of particle and air trajectories near a cylinder.

Brun and Mergler[30] used an analog computer to solve the linearized equations of motion for spherical particles impinging on a cylindrical collector. Figure 20 shows the curve of impingement efficiency versus several properties of the particle, air, and sampler which have been combined into two dimensionless parameters, K and ϕ.

$$\text{* Impingement efficiency} = \frac{\text{number of particles intercepted by cylinder}}{\text{number of particles initially in the volume swept out by the cylinder}} .$$

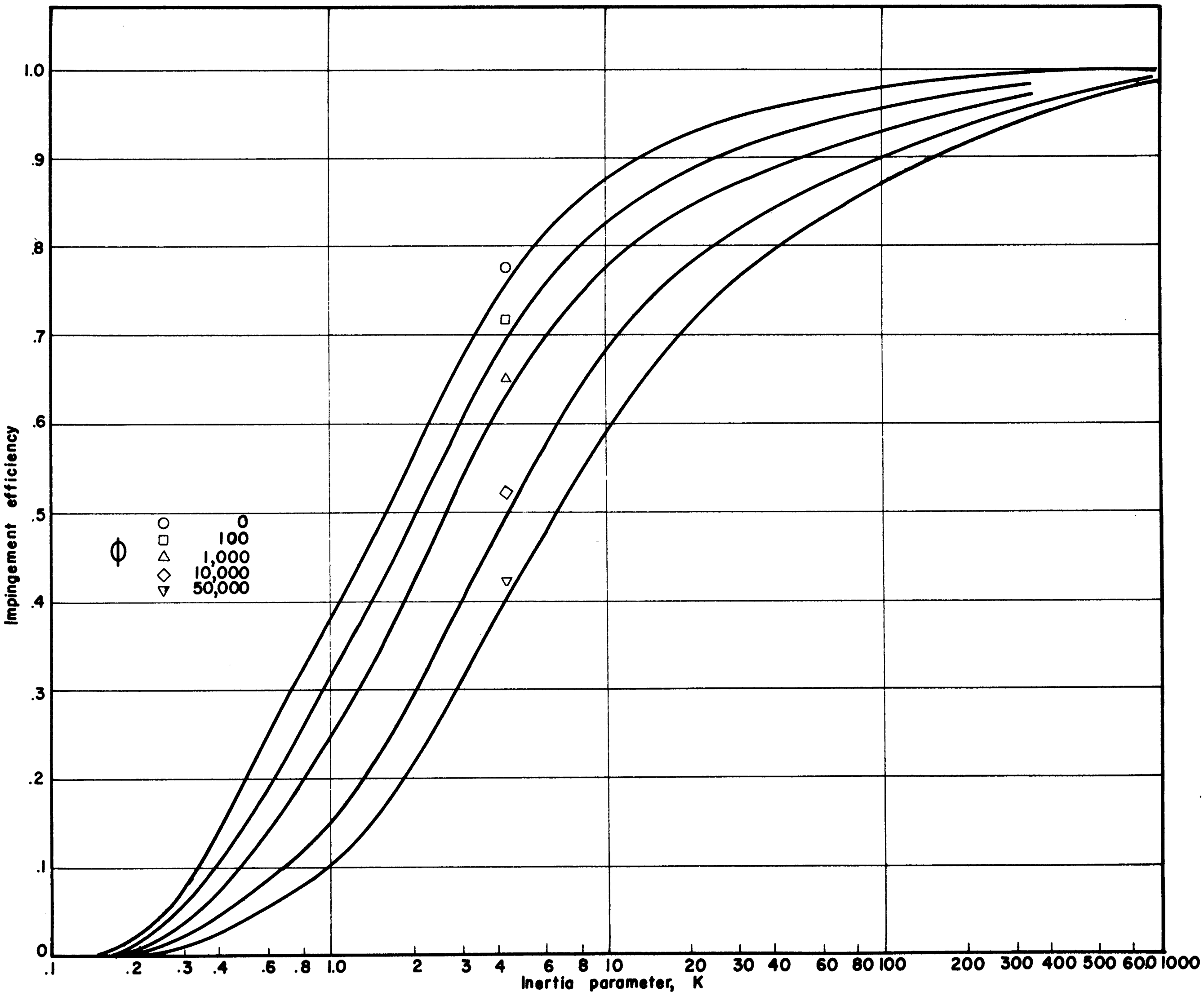

Fig. 20. Cylinder collection efficiency.

$$K = \frac{2}{9} \frac{\rho_p \gamma U}{\mu L} , \qquad \phi = \frac{18 \rho_a LU}{\mu \rho_p} ,$$

where ρ_p is the particle density, ρ_a the air density, γ the particle radius, L the sampler radius, U the free-stream velocity, and μ the air viscosity. (The equations and curve are included for those readers who may wish to compute collection efficiencies for particles or collectors different from those illustrated in the text.)

Figure 21 shows the impingement efficiency of a cylinder of 1 mm in diameter for particles of unit density and various radii as a function of wind speed. It will be noted that a 1-mm diam wire is a very satisfactory sampler for most of the common aeroallergens, when the wind speed exceeds 2 mph. For smaller particles or slower winds, a wire of smaller diameter must be used to maintain useful efficiencies.

The cylindrical samplers used by Gregory were satisfactory in the wind tunnel, but are impractical for use in the field. The curved surface of a cylinder is difficult to observe under a microscope, and if a removable coating is placed on the cylinder, at least the side along which the coating opens is unusable. These difficulties have been largely overcome with the invention of the flag sampler.

3.6 THE FLAG SAMPLER

Sakagami[31] has described a fast-response wind vane for use in micrometeorological studies. It consisted of a tiny paper vane turning about a vertical axis made of an ordinary household pin. The flag would follow up to 60 fluctuations in wind direction per second. By replacing the paper flag with double-coated cellulose tape, the Sakagami wind vane was converted into a flag sampler, shown in Fig. 22. The flag keeps the same edge constantly facing into the wind. Particles impinge on this edge and are collected with the efficiency of a cylinder whose diameter is equal to that of the pin.

The glass bearing is simply a 3/4-in. length of 1/8-in. glass tubing sealed at its lower end and fire-polished at the top to create a low-friction bearing. A variety of pins may be used depending on the size of the particle being sampled. For particles larger than 15 μ, a 2-in. pin 1 mm in diameter is satisfactory. A 2-in. length of double-coated, 1-in.-width cellulose tape is folded over the pin, and a thin piece of doubled paper, 1/4 in. wide, is inserted near the tail to facilitate separation of the tape after exposure. The leading edge of the sampler is coated with a dilute* solution of rubber cement. This procedure was adopted because wind-tunnel tests showed that rubber cement as a collecting surface is waterproof and has an adhesive efficiency three to eight times that of the original celulose tape surface.

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*One part rubber cement to five parts rubber cement thinner.

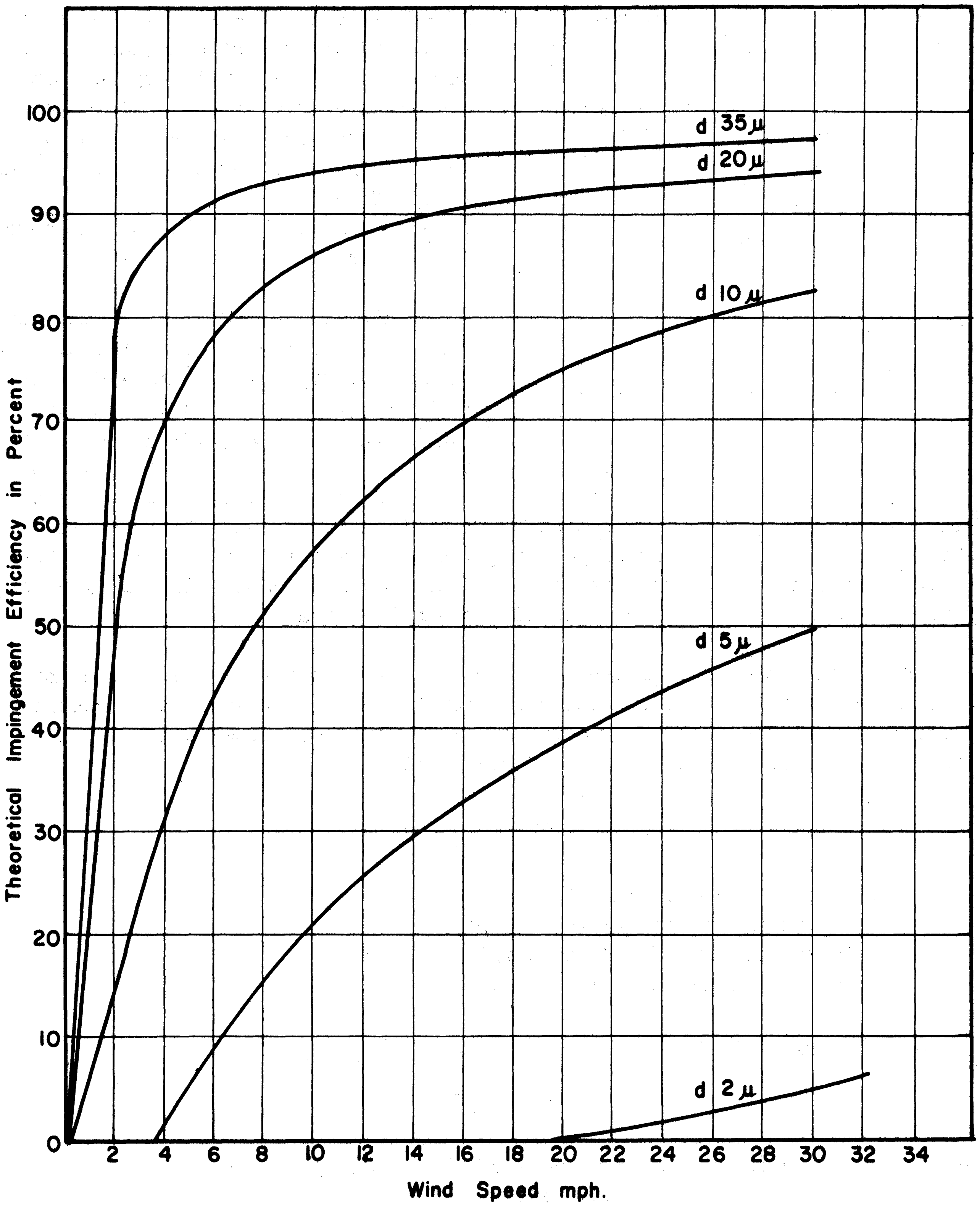

Fig. 21. Collection efficiency of a 1-mm-diam cylinder for particles of unit density and various diameters.

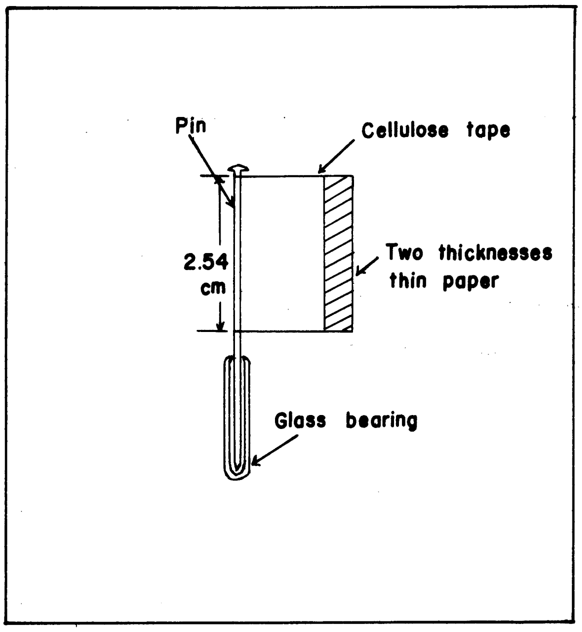

Fig. 22. The flag sampler.

The flag sampler mounted in its bearing may be exposed in the atmosphere wherever sampling is desired. After exposure, the flag may be stored to avoid future contamination of the sampling surface, or it may be mounted immediately on a standard microscope slide protected by a cover slip over the sample. If staining is desired, a thin strip of Calberla's solution* can be painted across the center of the cover slip before it is set in place.

Under laminar flow in a wind tunnel, a 1-mm pin collects all particles in a strip approximately 1/2 mm wide. In turbulent flow outdoors the sampler lags slightly in following the rapid fluctuations in wind direction. The catch is consequently spread over a band approximately 1-1/2 mm in width. This width corresponds to the field of view under an ordinary microscope using 100-power magnification. It is thus possible to count all particles collected on the flag sampler in a single longitudinal traverse by the microscope of the sampled area.

To compute the true concentration of a particulate from the flag count, it is necessary first to correct the count to the value that would have resulted had the sampler been perfectly efficient. Then the corrected count divided by the volume sampled yields the true concentration:

$$\chi_T = \frac{C}{EV} ,$$

where χ_T is the true concentration,
C is the actual particulate count,
E is the efficiency of the sampler,** and
V is the volume sampled.

The volume sampled is given by:

$$V = A\bar{u}t ,$$

where A is the cross-sectional area of the sampler,
$\bar{u}$ is the mean wind speed, and
t is the sampling period.

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*Calberla's solution: 1 part glycerine, 2 parts alcohol, 3 parts water, 1% basic fuchsin.

**$E = E_iE_a$, where E_i is the impingement efficiency and E_a the adhesive efficiency. In the following example E_a will be taken as 1.00 although a figure near 0.40 is indicated by the wind-tunnel experiments.

For example, a flag sampler 0.1 cm in diameter, 2.54 cm in height, with a wind speed of 220 cm/sec (5 mph) will sample

$$\frac{0.1 \times 2.54 \times 220 \times 3600}{10^6} = 0.2 \text{ m}^3/\text{hr} \quad .$$

If the particle being sampled has a density of 1 gm/cm^3 and a diameter of 20μ, the impingement efficiency, at 5 mph, will be 80% (see Fig. 21). Suppose that 100 particles are collected during the hour; the concentration

$$\chi = \frac{100}{0.2 \times 0.8} = 625 \text{ particles/m}^3 \quad .$$

The advantages of the flag sampler are:

1. It is sufficiently inexpensive to be useful in mass sampling programs.
2. Its efficiency is known and is satisfactory over a range of particle sizes which include the common aeroallergens.
3. The small sampling area minimizes the chance of accidental contamination after exposure.
4. Counting time is reduced significantly.

The disadvantages include:

1. The flag sampler is inefficient at very low wind speeds, approaching zero efficiency as the wind becomes calm (see Fig. 21).
2. The sampler is inefficient for very small particles (Fig. 21). It is possible to sample for smaller particles by reducing the pin diameter, but there is a practical limit to this reduction at about 1/2 mm because of the thickness of scotch tape and frailty of the pin.
3. The average wind speed must be known before the concentration can be calculated, and while the flag sampler itself may cost only a few cents, a recording anemometer is a great deal more expensive.

3.7 THE ROTO-BAR SAMPLER

The roto-bar sampler used at The University of Michigan is a modification of the roto-rod sampler originally designed by personnel of the Stanford University Aerosol Laboratory.[32] The principle of operation is much the same as for the flag sampler. A bar is rotated through the air at a constant speed. Particles impinge on the leading edge of the bar due to their inertia and are collected by a suitable adhesive (e.g., dilute rubber cement). The constant speed of rotation eliminates many of the disadvantages inherent in the flag sampler. Within reasonable limits the collection efficiency of the roto-bar sampler is independent of wind speed.

The sampler is a flat bronze bar (3.8 x 0.6 x 0.08 cm), notched on either end (see Fig. 23a). The length and depth were chosen so that 1-in.-wide, double-coated scotch tape could be applied to the bar and easily removed. The thickness was chosen so that the theoretical impingement efficiency would exceed 80% for particles 10μ in diameter and larger (Fig. 24). The sampler bar is held by a pair of phosphor bronze springs suitably notched and counter balanced (Fig. 23b). The springs are mounted on a shaft extension of 3600 rpm 110-v a-c synchronous motor as shown in Fig. 25.

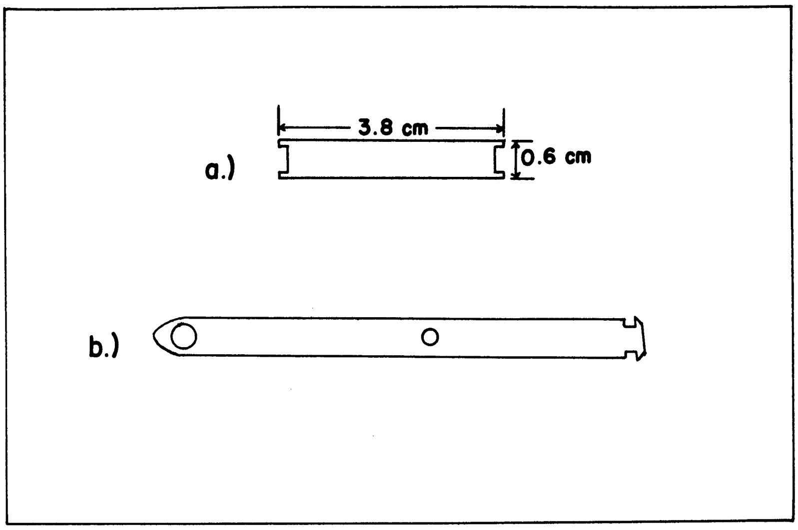


Fig. 23. Two components of the roto-bar sampler. (a) The sampling bar. (b) Phosphor-bronze spring holder for the sampling bar.

A "coolie-hat" (i.e., conical) rain shield has been designed so that the sampler can be used during adverse weather. During rain, only the smallest drops reach the sampler, and these do not appear to interfere with the collection efficiency.

As it is currently designed, the roto-bar unit samples nearly 1 m^3/hr. Conversion from count to concentration can be readily performed using the efficiency curve of Fig. 24, which was derived from Brun and Mergler's data.

The relationship between roto-bar efficiency and particle diameter, as shown in Fig. 24, must be used with reservation. The curve was drawn using Brun and Mergler's data for spherical particulates, and a right circular cylindrical collector and a perfect adhesive surface. In most cases none of these conditions is completely satisfied.

The sampling bar as currently designed is not cylindrical, but neither is it rectangular. During production, deburring slightly rounds the edges of the bar, and when the cellulose tape is applied, it springs slightly away from the leading edge of the bar. The net effect is to produce a curved impingement surface which may be considered to be of circular section.

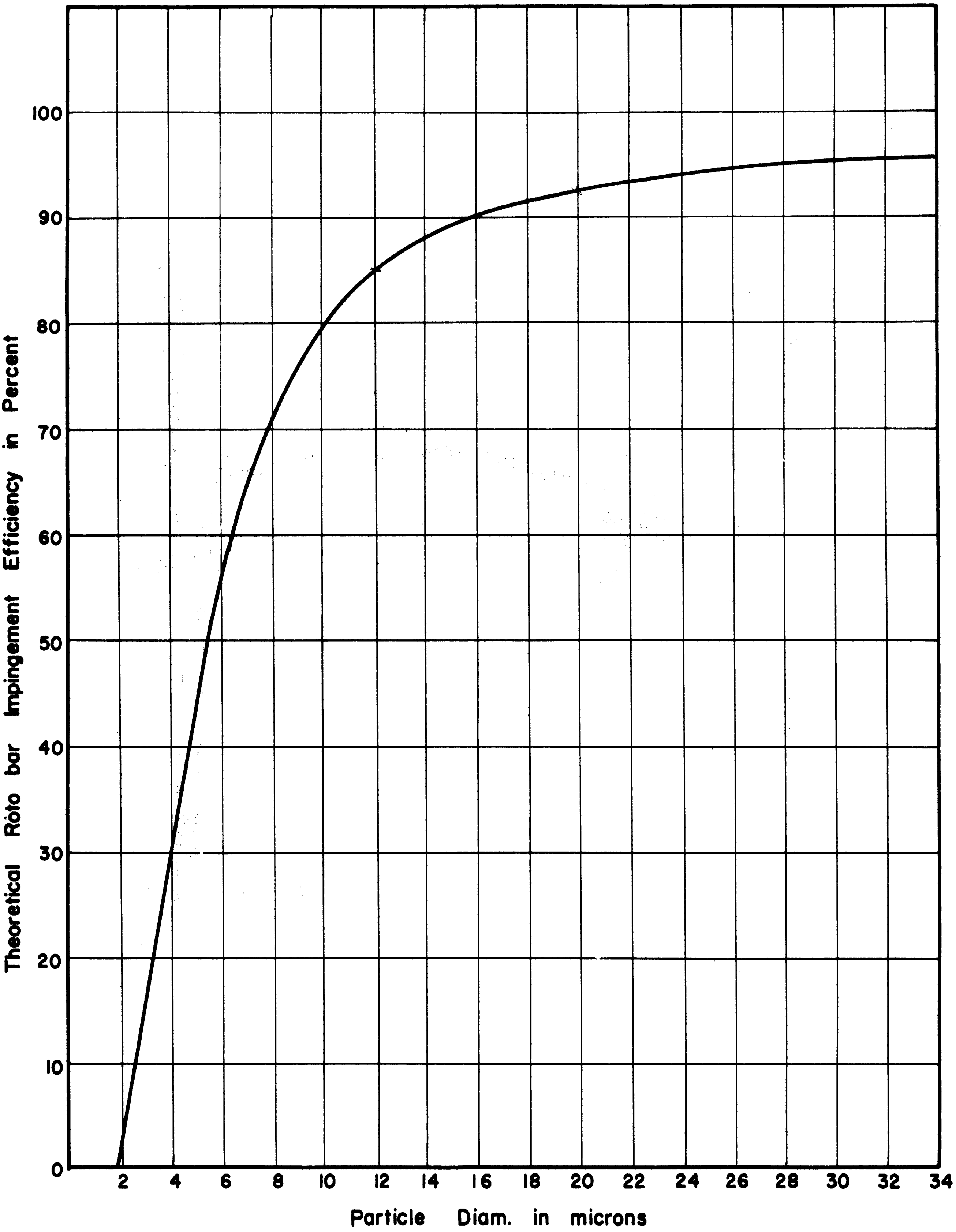


Fig. 24. Roto-bar efficiency for particles of unit density as a function of particle diameter.

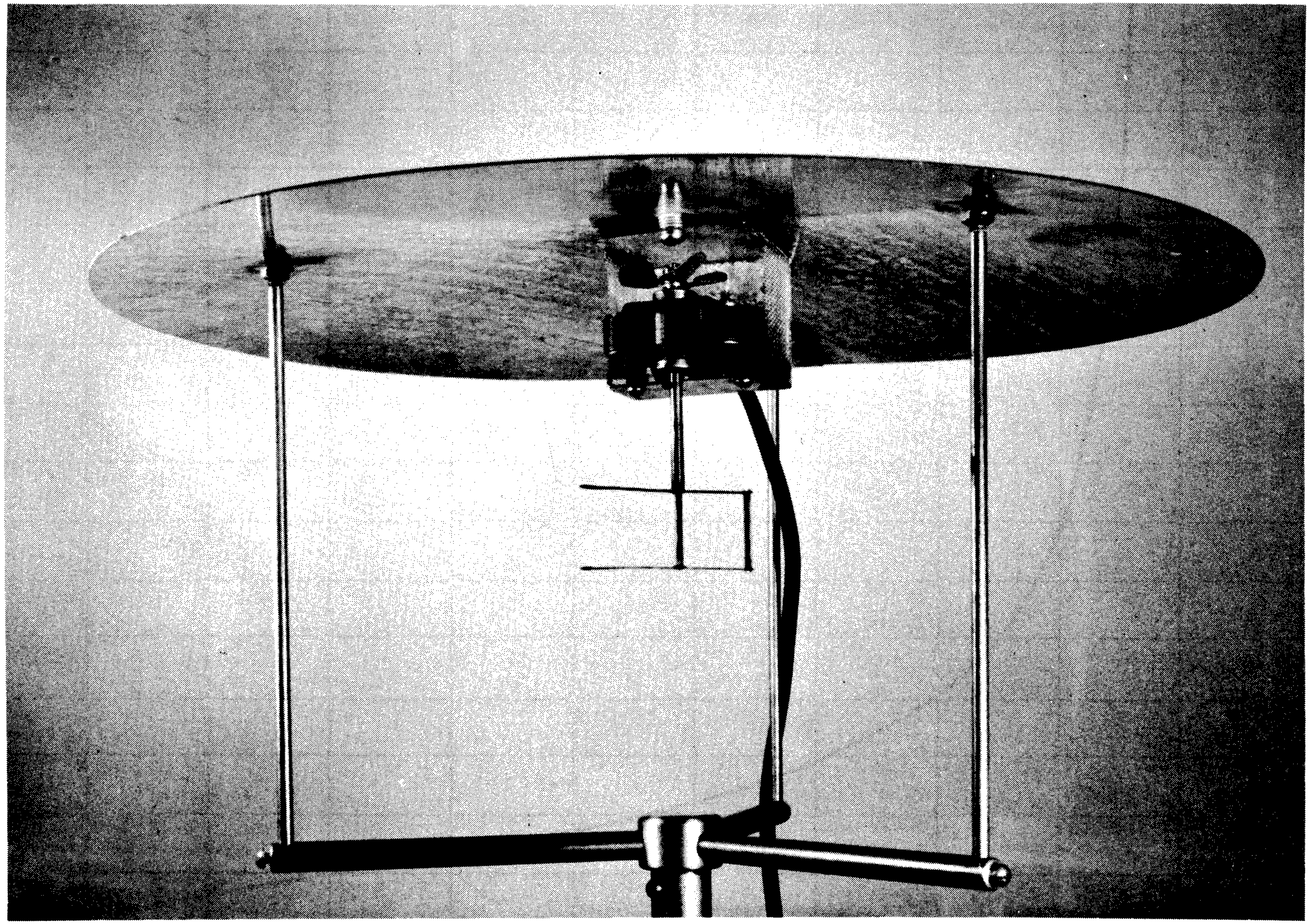


Fig. 25. A complete roto-bar sampling unit with rain shield.

Few of the common aeroallergens are spherical; Ambrosia pollen is probably the closest and Helminthosporium the farthest from that ideal. If great sampling accuracy is desired, an equivalent spherical size could be computed for the irregular particulates by utilizing their known densities and fall speeds.

At present, there is no perfect collecting surface for particulates as large as the common aeroallergens. Of the substances tested, dilute rubber cement seems the most promising. Wind-tunnel experiments are continuing both to measure the adhesive efficiency of rubber cement as a function of wind speed and to investigate other adhesive coatings.

The large volume sampled by the roto-bar can prove disadvantageous when a long sampling period is desired. For example, a roto-bar run continuously for 12 hours or more in a smoky or dusty atmosphere collects so many particles that counting and recognition of the various granules becomes difficult.

3.8 ELECTRONIC POLLEN COUNTER

It was reported in the last progress report that work has been done on an electronic pollen counter. A full discussion of this instrument follows.

In 1945 Guyton[33] constructed an electronic counting device to size aerosol particles. This device served as a model for the construction of the electronic pollen counter used by this project. The pickup and electronic components differ in form from that of Guyton's instrument but not in principles of operation.

The pickup probe (Fig. 26) consists of a capillary tube 2-1/2 in. long with a 0.8-mm bore fitted into a polystyrene tube. A vacuum of approximately 16 in. of Hg is maintained on this probe so that pollen particles are drawn through the capillary. These particles impinge upon or come into close proximity of a No. 26 wire which is placed 1 mm beyond the exit of the capillary. The No. 26 wire is attached to the grid of a cathode-follower-connected triode. It appears that the pollen grains pick up an electrostatic charge while traveling through the capillary. This charge is then transferred to the wire from the particles as they pass out of the capillary.

If it is assumed that the pollen grains are 20μ in diameter and the input capacitance of the grid circuit is 10 μμf, the maximum voltage pulse would be 3.3 mv. Guyton found empirically that $(\text{pulse voltage in } \mu v)^{1/2} = 3.04$ (particle diameter in μ). This gives a voltage of 3.7 mv. Observation on an oscilloscope showed that the average voltage was approximately 3.5 mv, which is in good agreement with the above values.

The electronic components consist of a power supply, a cathode-follower-connected triode, a triode amplifier, three pentode amplifiers, a mono-stable multivibrator and a power pentode (see Figs. 27 and 28). The power tube drives a mechanical counter. The two triode stages and capillary probe are housed as

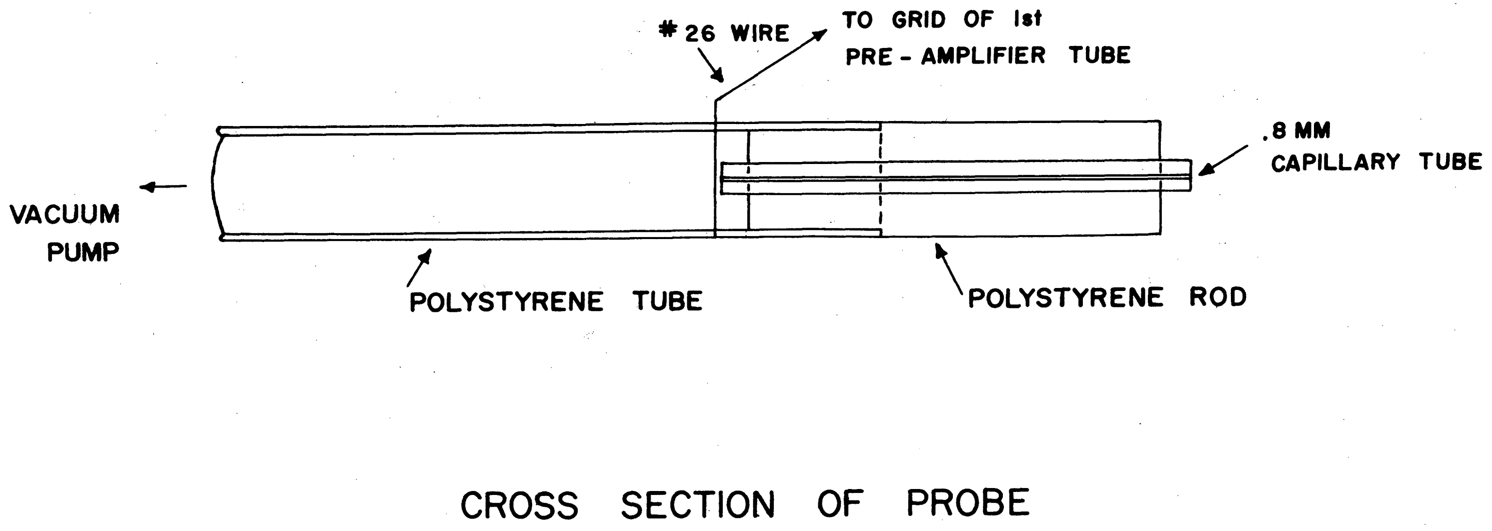


Fig. 26. Cross section of probe.

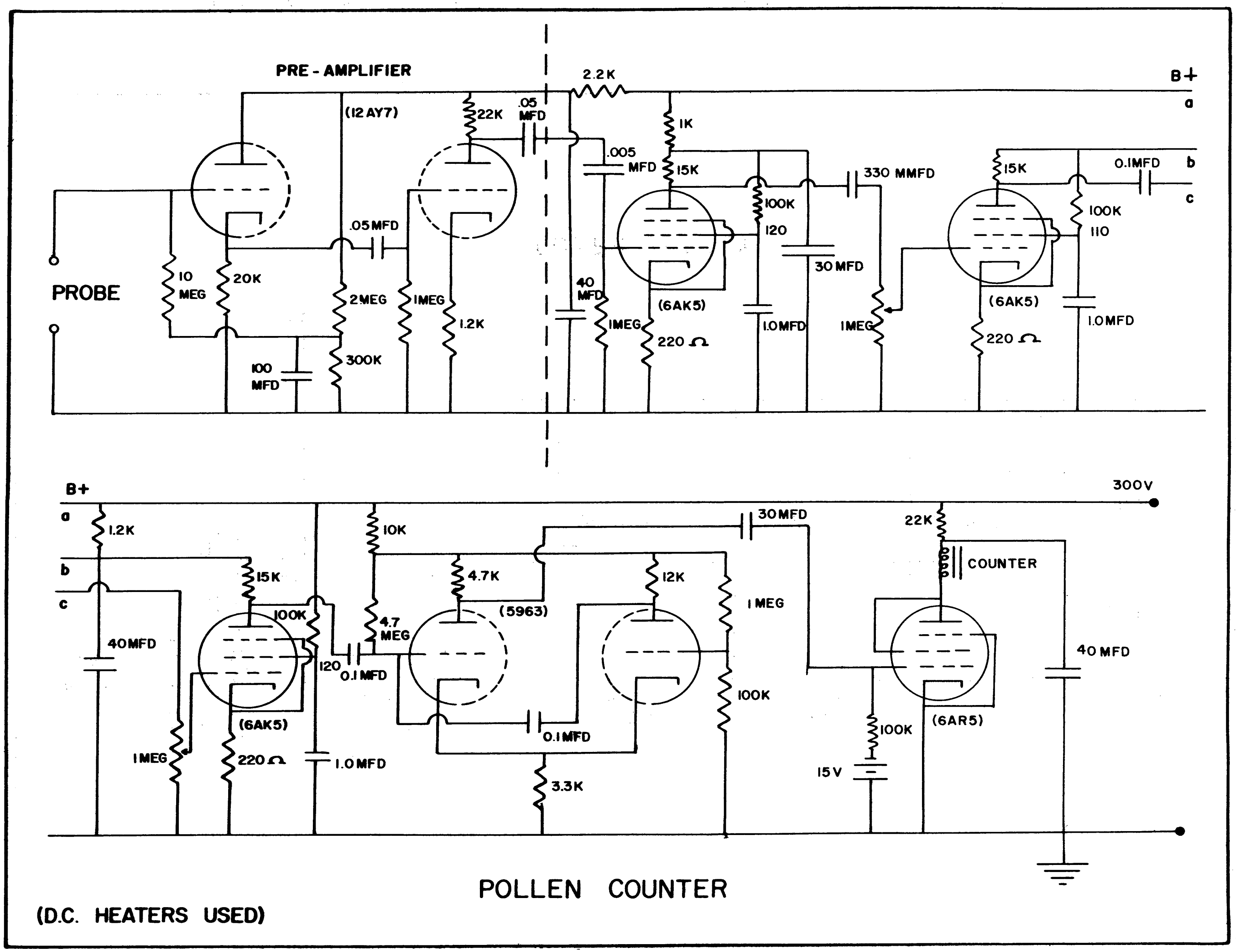


Fig. 27. Wiring diagram of electronic pollen counter.

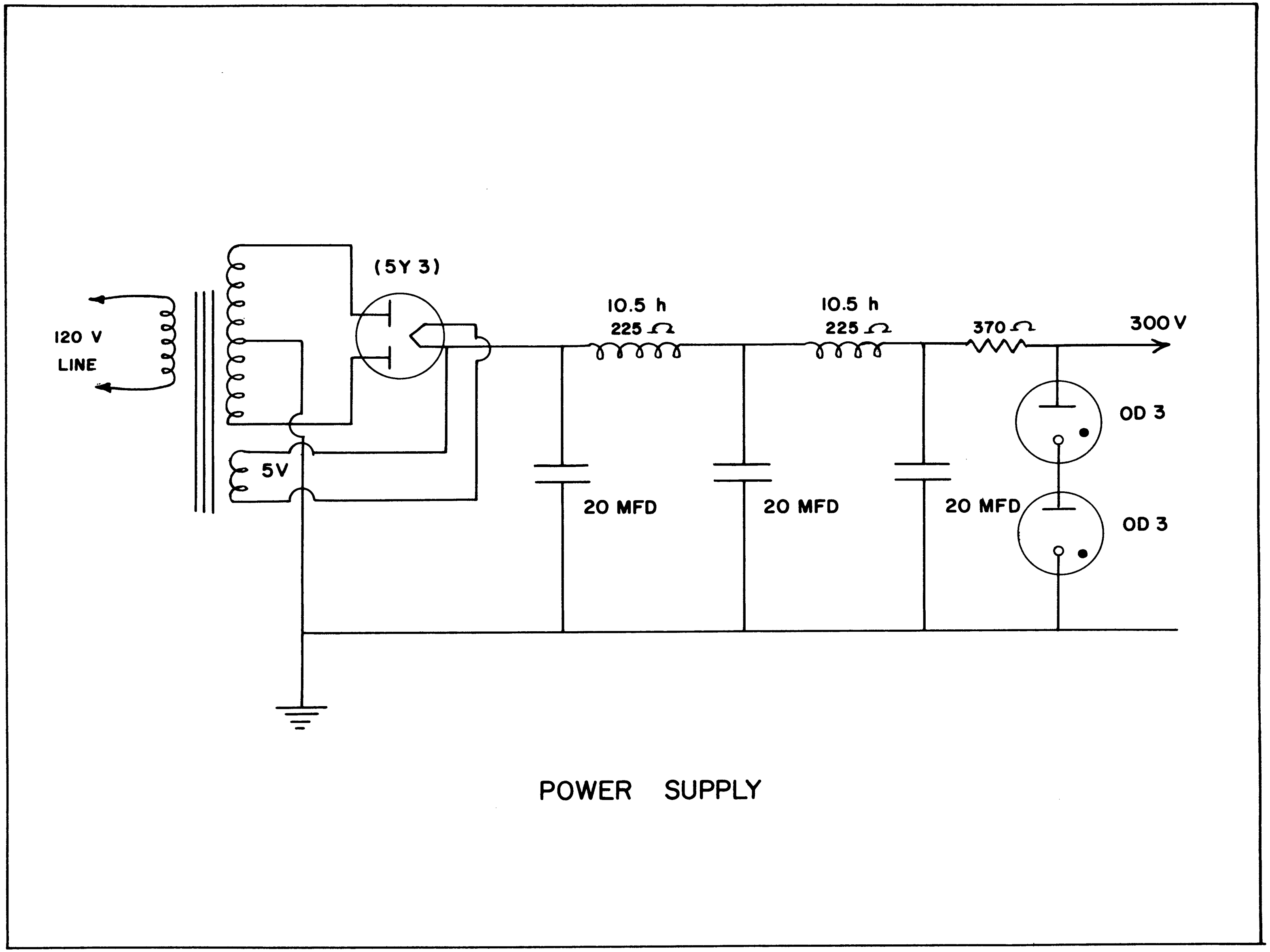


Fig. 28. Wiring diagram of power supply for pollen counter.

an individual unit or preamplifier pickup. The power supply and main amplifier are found on separate chassis.

In operation a constant volume of air (.134 cfm) is drawn through the pickup and the sensitivity is adjusted, by means of a potentiometer in the amplifier circuit, so there is no count in "clean" air. Each pollen grain that passes the probe wire causes a pulse, of about 3.5 mv, which is amplified and recorded by the mechanical counter. Since this device will count any particle in the 20-μ size range, it is designed for use in controlled experiments such as in the test chamber at the University Hospital where aerosol concentrations can be regulated.

At present the limitation of the pollen counter is the mechanical counter which has a frequency limit of 25 cps. This corresponds to approximately 4×10^5 pollen grains per m^3.

3.9 WIND-TUNNEL TESTS

Efficiency tests of several aeroallergen samplers were conducted in a wind tunnel of the closed duct or return flow type. Although the tunnel was originally designed for tests with aircraft models, wind speeds can be generated which are sufficiently low for meteorological applications. The test section is 4.5 m long, 1.8 m high, and 2.5 m wide.

Due to its closed duct construction, the tunnel is ideal for efficiency testing of the type described below. The pollen is placed in the tunnel through a small hole in the ceiling just downwind from the samplers. The pollen then passes through the fan, through the return ducts, through the turbulence- reducing meshes, and thence to the samplers. Concentrations were measured at 40-cm intervals horizontally and vertically across the test section and found exceedingly uniform except in the 60 cm nearest the tunnel walls.

The procedure followed in each experiment was as follows. Air in the tunnel was brought to the desired speed, and pollen samplers were placed in the test section. Pollen was then injected into the air stream, and roto-bars and pumps for filters and impingers were started. After 10 min the motors were stopped and the samples were protected from further pollution as quickly as possible. The samples were then removed and the wind speed was adjusted to a new value. The procedure was then repeated.

The possibility of error due to slight differences in exposure time of the various types of samplers was checked in the following way. A six-head millipore-filter unit, with the heads facing downward, was placed in the test section and the timer was adjusted for a 3-min cycle. Wind speeds were reduced as low as possible (to 3 mph) so that the catch would be reasonably large. One head was run prior to placing pollen in the tunnel and the remaining five in sequence for 3 min each. It was found that the concentration was reduced to

0.7% of its initial value in 10 min and to 0.07% in 15 min. After 15 min the concentration remained almost constant, probably due to reflotation from structural supports in the return duct and from the propeller. Thus, during removal of the samplers after exposure, short differences in sampling time had a negligible effect upon the pollen catches.

A six-directional millipore-filter unit was designed to measure the effect of orientation on collection efficiency (see Fig. 29). The unit consisted of six millipore-filter heads mounted so that the apertures faced into the wind, downwind, upward, downward, and to either side from an egg-shaped aluminum center piece 7.0 cm long by 5.0 cm in diameter. The unit was mounted in the center of the test section of the wind tunnel 45 cm above the floor.

To insure that the flow was equal through each head, heads, with millipore filter in place, were tested separately and only those whose volumes were within 5% of each other were chosen for each test. The heads were then covered with masking tape and screwed in place in readiness for the next test.

Six coated microscope slides, held on brass plates by small clips, were mounted on either side of the millipore-filter unit. Four slides, two facing upward and two downward, were placed at the level of the downward-facing millipore filter. Of the two slides in each direction, one was mounted with its long axis parallel, and the other normal, to the air flow. The other two samplers were about 6.5 cm higher and facing the wind (as shown in Fig. 29).

The collecting surface on the slide was double-coated cellulose tape, 2.54 cm wide, placed across the center of the slide. It was not realized until later that cellulose tape has a relatively low adhesive efficiency compared to dilute rubber cement.

Many tests were run at various wind speeds both with and without baffles. The purpose of the baffles was to induce turbulence in the air stream similar to that encountered outdoors. Later tests were run outdoors; hence the tunnel tests with baffles are not reported here. All tests showed much the same result; a typical one is illustrated in Table III.

The technique used to convert pollen count to concentration varied with each type of sampler. Horizontal gravity slides were assumed to sample a volume equal to the product of the fall speed of the pollen (1.6 cm/sec), the area of the sampling surface, and the time. Vertically oriented slides were assumed to sample a volume equal to the product of the area of the sampling surface, the wind speed, and the time. The millipore filters regardless of orientation were each assumed to sample 1/6 of the total volume of air drawn through the six-directional unit.

Three main conclusions can be drawn from the dramatic results displayed in Table III and reproduced in eleven other tests:

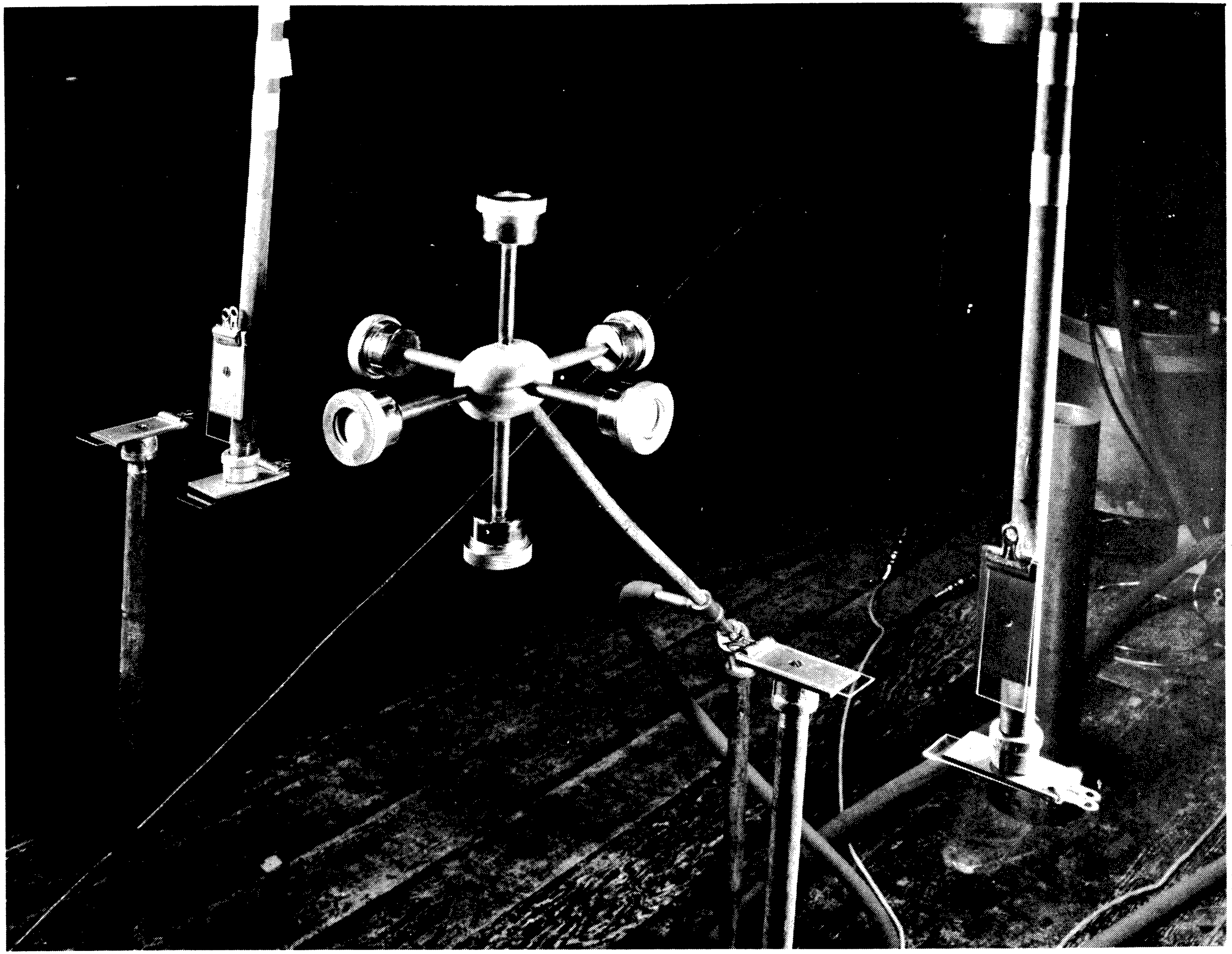


Fig. 29. Experimental wind-tunnel setup showing the six-directional millipore-filter unit with gravity-slide samplers on either side.

TABLE III

RESULTS OF A TYPICAL WIND-TUNNEL TEST DEMONSTRATING THE EFFECT OF ORIENTATION ON THE EFFICIENCIES OF GRAVITY SLIDE AND MILLIPORE-FILTER SAMPLERS IN NONTURBULENT FLOW

Test 8
Time: 10 min
Wind speed: 670 cm/sec (15 mph)
Total volume through six-directional unit: 0.645 cu m

Slides		Count	Computed Concentration in grains per cu m x 10^{-2}
Facing	Orientation of Long Axis		
Downward	Normal to wind	1	2
Downward	Parallel to wind	22	37
Upward	Normal to wind	17	29
Upward	Parallel to wind	530	900
Into the wind	Vertical	3034	12
Millipore Filters			
Facing left wall of tunnel		1	0
Facing right wall of tunnel		0	0
Facing upward		4	0
Facing downward		4	0
Facing into the wind		52080	6131
Facing downwind		7825	921

1. Horizontal microscope slides positioned in a nonturbulent air stream are poor samplers. Not only are they inefficient, but the efficiency varies markedly with orientation.

2. Millipore filters oriented normal to a nonturbulent wind have extremely low efficiencies except when the wind is nearly calm. For a curve showing millipore-filter efficiency as a function of wind speed,see Fig 30. Thus millipore-filter sampling is satisfactory indoors, as in the earlier prison-hospital sampling program, but unsatisfactory outdoors except in very light winds.

3. Filters facing into the wind are highly efficient, catching up to 10,000 times the number of particles caught by filters facing normal to the wind. Problems associated with this type of sampling have been mentioned earlier.

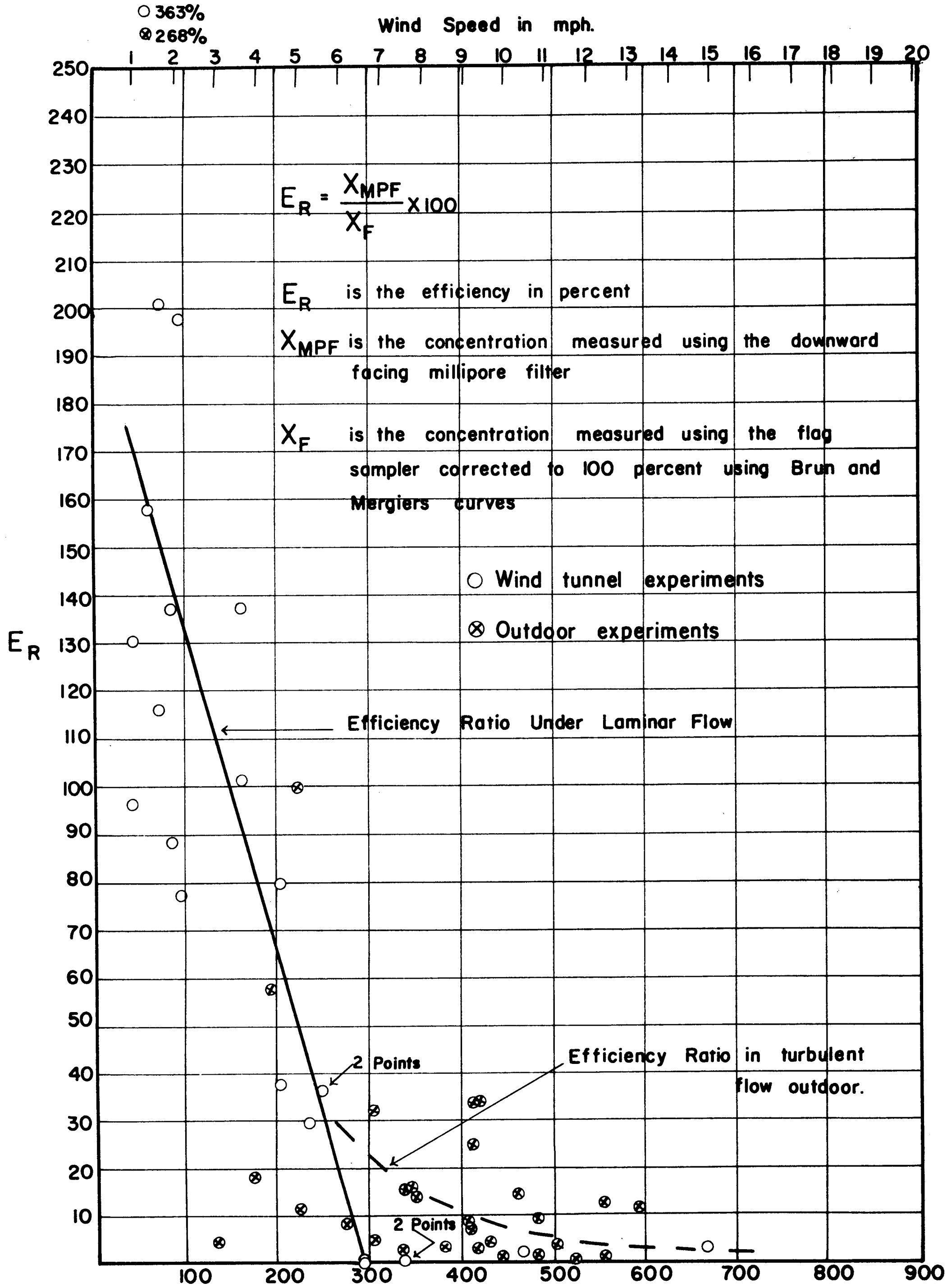


Fig. 30. Experimental millipore-filter efficiency as a function of wind speed. Efficiencies greater than 100% are the result of imperfect adhesiveness of the dilute rubber cement surface on the flag samplers.

The extraordinarily high counts on the forward-facing samplers led to experimentation with vertically oriented cylinders. Two sizes were used, pipe 17.44 mm in diameter and wire 1 mm in diameter. Both were wrapped with cellulose tape coated with dilute rubber cement. Dilute rubber cement was also applied to the cellulose tape on the microscope slides in these experiments. Typical results are shown in Table IV. No significance should be attached to differences in count between experiments since the amount of pollen injected into the tunnel varied from one experiment to another.

During tests 10, 11, and 12 a grid of lath 3.8 cm wide, 1.3 cm thick, spaced 20.3 cm apart was placed across the entire throat of the test section to induce small-scale turbulence in the air stream. A Gill bivane[34] was used to compare turbulence generated in the tunnel with normal turbulence outdoors. The bivane recorded a very low amplitude trace in the tunnel, while outdoors, even with stable air and light winds, the amplitude was two orders of magnitude larger.

The slight turbulence present during these three tests together with an increase in air velocity through the filters resulted in an increased catch on the normal facing filters relative to the forward facing filter. Otherwise the results for gravity slides and filters closely resemble those for test 8 shown in Table III.

Concentrations computed from the catch on the 1-mm wire sampler were of the same order of magnitude as those computed from the catch on forward facing filters.

Further experiments were conducted to determine the reliability of the wire sampler. A typical test in which five samplers were exposed at the same height across the center of the wind tunnel test section is illustrated in Table V.

3.10 OUTDOOR TESTS

Outdoor experiments were conducted to measure the effect of natural turbulence on sampler collection efficiency. Two series of tests were run, the first using commercial pollen dispensed from a tube by compressed air, and the second conducted during the regular ragweed season.

During the first series of experiments the samplers were mounted 0.6 m above ground level in an array 3.0 m long by 1/2 m wide. Pollen was dispensed 30.0 m upwind from the array so that the cloud of pollen passing the array would be uniformly dispersed. A plume of smoke, made by mixing ammonia and sulfur dioxide gases, made it possible to follow the pollen plume visually.

Meteorological instrumentation included the following:

TABLE IV

RESULTS OF THREE WIND-TUNNEL TESTS COMPARING EFFICIENCIES OF GRAVITY SLIDES, MILLIPORE FILTERS, AND CYLINDERS UNDER SLIGHTLY TURBULENT FLOW

		Test:	10		11		12	
		Time, min:	10		10		10	
		Wind, cm/sec:	223		447		670 (15 mph)	
		Vol, cu m:	1.55		1.42		1.55	
			Count	Computed Concentration in grains per cu m x 10^{-2}	Count	Computed Concentration in grains per cu m x 10^{-2}	Count	Computed Concentration in grains per cu m x 10^{-2}
	Slides							
	Facing	Orientation of long axis						
1.	Downward	Normal to wind	0	0	28*	-	0	0
3.	Downward	Parallel to wind	2	3	1	2	0	0
2.	Upward	Normal to wind	8	14	3	5	1	2
4.	Upward	Parallel to wind	744	1265	186	316	45	77
5.	Into the wind	Vertical	3850	58	1118	8	441	2
	Millipore Filters							
7.	Facing left wall of tunnel		3653	307	21	2	21	2
9.	Facing right wall of tunnel		9442	794	89	8	53	5
11.	Facing upward		5859	493	61	6	15	1
12.	Facing downward		4066	342	59	5	32	3
8.	Facing into the wind		73500	6180	6257	566	1975	166
10.	Facing downwind		23100	1942	945	85	236	20
	Cylinders							
	Pipe 17.4 mm diam		5470	92	4871	42	485	3
	Wire 1.0 mm diam		3310	971	1298	190	826	81

*High count appears to be due to accidental pollution.

TABLE V

THE CATCH ON FIVE 1-MM-DIAM WIRE SAMPLERS PLACED AT THE SAME ELEVATION ACROSS THE WIND-TUNNEL TEST SECTION

Counts	1809	1862	2007	1992	1822
Mean	1899		Standard Deviation 70		

1. Thermocouples at 1/2, 1, 2, and 4 m.
2. Anemometers at 1/2, 1, 2, and 4 m.
3. A bivane at 1 m.

Accurate measurement of lapse rate, wind speed, and turbulence was possible.

The second series of experiments was conducted during the regular ragweed season. A flag, roto-bar and downward facing millipore filter sampler were mounted 3.7 m above a weedy field. An anemometer at the same level continuously recorded the wind speed. The three samplers were operated simultaneously for a 2-hr period from 10.00 to 12.00 EST on 13 days beginning September 1, 1958.

The results of the tests corroborate the wind-tunnel findings mentioned earlier. Millipore-filter efficiencies were higher than indicated in wind-tunnel studies as shown in Fig. 30, but still much below those of the flag and roto-bar samplers, except at very low wind speeds. The increased efficiency of the millipore filter outdoors is apparently due to the effect of impaction of pollen on the samplers by turbulent eddies. The dependence of millipore-filter catch on wind speeds is clearly indicated by the results of many experiments, four of which are illustrated in Tables VI and VII.

A comparison of roto-bar and flag-sampler results showed a surprising agreement in the calculated concentration (Table VII). Since no correction for impingement efficiency has been made, it was expected that the roto-bar, with its high speed of rotation and regulting high impingement efficiency, would indicate higher concentrations than the flag sampler, particularly at low wind speeds. This expectation was not born out by the observations. The diminution of the roto-bar catch is likely the result of decreasing adhesive efficiency at high wind speeds. Nevertheless, both flag and roto-bar samplers are seen to give uniform and reliable measurements of pollen concentration.

3.11 RESULTS FROM TESTS

1. The gravity slide is not a suitable instrument for measuring concentrations of particulates in the air. If gravity slides must be used, they

TABLE VI

RESULTS OF TWO TESTS CONDUCTED OUTDOORS OVER LEVEL GROUND USING COMMERCIAL RAGWEED POLLEN

Time:	10		10	
Wind, cm/sec:	58 (1.3 mph)		460 (10.3 mph)	
Vol, cu m:	0.26		0.05	
	Count	Computed Concentration in grains per cu m x 10^{-2}	Count	Computed Concentration in grains per cu m x 10^{-2}
Glass slide, long axis normal to the wind	760	1238	15	26
Glass slide, long axis parallel to the wind	1129	1852	67	109
Downward facing millipore filter	9793	376	11	2
Flag sampler	582	66	97	14

should be mounted level with the ground where wind speeds are virtually zero.

2. Samplers which draw air through an aperture are not recommended for outdoor sampling unless the particle being sampled is extremely small (i.e., at least under 5μ).

3. The flag sampler is an excellent instrument for use in large-scale sampling programs or at other times when a roto-bar sampler is not available. Its limitations in very light winds or for very small particles must be considered in calculating true air concentrations.

4. The roto-bar sampler has very distinct advantages over previous outdoor sampling techniques and is highly recommended for pollen or spore sampling programs. Its efficiency is semi-independent of wind speed so that roto-bar counts can be used directly to calculate concentrations without recourse to an anemometer record.

TABLE VII

RESULTS OF TWELVE TESTS CONDUCTED OUTDOORS 3.7 METERS ABOVE A WEEDY FIELD DURING THE NORMAL RAGWEED SEASON

Sampler	Wind Speed		Count	In Grains per cu m	Wind Speed		Count	In Grains per cu m
	mph	cm/sec			mph	cu/sec		
M.P.F.	3.0	134	8	18	9.9	443	12	28
Flag	3.0	134	46	187	9.9	443	964	1190
Roto-bar			170	92			790	424
M.P.F.	5.4	241	68	148	10.8	483	63	134
Flag	5.4	241	288	653	10.8	483	987	1119
Roto-bar			1232	660			2529	1353
M.P.F.	8.5	380	7	14	12.1	541	7	14
Flag	8.5	380	195	279	12.1	541	2209	2232
Roto-bar			272	145			3266	1748
M.P.F.	9.1	407	128	286	12.4	554	3	7
Flag	9.1	407	2573	3289	12.4	554	434	427
Roto-bar			5824	3115			399	212
M.P.F.	9.3	416	172	371	13.2	590	40	88
Flag	9.3	416	650	855	13.2	590	697	646
Roto-bar			1319	706			930	498
M.P.F.	9.3	416	5	11	18.1	809	6	14
Flag	9.3	416	214	279	18.1	809	2256	1526
Roto-bar			435	233			2773	1483

Duration of each test - 210 min
Filter volume for each test was .45 ± .01 cu m

3.12 THE PRESEASONAL EXPERIMENTS

Analysis of the 1957 Data.—The 1957 preseasonal experiment has been described in a previous report.[5] Additional work has been done with these data to determine the effects of wind direction and speed upon pollen transport as shown by the coated slides at the 2-ft level.

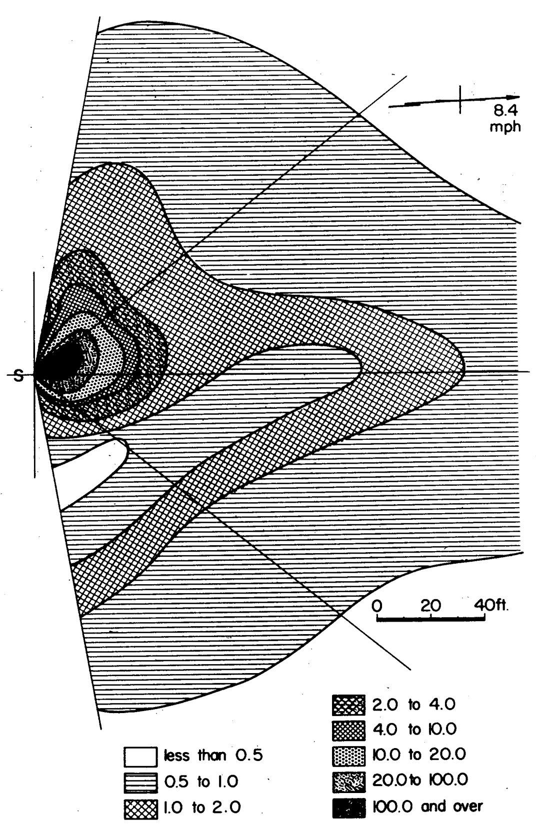
In studies of the initial "pilot-preseasonal" experiment conducted under this project in 1956, peculiar relationship of wind direction to the direction of largest catch of pollen on coated slides was observed.[24,35] To pursue further the questions raised by this early study, the 1957 preseasonal data were assembled in a similar manner.

In the case of the 1956 data, the wind speed and direction were measured at 8 ft above the ground. Selection of the data, using the 5-mph minimum wind speed criterion so that the direction would be representative, reduced the total to 31 hr of sampling time. The result of this summarization is reprinted in Fig. 31 for convenience.

Turning now to the 1957 preseasonal experiment and selecting data on a comparable basis, some immediate difficulty is encountered. The wind speed and direction recorders for the desired level were inoperative because of mechanical and electrical difficulties a large part of the time; hence it was necessary to use the 12-ft level for wind speed and the 8-ft level for direction. Again using the 5-mph minimum speed criterion, 52 hr of data were selected. These data were then combined on the basis of the mean wind vector for the respective sampling period as follows.

(1) The mean wind vector for each 4-hr sampling period was determined by vectorial addition of the eight 30-min mean wind vectors for the period. These were in turn determined directly from the wind records by visual averaging.
(2) The array of sample counts for each period was oriented so that the sampling ray nearest the mean wind vector for the period lay along the positive X-axis.
(3) The samples were then summed point-by-point for the entire 52-hr period.
(4) The mean departure of the wind vector from the positive X-axis for the composite sampling period of 52 hr was obtained by vectorial addition of the individual 4-hr vector departures.

Figure 32 shows the results obtained. Note that the scale of Fig. 31 is twice that of Fig. 32. Because of differences between the pilot experiment (Fig. 31) and the later full-scale experiment, the numerical values represent the pollen catch per cm^2 in 6 hr in Fig. 31, whereas in Fig. 32 they give the pollen catch per cm^2 in 4 hr. Nominally, therefore, one might expect to find a 3:2 ratio between the counts in Figs. 31 and 32, respectively, for a constant distance from the source. The respective counts, on the X-axis of the composite figures, are given in Table VIII.



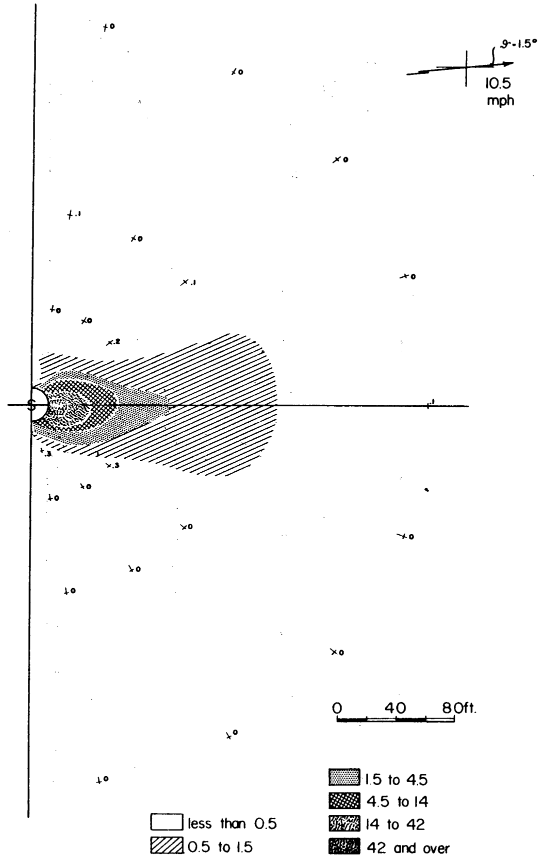


Fig. 31. Composite of ragweed pollen distribution for 1956 at wind speeds greater than 5 mph as constructed from impaction samples taken 2 ft above the ground. Source S was a 4-ft-square bed of hot-house-forced Ambrosia elatior set out in a mown meadow. Deposition values are in grains per cm^2 in 6 hr. Wind direction and speed measured 8 ft above the ground.

Fig. 32. Composite of ragweed pollen distribution for 1957 at wind speeds greater than 5 mph as constructed from impaction samples taken 2 ft above the ground. Source S was a 13-ft-diam circle of hot-house-forced Ambrosia elatior set out in a tomato field. Deposition values are in grains per cm^2 in 4 hr. Wind speed measured 12 ft above the ground. Wind direction measured 8 ft above the ground.

TABLE VIII

POLLEN CATCH AT 20, 40, 80, AND 160 FT FROM THE SOURCE IN THE DOWNWIND DIRECTION: 1956, COUNTS PER CM^2 IN 6 HR AVERAGED FOR 31 HR OF DATA; 1957, COUNTS PER CM^2 IN 4 HR AVERAGED FOR 52 HR OF DATA

Year	Distance, ft					
	10	20	40	80	160	320
1956	259.3	20.9	2.2	0.6	1.0	---
1957	---	67.2	15.4	2.2	0.6	0.1

If the data are smoothed by averaging at a constant radius over the 160° downwind sector of each of the composite diagrams, the figures of Table IX are obtained.

TABLE IX

AVERAGE POLLEN CATCH FOR ALL SAMPLERS WITHIN 80° OF THE DOWNWIND DIRECTION AS A FUNCTION OF DISTANCE FROM THE SOURCE, COUNTS INTERPRETED AS IN TABLE VIII

Year	Distance, ft					
	10	20	40	80	160	320
1956	62.7	9.6	1.4	0.9	0.5	--
1957	--	24.1	4.9	0.5	0.2	0.01

Both tables reflect the marked difference in extent of the source between the two experiments. It appears that this effect reaches to at least 5 radius lengths, or about 10 ft in the earlier and about 65 ft in the later experiment. Theoretical investigations of the effect of an extended source are presented below (Section 3.14).

It has been noted earlier that the close-in deposition figures showed a deviation of the maximum to the left of the vector mean wind direction. The 1957 data exhibit a small but significant deviation of the maximum deposition to the right of the vector mean wind direction. In both cases the directional deviation of the maximum appears to extend to or beyond about 80 ft, but it gives way to nearly perfect symmetry at 160 ft and beyond.

Interpretation of Pollen Counts.—Some examination of the sampling technique is required to construct a reasonable interpretation of the above data. Considering that the coated slides for the entire array are simultaneously exposed for each sampling period, and that hence all are exposed to similar wind and other environmental effects, it is reasonable to assume that the distribution of pollen counts so obtained represents the relative distribution of pollen concentration, χ, in the air moving over the slides during any particular sampling period. Summarizing these over a sufficient number of periods will give a similar result. It is not yet clear that the summarizations presented (Figs. 31 and 32) contain a large enough number of *periods* to be representative of the relative values of χ, and, in fact, the great differences between these figures may indicate to the contrary.

Assuming that χ may be represented by these figures, it is necessary to study the effect of wind and distance upon χ. Given a certain "source strength," Q, which expresses the total number of pollen grains per unit of time coming from the source, it is clear that

$$\chi(x) \propto \frac{Q}{v\ x^{m}}$$

where v is the wind speed, m is a positive number, and x is the distance downwind from the source.

In the case of ragweed pollen, it is also clear that "Q" depends upon "v" to some extent, in addition to other variables important in pollen production. It appears that an excellent biological index for "Q" would be the time rate of opening of male flowers but this is not available for the present analysis.

Assuming, then, that

$$Q \propto v^{k}$$

where k is a positive number, the expression for $\chi(x)$ becomes

$$\chi(x) \qquad \frac{v^{k-1}}{x^{m}} \ .$$

Thus it is anticipated that, at any instant of time, the concentration of pollen in an air sample downwind from the source will depend upon the wind speed and the distance upwind to the source, and hence the rate of deposition of pollen upon the coated slides must depend upon these variables.

This offers a clue as to the proper manner of summarization of the date, and suggests that the summarizations of Figs. 31 and 32 are the most valid possible only if k = 2. Because these figures are reasonable in most respects, and because there are independent mechanical reasons to expect that k ≐ 2 for the range of velocities encountered, this value of "k" may be assumed adequate for the present.

Interpretation of Wind Data.—Continuous records of wind speed and direction recorded using a relatively fast-response anemometer and wind vane suggest immediately an additional problem regarding the time variation of "Q," the source strength. Roughly, it may be said that the instantaneous wind speed varies from 20% below to 20% above the average for a period of the order of 15 min. If indeed $Q \propto V^2$, the instantaneous values of "Q" may vary, in such a case, from

$$Q \text{ min} \propto (.8\overline{V})^2$$

to

$$Q \text{ max} \propto (1.2\overline{V})^2$$

and hence

$$\frac{Q \text{ max}}{Q \text{ min}} = \frac{1.44}{0.64} = 2.25$$

if all other factors controlling "Q" remain constant. Thus if it were possible to reduce the wind record to wind vectors representative of the very short intervals of time required to detach pollen from the source plants, one would find several short periods, lasting a few tenths of a second, of high wind speed and a comparable number of short periods of low wind speed in the same 15-min interval of time.

Considering the process of turbulent mass exchange which is responsible for the observed fluctuations of wind speed near the ground, it is reasonable to anticipate a systematic directional difference between the high-speed winds (gusts) and the lulls. Assuming that the gusts are produced by elements of air that descend from higher levels, and that the wind direction shifts to the right with increased elevation following Taylor,[36] a deviation of the axis of maximum pollen concentrations to the right of the low-level mean wind direction is to be anticipated. This is essentially the result observed in Fig. 32.

It is, however, not satisfactory to accept this interpretation of Fig. 32 without further evidence of the systematic turning of the gusts with respect to the lulls for the periods in question. For some of the period, the wind speed and direction at the 2-ft level were recorded continuously using the Beckman-Whitley wind unit. An analysis of the peak gusts over a period of several hours does not confirm the hypothesis that the wind direction at the time of the gust is always to the right of the mean wind direction, nor does an analysis of the lulls show that the wind direction associated with each lull is to the left of the mean wind direction.

Thus, at this time, there can be no valid conclusion drawn about the reason or reasons why the 1956 data differ from the 1957 data.

Description of the 1958 Layout.—The plot and array were located at Willow Run Airport on a stretch of mown quack grass and stubble land. Here the land was extremely flat with no obstructions for over a mile to the west, north, and south. To the east lies a wide concrete apron and, a quarter of a mile away, a row of hangars.

The plot of artificially grown ragweed was similar to that of 1957 except that an 8-ft-diam space was left in the center to accommodate instruments and samplers. A path 30 in. wide extended from the periphery to the open center on the southeasterly side. This side was chosen for the path because winds from this direction are infrequent.

The array was laid out as in 1957 with markers every 20° on arcs at 20, 40, 80, 160, and 320 ft from the center of the plot. This geometry provides a basis for comparison between the 1956, 1957, and 1958 preseasonal experiments.

Instrumentation.—Certain highly specialized measuring equipment was already available at the site, having been used for the meteorological phase on Project Shimmer, UMRI Project 2144-002. Lapse rates were measured by sensitive thermocouples at heights of 1/2, 1, 2, and 4 meters. Wind speed was also measured at these levels. Radiation data were recorded using a Beckman and Whitley net radiometer and an Eppley pyrheliometer. A Gill bivane was used to record the wind direction and the lateral and vertical gustiness. Temperature and humidity in the plot were measured using the Bellaire Tetraskelion, a wet and dry thermocouple device. In addition, conventional hygrothermographs were located some distance away from the plot.

Four pollen sampling techniques were used. During the botanical-meteorological phase, millipore filters,flag samplers, and a roto-bar sampler were used to sample in the ragweed plot. The millipore filter has been found to have a decreasing efficiency with increasing wind speed, but is quite accurate at speeds below 2 mph. The flag sampler is most efficient at wind speeds above 2 mph and has zero efficiency when the wind is calm, i.e., when the millipore filter is at peak efficiency. The roto-bar has a uniformly high efficiency at speeds below 20 mph. Flag samplers were used on masts during the vertical-diffusion phase to measure concentration at various heights. Gravity slides were placed on blocks at the base of each mast to measure deposition. The blocks were worked into the ground so that the top of the slide was approximately at Z_o, the level at which the wind becomes zero.

Botanical-Meteorological Phase.—The life history of a pollen grain may be divided into roughly four phases: pollen production, emission, transmission through the air, and finally settling on the ground with subsequent destruction of the pollen grain. During the preseasonal experiment, the meteorological factors involved in pollen emission and pollen transmission through the air were studied in detail. This investigation was carried out jointly by the botanical and meteorological groups. The details of emission were studied by a botanist who marked an inflorescence on each of several plants. The number of projecting florets on each involucre were counted and recorded at regular intervals and observations made as to whether pollen was being emitted.

<u>The Vertical Diffusion Stage</u>.—Most current diffusion theories suggest that the axis of a plume of gases or particulates remains at ground level. In addition, several of the most widely accepted theories assume no deposition at ground level. If we accept the first hypothesis and reject the second as Gregory[29] has done, we have a plume whose axis stays at ground level and in which deposition occurs. The result is a very rapid deposition of the plume. Gregory suggests perhaps as high as 90% attenuation due to deposition within 100 meters. On the other hand, if deposition results in a gradual lifting of the plume axis, a vastly greater amount of pollutent will remain in the air.

To test this hypothesis and to gather other important information regarding diffusion, fifteen 20-ft masts were erected. The masts were placed at various distances from the source on 40° arcs. At a distance of 320 ft from the center of the source, 7 masts were placed each with six samplers placed at heights of 2, 4, 8, 12, 16, and 20 ft. At 160 ft from the center five masts were placed each with seven samplers at 2, 4, 6, 8, 12, 16, and 20 ft. At 40 ft, three masts, each with eight samplers were located at 1, 2, 3, 4, 6, 8, 12, and 20 ft. The samplers used on the masts were of the flag type. Sampling was done only during suitable weather for a period of time ranging from 1 to 2 hr each day.

<u>Summary</u>.—The 1958 preseasonal experiment was designed to answer the following questions.

1. How are emission and transmission of ragweed pollen related?
2. What meteorological variables control emission and what is the lag between change in a variable and emission of pollen?
3. How are pollen concentration and deposition related?
4. What is the rate of attenuation of a pollen plume?
5. How much pollen actually gets into the atmosphere?
6. Does the axis of the pollen plume rise downstream from the source?

The experiment was carried out as planned and analysis of the data has been begun following the abstraction of the data from chart rolls and the counting of samples. It is too early yet to say whether the questions posed above have been completely answered.

3.13 THE IN-SEASON EXPERIMENTS

The in-season experiment of 1958 was divided into three major sections: a continuation of past years sampling at the Jackson Prison Tower; a sampling program on the roof of the East Engineering Building at The University of Michigan; and a roadside experiment.

At the Jackson Prison Meteorological Tower, six-head millipore-filter units were mounted at 6, 25, 50, and 100 ft. Sampling was continuous, each sampler operating for a 4-hr period. Wind speed was recorded continuously at 12, 25, 50, and 102 ft while wind direction was recorded continuously at the 50-ft level. Temperature differences were recorded between 1-4 ft, 4-25 ft, 4-51 ft, and 4-102 ft. Temperature, humidity, and rainfall were recorded at the ground.

The data from this experiment will be comparable with those taken at the same heights and the same location in 1956 and 1957, thus providing continuity. Some field tests were also run in an effort to calibrate the millipore-filter and roto-bar counts. This section of the experiment ran from mid-August to mid-September.

The sampling program on the top of the East Engineering Building was divided into two separate parts. The first part consisted of placing a six-head millipore-filter unit 150 ft above the street level on the meteorological tower on top of the roof of the building. Although mechanical and electrical difficulties hampered the operation, it is anticipated that the data collected there, as well as at Jackson Prison, can be correlated with synoptic weather patterns in an effort to aid in forecasting pollen concentration. Although the marked limitations of the millipore filter for outdoor sampling were recognized, it was hoped that a calibration technique might be evolved which would permit some use of the data obtained.

A roto-bar sampler was also mounted on the top of the East Engineering Building, as well as on the roof of the Kresge Medical Research Building. Ragweed pollen, spores, and other pollens were collected and identified until early in December, 1958. During the peak of the ragweed pollen season, the roto-bar was changed every hour from 8:00 a.m. until 5:00 p.m. Changes in synoptic situation can be identified, indicating that perhaps the roto-bar counts may be more useful in a synoptic correlation than the millipore-filter counts.

The roadside experiment, too, was divided into two separate parts. In the first part, a flag sampler was mounted on the automobile which went back and forth to Jackson Prison each day. This flag sampler was placed on an 18-in. aluminum rod that extended out of the right-hand side of the automobile. Samples were taken at seven separate 1-mile intervals enroute to and from Jackson Prison. Samples were made during the morning on the way to the prison and about noon on the return trip to Ann Arbor. Figure 33 shows the sampling sites and the route traversed. Data were collected from 17 August to 14 September 1958. All the counts have been reduced to grains/m^3. Because the flag sampler needs associated wind-speed measurements, the wind-speed measurements from the tower at Jackson Prison were used. Again, it is anticipated that the counts can be correlated with the weather patterns to be used in forecasting pollen concentration.

The second part of the roadside experiment consisted of similar instrumentation mounted on another car which traveled a prescribed 43-mile route in and around Ann Arbor. Figure 34 shows the detail of this route. The flag sampler

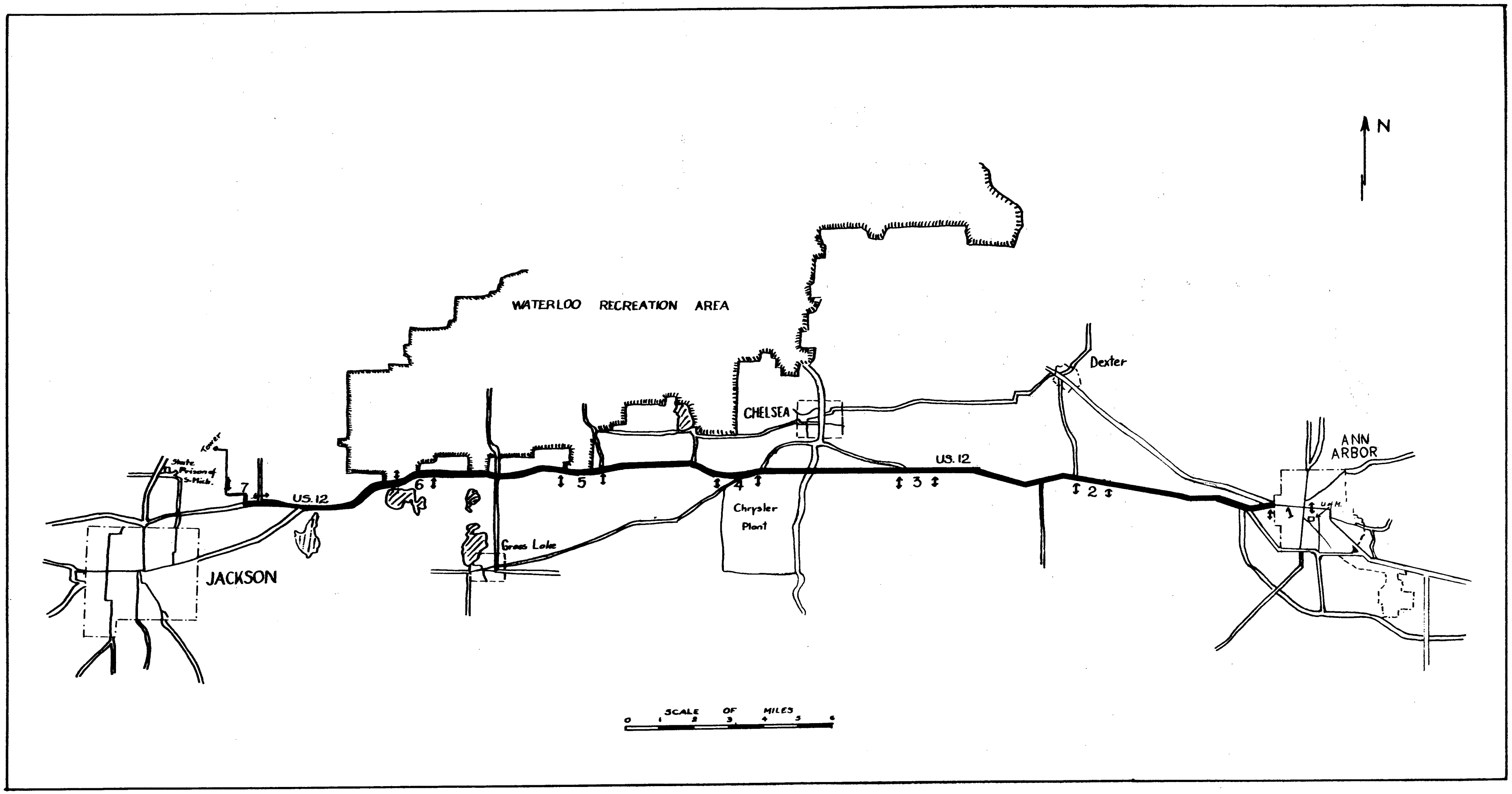


Fig. 33. Sampling sites and route traversed from Ann Arbor to Jackson Prison meteorological tower, 17 August to 14 September 1958.

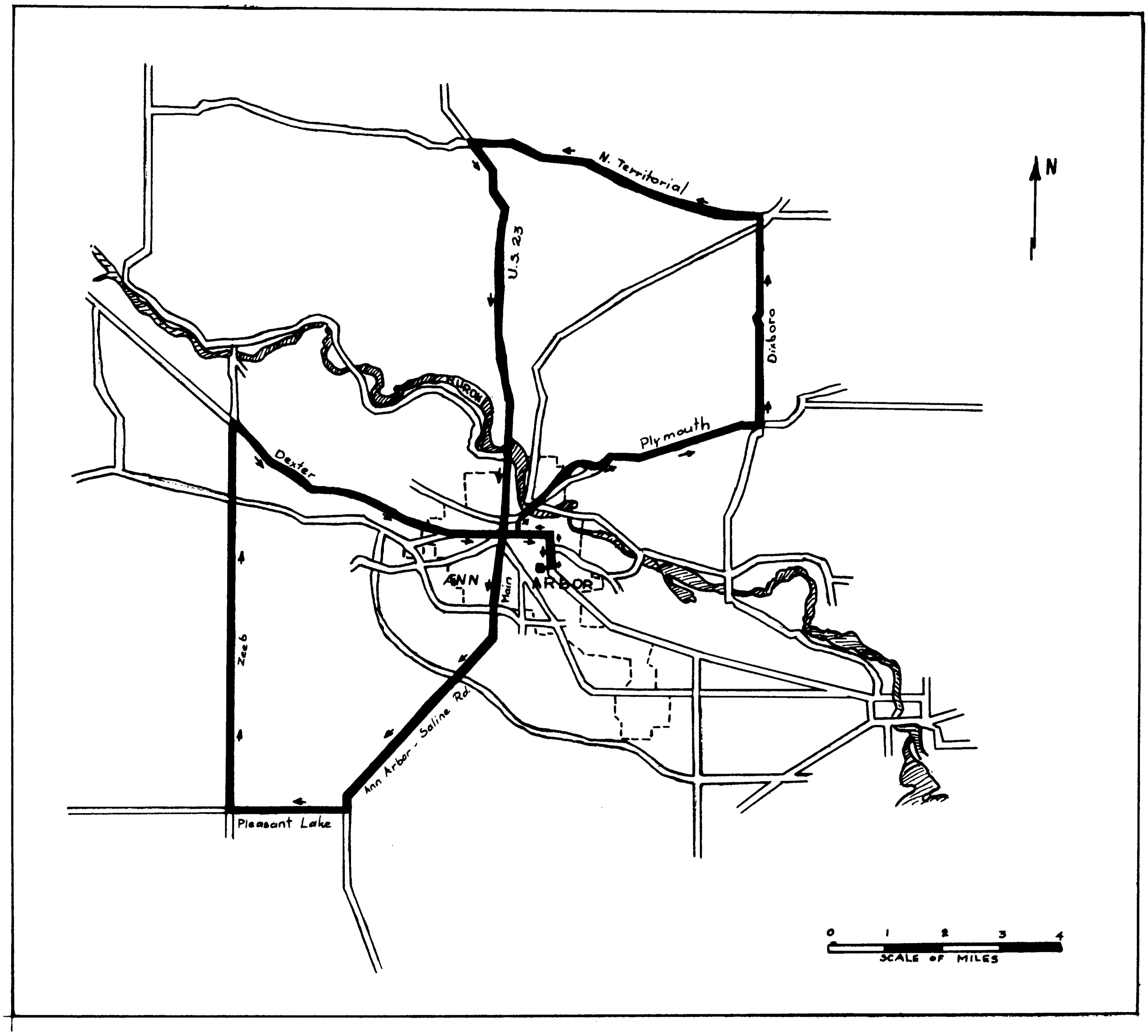


Fig. 34. Details of the route for the roadside experiment in and around Ann Arbor, 18 August to 12 September 1958.

was changed each mile. Because of the unavailability of a suitable anemometer, no wind-speed records were obtained on these trips. The car always moved at a constant speed of 35 mph except over the poorer roads when the speed was reduced to 20 mph. The average speed of the automobile was recorded during each mile interval. Since it is difficult to assess the wind speed, concentration will probably not be computed although relative dosages will be available. Sampling was carried out from 18 August to 12 September at a rate of from one to five trips per day. Normally a trip was made in mid-morning, early afternoon, and early evening.

The aim of both of the roadside experiments was to establish the extent of exposure that a person might encounter while driving back and forth to work in addition to comparing the variability in rural and urban conditions.

3.14 THEORETICAL STUDIES OF DIFFUSION FROM A CONTINUOUS SOURCE

This is the first attempt to obtain a reasonable estimate of the effect of eradicating ragweed plants within an area to reduce the pollen concentration at ground level downstream.

The problem is a particularly important one for communities wishing to alleviate the suffering of hay fever and asthma victims by carrying out a ragweed eradication program. These communities are likely to ask such questions as the following:

(1) In how large an area must ragweed be destroyed to reduce pollen concentrations in our community by 50%?
(2) Will it be worthwhile eradicating ragweed within a 3-mile radius from our city limits?
(3) We have attempted to destroy all ragweed plants within our city and have noticed no appreciable change in pollen concentrations. Has the eradication program been incomplete, or is the pollen arriving from a distant source?

The problem is being approached in two ways. First, the best diffusion theories are being adapted to the exceedingly complex problem of computing pollen concentrations downstream from an area source. Secondly, the mathematical model will be tested during the 1959 ragweed season using some ragweed-free area.

Although a mathematical solution to the problem is far from complete, a first approximation has been made using the following assumptions:

(1) The source strength, Q, expressed as the number of particles emitted per unit time from a unit area, is constant and the same everywhere in the source region.
(2) Diffusion is assumed to take place as if no ground surface were present.
(3) Diffusion parameters were considered to remain constant.

The theory developed by O. G. Sutton[26] in which atmospheric turbulence is believed to be the main agent in dispersing air-borne materials has been followed in this work. The diffusive property of atmospheric turbulence is described by a number of macroscopic parameters such as the index of the vertical wind profile "n" and the coefficients of diffusivity, "C_y" and "C_z." According to the theory, the concentration at a point P'(o,o,o) due to a point source of constant strength "Q" at another point, P(x,y,o) is given by

$$q_P(o,o) = \frac{Q}{\pi\ C_yC_zU\ x^{2-n}} \exp\left(-x^{2-n}\frac{y^2}{C_y^2}\right),$$

when there is a mean wind of speed U blowing along the x-direction. The concentration due to an area source is a direct extension of this formula and is obtained by integrating the formula over the area source.

The following formulae are obtained for concentrations due to various area sources:

(1) Infinite strip across the mean wind extending a distance x upwind:

$$q_{\infty,x}(o,o) = \left(\frac{Q}{\sqrt{\pi}\, C_z U}\right)\left(\frac{2}{n}\right) x^{n/2}$$

(2) Rectangular source of dimension x by $2y_o$:

$$\begin{aligned}
q_A(o,o) \\
= \left(\frac{Q}{\sqrt{\pi}\, C_z U}\right)\left(\frac{2}{n}\right) \Bigg\{ & x^{n/2}\, \Phi\left(\sqrt{2}\, \frac{y_o}{C_y}\, x^{(n/2)-1}\right) + \frac{1}{\sqrt{\pi}}\left(\frac{y_o}{C_y}\right)^{n/(2-n)} \Gamma\left(1 - \frac{1}{2-n}\right) \\
& - \frac{1}{\sqrt{\pi}}\left(\frac{y_o}{C_y}\right)\left(\frac{2-n}{1-n}\right) x^{n/2}\, \frac{\exp\left[-\dfrac{y_o^2}{C_y^2}\, x^{(n-2)/2}\right]}{x^{1-(n/2)}} \\
& + \frac{y_o^2}{C_y^2} \int_o^{x^{n-2}} z^{\left(\frac{1-n}{2-n}\right)} \exp\left(-\frac{y_o^2}{C_y^2}\, z\right) dz \Bigg] \Bigg\}
\end{aligned}$$

where

$$\Phi(x) = \int_{-x}^{x} e^{-z^2}\, dz \; .$$

(3) Infinite strip of width $2y_o$ along the direction of the mean wind:

$$q_{2y_{o,\infty}}(o,o) = \left(\frac{Q}{\pi C_z U}\right)\left(\frac{2}{n}\right)\left(\frac{y_o}{C_y}\right)^{\frac{n}{2-n}} \Gamma\left(1 - \frac{1}{2-n}\right)$$

where Γ = the gamma function.

Figure 35 presents some results computed from the equations listed above. The concentration due to any finite rectangular area source is expressed as a fraction of what one might expect from an infinite strip across the mean wind extending upwind 20 km. The value of "n" was chosen to be 0.25 after O. G. Sutton[26]. The value of the coefficient of horizontal diffusivity, C_y on a warm afternoon with a moderate wind and an unstable lapse rate has been reported to be about 0.4 $\mathrm{cm}^{1/8}$ or 0.095 $\mathrm{km}^{1/8}$.[26] Thus, under such conditions as described

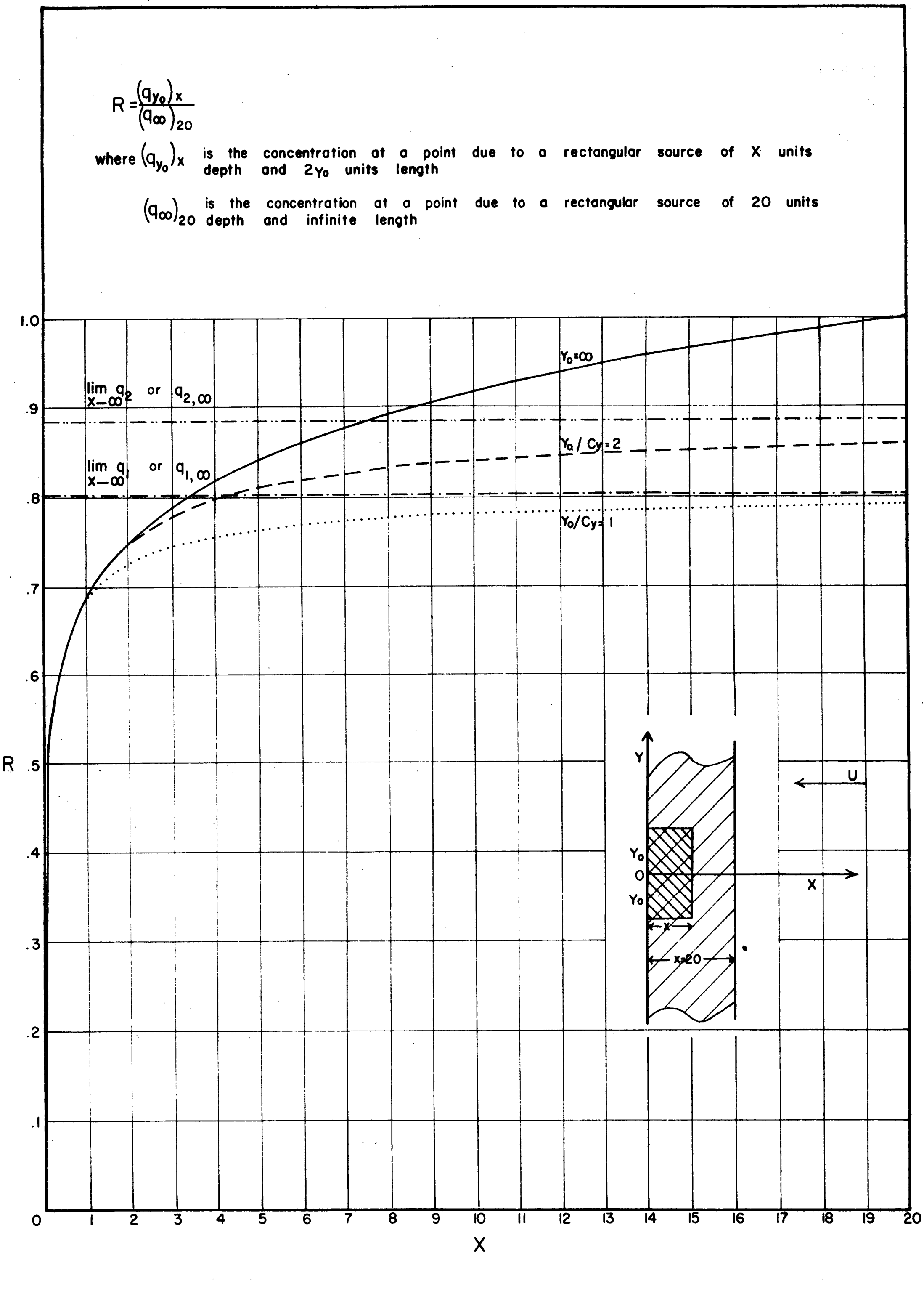


Fig. 35. Plot of the relative concentration at point 0 from an area source whose dimensions are x by 2y$_o$. The ordinate is the ratio of the concentration at a point due to a rectangular source x by 2y to the concentration at the same point due to a rectangular source 20 units by infinite length.

by the values of the meteorological parameters given above, one sees from Fig. 35 that by clearing an area of approximately 0.2 km in width and 1 km in depth out of a total area of 20 km in depth with an infinite width, the concentration would be reduced by 69%. By increasing the depth of the clear area to 2 km, one decreases the concentration by 73%. Table X shows the percentages of the concentration due to an infinite strip across the mean wind with various areas in the vicinity of the spot under consideration cleared of ragweed.

TABLE X

PERCENTAGE CONCENTRATION FROM A SOURCE OF INFINITE WIDTH AND 20-km DEPTH WITH A CLEAR AREA OF $2y_0$-km WIDTH AND x-km DEPTH

$2y_0 \diagdown x$	1	2	5	10	20
0.2	31	27	24	22	21
0.4	31	25	19	16	14

Figure 35, on the other hand, may also provide us information regarding the concentrations one could expect at various distances downwind from a fixed area source. For example, suppose we have an area source 2 km deep and 0.2 km wide and another 2 km deep and 0.4 km wide. If one takes the concentration at the edge of the former area source as 100, one would expect the percentage concentrations at various distances downwind as given in Table XI.

TABLE XI

PERCENTAGE CONCENTRATION AT VARIOUS DISTANCES, x_1 km, DOWNSTREAM FROM AN AREA SOURCE OF $2y_0$-km WIDTH AND 2-km DEPTH

$2y_0 \diagdown x$	0	1	2	3	4	5	6	7	8	9	10
0.2	100	6.6	3.7	2.5	1.6	1.2	0.8	0.5	0.4	0.3	0.3
0.4	102	12.6	6.4	4.1	3.2	2.3	1.8	1.5	1.1	0.8	0.7

Figure 35, together with Tables X and XI, show that the concentration at a point is really governed by the sources existing in its neighborhood. Under extremely unstable lapse conditions, the value of C_y is known to be as high as 0.8 $cm^{1/8}$ or twice as great as the value cited above. To achieve the same effect of reduction of the concentration as given in Table X, one would have to increase the width of the cleared area by twice the given values.

The present model is a very simple one with several extremely idealized assumptions. Perhaps a more careful study of the effect of the ground and deposition will improve the accuracy of the estimates. The effect of deposition by gravitational settlement may be taken into account by incorporating an exponential decay, the decay constant being proportional to the terminal velocity and the inverse of the vertical diffusivity, C_z. The effect of deposition reduces the concentration downstream, although it seems rather small.

3.15 STATUS OF THE TEST CHAMBER

The Engineering Section of the University Plant Department completed their investigation of costs for various types of construction for the chamber. After a number of conferences to insure that the design would meet the needs of the project, but at a minimum cost, working drawings were approved and a construction order was placed.

The basic design concept of the chamber remains unchanged, and the equipment purchased and delivered last year was incorporated into the design as planned. Walls of the chamber have been built of panels which are bolted together and may be disassembled if it is desired to move the chamber at some future date. The inside of the chamber walls have a painted plywood surface with a smooth finish for easy cleaning. The space under the chamber floor is used to obtain even pollen distribution so that it is necessary to remove the floor from time to time for adjusting the air distribution tubes and for cleaning. The material finally selected for the floor was welded aluminum subway-type grating. The floor sections were made in limited size panels for ease of handling.

The ceiling height of the room in which the chamber was placed was not as high as could be desired, and it was necessary to reduce the inside height of the chamber to 7 ft 4 in. The floor size of 9 by 8 ft remained unchanged.

All ductwork for the chamber was fabricated with joints which facilitate taking the ductwork down for cleaning. Return air ducts over the top of the chamber are constructed of flexible tubing.

The air-filtering and -conditioning equipment has been installed and connected to the chamber. The installation will be complete before 1 July 1959. Test runs have already been made on some of the air-conditioning equipment.

Figures 36 and 37 show schematic plans of the chamber and its component parts.

3.16 PLANS FOR 1959

A large amount of data collected during the 1957 and 1958 pollen season remains in a state of only preliminary analysis and in some cases has not been subjected to analysis. Data collected during the same two preseasonal experiments

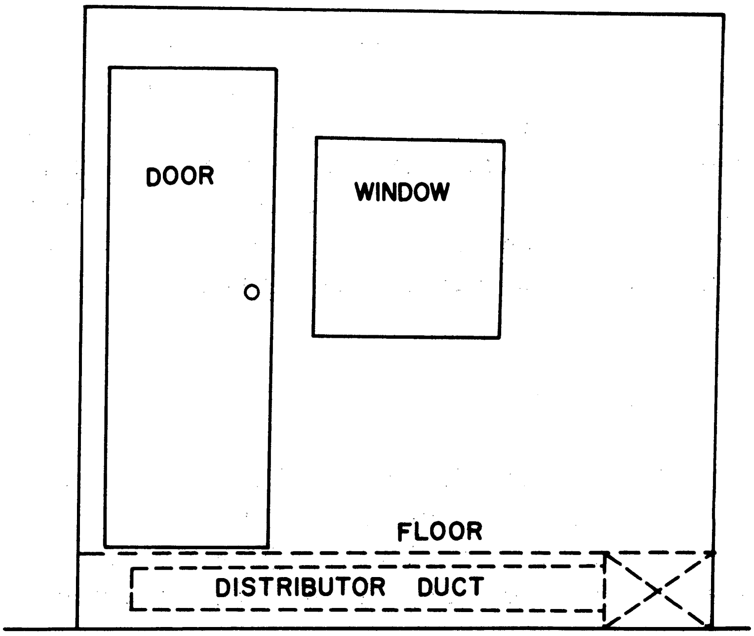


Fig. 36. Front view of the test chamber.

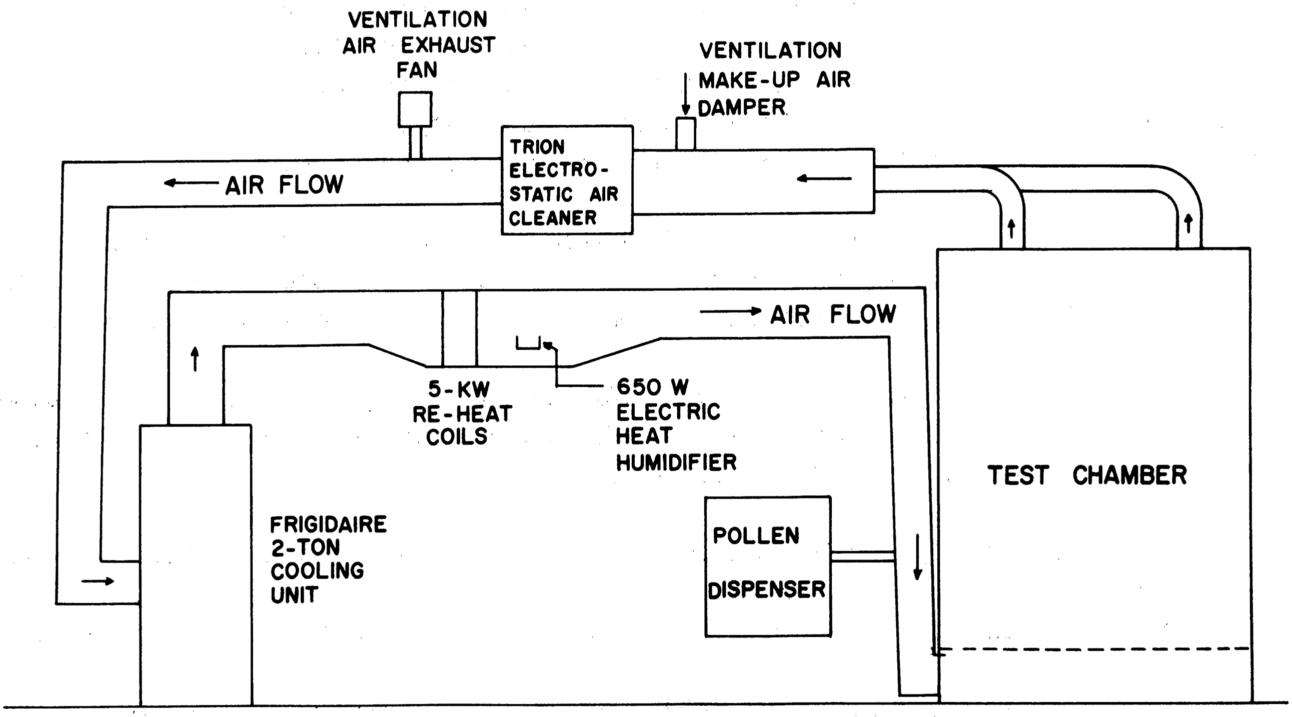


Fig. 37. Schematic diagram of the component parts of the test chamber.

are also believed to contain much information obtainable by further analysis.

Plans for 1959 will therefore allow for accumulation of a smaller amount of additional new data and in this way will permit a greater emphasis on data reduction and analysis. The experimental program for 1959 will be designed primarily to obtain data required to fill gaps in existing knowledge as indicated by results of analysis to date. Thus it is not planned to devote great efforts to repetition of experiments such as carried out in prior years except where a definite lack of data is indicated. An effort will be made to obtain experimental data for evaluation and verification of some of the theoretical work completed during 1958.

Preseasonal Experiment

The preseasonal experiment will be similar to that of 1958 but conducted on a smaller scale and with a more intensive program of measurement over a shorter period. A plot of ragweed forced to maturity in late June will again serve as a pollen source at a time when natural ragweed pollen is not present in the air. An array of masts supporting flag samplers at several levels will be exposed at several radial distances from the source across the mean wind. The number of samplers will be increased over that employed in 1958 but measurements will be conducted on fewer days. Strength of the source or total amount of pollen emitted per unit time will be measured by instrumentation to be developed. More detailed measurements of rate of pollen emission will be conducted to resolve undetermined effects of certain meteorological parameters upon pollen emission.

The goals of the preseasonal experiment will be:

(1) To determine the rate of emission of pollen as a function of meteorological parameters, and to verify the tentative results obtained during the 1958 experiment.
(2) To determine the source strength of pollen and to employ this value together with micrometeorological data in diffusion models.
(3) To make measurements of the diffusion rate downwind from the source for verification or modification of the models.

In-Season Experiments

City Survey.—Roto-bar pollen samplers will be located at selected sites throughout the city of Ann Arbor and operated throughout a period of about one week near the peak of the ragweed pollen season. Exposure will be chosen so as to eliminate very local effects except in certain cases where study of such effects is desired. The goal of this study will be to determine the effect of an urban area upon pollen concentration and to determine local variations of concentration within the city.

Effect of a Ragweed-Free Area Upon Pollen Concentration.—During 1958 a theoretical study* was conducted to determine the magnitude of reduction in pollen concentration that would result from eradication of ragweed from areas of certain dimensions. This experiment will be conducted in an attempt to verify the theoretical findings.

A ragweed-free area consisting of a lake or open field will be located. Pollen concentrations will be measured by means of balloon-borne samplers to an altitude of about 500 ft both upwind and downwind from the ragweed-free area. Thus any change in pollen concentration due to the clean area will be determined.

Forecasts of Airborne Ragweed Pollen Concentration.—Our knowledge of the ecology of the ragweed plant, its means of pollen production and dissemination, and of the atmospheric processes of pollen diffusion and transport has been vastly increased through the findings of the research effort of the past periods. While many areas in each of the phases of study remain in question, it may be possible that sufficient information has now been obtained to permit fruitful returns from an effort directed toward development of pollen concentration forecast methods on both a seasonal and short-range basis. Accordingly, plans are being formulated to conduct a preliminary study using pollen concentration measurements from the 1957 and 1958 seasons for correlations with synoptic weather observations. This study should serve as a guide to planning of the observational program during the 1959 pollen season so that satisfactory data will be available for a more comprehensive development of short-range forecast methods.

Seasonal forecasts of the severity of the pollen season will depend in large measure upon knowledge of the phenology of the ragweed plant. This phase of study will receive the combined attention of the botanical and meteorological groups during the 1959 season. The goal will be to determine a relationship between the magnitude of the pollen crop and the environmental conditions experienced by the plants prior to maturity and pollen production.

3.17 SUMMARY

Following extensive research on various types of samplers both in the wind tunnel and under natural conditions, the meteorological group has concluded that:

(1) The well-known gravity slide is reliable only in calm wind conditions.
(2) Impaction-type samplers give their best performance indoors or when the particles being sampled are smaller than 5 μ.
(3) The newly developed flag sampler used in conjunction with an anemometer record is an efficient sampler for use in a large-scale sampling program.
(4) The modified roto-bar sampler can be used to calculate concentrations directly since its efficiency is semi-independent of wind speed.

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*See Section 3.14 above.

Results of analysis from the preseasonal experiment of 1957 show only that the pollen distributions along the mean wind direction were different from those obtained from the 1956 data. Although no reason is apparent, the manipulation of the data must be examined before any valid conclusion can be drawn from this analysis.

Data have been abstracted from the preseasonal experiment and the in-season experiment of 1958 and are ready for analysis. Preliminary analyses have begun, but it is too early to state any definite findings.

The theoretical study of diffusion from a continuous area source shows that it does pay to clear an area of ragweed between the source and the point under consideration. This study gives an estimate of the size of the area that would have to be cleared to achieve a specified reduction in concentration. Testing of this theory will be started during the 1959 ragweed season, using some large natural ragweed-free area.

The test chamber has been completed and test runs of some of the equipment have been made. Plans are being readied for beginning actual tests using human ragweed-sensitive patients.

Plans for 1959 have been made to fill the gaps in existing knowledge. Major emphasis will be placed on the analysis of data already obtained rather than gathering new data. Progress in the field of forecasting ragweed pollen concentration has been made; more emphasis will be placed on that phase in the future.

4. STATISTICAL PHASE

by

F. M. Hemphill

4.1 INTRODUCTION

Statistical activities during the year have continued to consist primarily of assisting in the planning and evaluation of current studies in specific areas of the overall investigation. Supplementary funds for reinforcing this phase of our work became available in September, 1958; considerable progress has been made since then toward culminating plans for getting backlogs of statistical analyses reduced, and in reviewing, editing, and coding segments of the accumulated data in preparation for mechanized processing of the information. Mr. Eugene Bierly returned to the project as acting coordinator of this phase in December, 1958, and has been instrumental in making progress in preparation for an orderly procedure of managing information on a current basis. Our expectation is that a more permanent arrangement for the coordination of our statistical work will be an integral part of our operations by September, 1959.

References to studies in which statistical planning and evaluation were involved are included in papers and reports in the sections on allergy and meteorology. Brief descriptions of analytical work during the year follows in Sections 4.2 and 4.3. Plans for the coming year are given in Section 4.4.

4.2 MEDICAL DATA FROM THE 1956 IN-SEASON STUDIES

These data were thoroughly and exhaustively evaluated by Dr. James McLean of our Department of Allergy, assisted by Dr. Robert Lewis of the Department of Public Health Statistics. Their report is given in the section on allergy. Their studies led to the plan of using human subjects in the out-of-season experiment of 1958, likewise reported in the section on allergy.

Limited information was gained from some phases of the study of the symptoms and signs accumulated in the study of prisoner-patients. However, selected experiences from the 1956 and 1957 clinical study of patients plus the clear-cut findings in the 1958 out-of-season clinical observation of known allergic volunteers are quite promising for successful use of the chamber as an experimental device.

4.3 MEDICAL AND METEOROLOGICAL DATA FROM THE 1957 IN-SEASON STUDIES

These data were collected for the same purposes as were the similar data during 1956 and reports have been made on the broad findings of the medical findings and gross relationships of these with selected meteorological recordings. More detailed analyses are contemplated. The data for these analyses are now in their final stages of coding and preparation for IBM processing and tabulation.

4.4 PLANS FOR 1959

Findings from the experiences, both meteorological and medical, at Jackson prison, together with the encouraging clinical leads found during 1958, will be utilized for planning the studies in 1959. Our improved sampling techniques for estimating quantities of airborne pollen have suggested improved designs for studies of in-season relationships between meteorological measurements, medical data, and quantities of airborne ragweed pollen. Selected botanical data may be investigated simultaneously.

As readily as the data can be made amenable to machine processing, statistical investigation of two voluminous sets of information is contemplated: (1) studies of the relationship of "flag sample" pollen collections made by car from Ann Arbor to Jackson and return (1958), and (2) similar statistical study of data from the "flag samples" taken in Ann Arbor and vicinity (1958).

Additional statistical studies of the out-of-season data for 1957 and 1958 are contemplated when the information has been processed to a stage where IBM machines may be utilized to manage the information.

Much planning has gone into constructing and equipping the chamber for use in experimental work. Drs. James McLean and Richard Remington have worked with Mr. Bierly on preliminary plans for study of subjects in the chamber. Much remains to be accomplished in the design of the chamber experiments even after its instrumentation and pollen-feeding and pollen-sampling procedures have been calibrated and evaluated. Toward the end of assisting the researchers with developing experiments designed to fulfill specified objectives, the statisticians associated with the project have placed themselves "on call" to the principal investigators and the coordinator. These statisticians expect to devote much time during 1959 to the planning of chamber experiments and the analyses of data emanating from these studies.

A phase of botanical studies which aims to evaluate effects of selected variables on development and germination of pollen grains may receive additional statistical effort if there arises a desire to quantitate the relative relationships of several factors and to assess their interaction effects.

4.5 SUMMARY

Statistical efforts during the year have continued to be largely on an "immediate" basis for planning and gross summary. However, progress is being made toward processing the data which have accumulated and getting into an improved current status of analytical work. Increased opportunities for utilization of statistical acumen in planned and orderly investigation and analysis should become available in 1959. Such opportunities have been made possible by past experiences of the project and by increased efficiency of instrumentation and measurement, together with the devotion of adequate time to the statistical phase of the studies and the coordination of related information from the various phases of the project.

5. PUBLICATION OF RESULTS

The following papers have either been published, are in press, or have been submitted for publication.

1. Dingle, A. N., "Ragweed Pollen Concentrations in Relation to Meteorological Factors," *Proc. Air Pollution Control Assoc.*, Semi-Ann. Tech. Conf., 18-19 November, 1957, pp. 148-177.

2. Wagner, W. R., Jr., and T. F. Beals, "Perennial Ragweeds (*Ambrosia*) in Michigan with the Description of a New Intermediate Taxon." *Rhodora, 60*, 177-204 (1958).

3. Hewson, E. W., J. M. Sheldon, *et al.*, "Air Pollution by Ragweed Pollen," submitted for publication in *Geographical Review*.

4. Patterson, R., Correa, J. N., and Mathews, K. P., "Studies on the Neutralizing and Eliciting Activity of Altered Ragweed Antigen," *Annals of Allergy*, in press.

5. Dingle, A. N., Gill, G. C., Wagner, W. H., Jr., and Hewson, E. W., "The Emission, Dispersion, and Deposition of Ragweed Pollen." *Advances in Geophysics*, *6*, 367-387 (1959).

6. Wagner, W. H., Jr., "The Hybrid Ragweed, *Ambrosia Artemisiifolia* X *Trifida*," *Rhodora*, *60*, 309-315 (1958).

7. Bianchi, D. E., Schwemmin, D. J., and Wagner, W. H., "Pollen Release in Common Ragweed (*Ambrosia Artemisiifolia*)," submitted for publication in *Botanical Gazette*.

8. Wagner, W. H., Jr. (ed.) "Bibliography of Ragweed (Ambrosia)," *Quarterly Review of Allergy and Applied Immunology*, accepted for publication.

9. Harrington, J. B., Gill, G. C., and Warr, B. R. "High Efficiency Pollen Samplers for Use in Clinical Allergy," *Journal of Allergy*, accepted for publication.

10. Patterson, R., and Correa, J. N. "The Demonstration of a Quantitative Relationship Between Skin-Sensitizing Antibody and Antigen," *Intern. Archive of Allergy and Applied Immunology*, accepted for publication.

11. Patterson, R., "Ragweed Allergy in the Dog," submitted for publication in *Journal of the American Veterinary Medical Association*.

12. Dingle, A. N., "Comments on the Diurnal Variation of Airborne Ragweed Pollen as Determined by Continuous Recording, Particle Samples, and Implication of the Study by R. D. Smith and R. Rooks," *Journal of Allergy*, 1958.

6. TRIPS AND TALKS BY MEMBERS OF THE TEAM

TRIPS

August, 1958 - Mr. Gerald C. Gill to Nashville, Tennessee, at the request of Dr. Jeanne Morton, USPHS, to aid Dr. Sidney Sussman of Vanderbuilt University Hospital.

August, 1958 - Professor E. Wendell Hewson and Dr. John M. Sheldon to Oxford, England, to attend the International Symposium on Atmospheric Diffusion and Air Pollution.

November, 1958 - Professor E. Wendell Hewson and Mr. Paul Giever to Washington, D. C., to attend the National Conference on Air Pollution.

December, 1958 - Professor W. H. Wagner, Jr., and Dr. James McLean to Detroit to attend the Midwest Forum on Allergy.

January, 1959 - Professor W. H. Wagner, Jr., to New York City to attend the Northeastern Weed Control Conference.

January, 1959 - Mr. Eugene W. Bierly to New York City to attend the 39th annual meeting of the American Meteorological Society.

TALKS

February, 1958 - "Air Pollution and Allergy," Professor E. Wendell Hewson, 2nd Air Pollution Research Planning Seminar, Cincinnati, Ohio.

March, 1958 - "The Ragweeds and their Pollens," Professor W. H. Wagner, Jr., Interdepartmental Seminar on Applied Meteorology: Engineering, The University of Michigan.

March, 1958 - "Pollen Dispersion and Deposition," Professor A. N. Dingle, Interdepartmental Seminar on Applied Meteorology: Engineering, The University of Michigan.

April, 1958 - "Atmospheric Pollution by Aeroallegens; Medical Aspects," Dr. J. A. McLean, Interdepartmental Seminar on Applied Meteorology: Engineering, The University of Michigan.

May, 1958 - Panel on Aeroallergens, I. Prof. F. H. Hemphill—Public Health Statistics, Prof. A. N. Dingle—Meteorology, Prof. W. H. Wagner, Jr.,—Botany Class for Doctor of Public Health candidates, The University of Michigan.

July, 1958 - "Atmospheric Pollution by Ragweed Pollen," Prof. E. W. Hewson, Allergy Assoc. of Northern Calif., Berkeley, California.

August, 1958 - "The Emission, Dispersion and Deposition of Ragweed Pollen, Prof. E. W. Hewson, International Symposium on Atmospheric Diffusion and Air Pollution, Oxford, England.

November, 1958 - Effects of Topographic and Meteorological Conditions on the Distribution of Air Pollutants," Prof. E. W. Hewson, National Conference on Air Pollution, Washington, D. C.

December, 1958 - "Ragweed Pollen: Production, Emanation, Dissipation, and Destruction," Prof. W. H. Wagner, Jr., Midwest Forum on Allergy, Detroit, Michigan.

January, 1959 - "Botanical Research on Atmospheric Pollution," Prof. W. H. Wagner, Jr., Northeastern Weed Control Conference, Public Health Section, New York.

February, 1959 - "Botanical Aspects of the Hayfever Problem," Prof. W. H. Wagner, Jr., Undergraduate Botany Club, The University of Michigan.

March, 1959 - Panel on Aeroallegens, II. Prof. F. H. Hemphill—Public Health Statistics, Dr. J. M. Sheldon—Allergy, Prof. W. W. Hewson—Meteorology, Class for Doctor of Public Health candidates, The University of Michigan.

March, 1959—"Hayfever and the Botany of Ragweed," D. E. Bianchi and T. F. Beals, Elementary Botany Seminar, The University of Michigan.

7. ACKNOWLEDGMENTS

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ADDENDUM

Since the preparation of this report was begun, the technique mentioned in Section 2.9 has been described in print: Mathews, K. P., "Adaptation of the Antiglobulin Test for Use with Tanned and Treated Erythrocytes," J. Immunol., 82, 279 (1959).

APPENDIX

Abbreviated* Minutes of the Regular Meetings of The University of Michigan Research Team on Atmospheric Pollution by Aeroallergens

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*Reports presented at the meetings that appear in the main body of this report have been deleted from the minutes.

MEETING OF 24 APRIL 1958

The following were present: Baynton, Dingle, Elder, Gill, Harrington, Hardy, Hemphill, Hewson, Jones, Patterson, Sheldon, Wagner, Warr, Yang. Guests: Dr. E. C. Ogden, New York State Museum and Science Service; Mr. G. Raynor, Brookhaven National Laboratory; and Mr. R. Brown, Brookhaven National Laboratory.

METEOROLOGICAL PHASE

Mr. Gil Raynor discussed experiments with pollen sampling at Brookhaven National Laboratory. The meteorology group at this facility was first established to study the distribution of radioactive effluents. When these studies were completed, the techniques used were then applied to the study of pollen dispersion. A cooperative project between the botanists and meteorologists was then begun using the 400-ft tower available.

In the first year slides were placed at each level of the tower. Two gravity slides at each level were used, one placed crosswise and the other lengthwise. In addition, hourly samples and some 24-hr samples were collected at 3 levels using millipore filters. Studies were made correlating the count with height. Meteorological data such as mean hourly wind, gustiness, mean hourly lapse rate, temperature, incoming solar radiation, humidity, and the time, amount, and type of precipitation were also studied.

The results of the first year showed how poor the present sampling methods were. Gravity-slide counts showed a great increase in pollen with height, but the millipore-filter counts showed a decrease in pollen counts with height. The north-south oriented slides showed more pollen than the east-west slides. The only conclusions drawn were that the catch on the slides is probably a function of the amount of pollen in the air and also of the amount of air that flows over the slides. Also, the gravity-slide holder apparently affects the pollen count by some aerodynamic mechanism.

In 1956 wind-tunnel experiments were undertaken with two tunnels utilizing two different air speeds. The studies showed that a higher count appeared on the slides exposed to a higher wind speed and that the slides were so close to the end of the tunnel that they were influenced by outside air speed. Larger tunnels were used in 1957 so that the influence of the outside air speed was minimized. Tunnel wind speeds used were 4.2 and 8 mph. Results showed that the percentage increase in pollen catch was roughly half the percentage increase in the wind speed. Two and a half times as much pollen was found on slides oriented lengthwise to air flow as was found on slides oriented crosswise. The studies indicated that slides that are oriented at random in regard to wind direction and with no correction for wind speed are inaccurate. A round slide avoids the error of the directional aspect, but not the wind-speed error. Studies to find a better method of gravity counting are underway.

Millipore filter counts of pollen collected in the tunnel showed that the pollen counts collected at a wind speed of 4 mph were four times greater than those collected at a wind speed of 8 mph. The explanation for this is thought to be that the pollen grains do not always follow the air as it is pulled into the filters and that this phenomenon becomes more exaggerated at higher wind speeds. This is also thought to be the explanation for decreasing millipore filter counts at greater heights on the tower.

Samplings have been made before and after temperature inversion and before and after showers, but results are too few to warrant conclusions as yet.

An additional study was done in which a technician counted the pollen on the front and back sections of slides which had been exposed longitudinally in the wind tunnel. Counts were 70% greater at the back end of the slide. A possible explanation for this is that air hits the front end, rises, and does not drop down again until it reaches the back end of the slide, thereby depositing more pollen at the back end of the slide.

Future studies of this problem will include studies in wind tunnels to determine air flow around samplers. A sampler which will be effective and simple, yet avoid the inaccuracies resulting from variable wind speed and variable orientation of the slide to wind directions, will also be considered. An upright cylinder with the slide attached to the front, oriented into the wind by a vane, with a wind-speed recorder in the other end of the device, is presently being considered. Work is in progress to develop an isokinetic sampler which could be used as a standard. This standard sampler would then be used to calibrate other methods of sampling.

A separate study includes the preparation of particles in the size range 20 to 30 microns. Investigation is then to be conducted in regard to measurement, dispersion, collection, and deposition of these particles.

Dr. Ogden discussed plans to study the travel of ragweed pollen. Of course, the pollen must come from a known source. Tagging of pollen with radioactive phosphorus is planned. In addition, other methods of tagging, such as with dyes, are planned. Initial work will consist mainly of the development of techniques to measure accurately pollen traveling from a known source. It is of interest to determine the distances that pollen grains may travel because it has been suggested that pollen which has been in the air a long time or traveled a great distance may not be allergenic.

GENERAL DISCUSSION

The discussion which followed these presentations concerned the effect of air turbulence above a gravity slide and the effect of aerodynamics on the slide attached to the cylinder. It was thought that the pollen might not follow the course of the air around the cylinder and not impact on the slide at all. It was emphasized that we need both an effective, easy method of pollen counting suitable for clinical usage and an exact method of pollen counting which can be used for experimental study and for calibration and correction of the simpler, more inaccurate methods.

A discussion of the radioactive phosphorus labeling of ragweed pollen dealt with the ease of obtaining the P^{32}, the danger of the radioactivity, and the background counting. Dr. Ogden believed that the danger from radioactivity is negligible because of the small total amount used and that most of the radioactive pollen will fall a short distance from its source. It has been found that carrying radioactive materials on clothing is a real problem. Another major problem which may seriously interfere with this study is that of background counts of a significant magnitude. Such background counts could stop the study.

Further discussions dealt with the question of antigenicity of pollen at high levels, the effect of turbulence upon roadside pollen, and why such marked variations in pollen counting may be found on slides set out in nearby areas. Dr. Ogden commented that further ecological studies are planned by their group in which various studies on individual ragweed plants will be made. It has been suggested that perhaps it is not advisable to pull up ragweed plants since this plant is a product of a disturbed environment. Cutting or spraying the plants might be more advisable.

The next discussion concerned observations on ragweed plants in Louisiana and Oregon and the plans in New York State to control ragweed.

Mr. Brown of Brookhaven National Laboratory described the sampler used at Brookhaven to sample oil fog. This device utilized two model airplane engines to supply a negative pressure to sample oil fog through a millipore filter. A movie demonstrated the sampler in action. A newer method used Freon cans to supply the vacuum.

The next discussion concerned the importance of stressing the problem of air pollution by aeroallergens and other biological entities which constitute air pollution in addition to ragweed, such as mold spores, grass pollen, tree pollen, rusts, smuts, and bacteria.

The problem of allergy in the population in general was mentioned. More than 4% of the population state that they have had some allergic manifestation at some time. Further studies along this line in population samples seem of interest.

The tentative date of the next meeting is Thursday, May 15.

MEETING OF 15 MAY 1958

The following were present: Akerman, Giever, Gill, Hardy, Harrington, Hemphill, Hewson, McLean, Patterson, Ruffner, Sheldon, Wagner, and Warr.

The first discussion centered around the second annual report which has now been completed and was passed out to the members who were there. Drs. Sheldon and Hewson congratulated the group on the report.

METEOROLOGICAL PHASE

Mr. Harrington then discussed wind-tunnel experiments in which several methods of pollen sampling were tested. He gave the group the numerical results and a diagram explaining the different types of procedures used. The relative efficiencies of gravity slides, millipore filters, impingers, vertically mounted slides, and two cylinders (diameters 1 mm and 18 mm, respectively) were tested.

Dr. Hewson wondered if the turbulence of the natural atmosphere could be compared with that in the tunnel. It was noted that atmospheric turbulence could not be adequately duplicated in the wind tunnel. Several baffles were used in the wind tunnel to induce turbulence, but the turbulence patterns were quite different from those occurring naturally.

Dr. Hewson asked if the equipment had been centered in the tunnel. It was reported that the samplers were 18 in. above floor level or approximately one quarter of the distance from floor to ceiling. Mr. Warr mentioned that the air flow was very uniform in the test section of the tunnel.

The next discussion concerned the counts taken with the millipore filters. A six-directional millipore-filter unit was used to test the effect of orientation on the collection efficiency of the filters.

Various materials were tested for adhesiveness as far as ragweed pollen is concerned. Glycerine jelly was assumed to be 100% efficient, vaseline was 90% efficient relative to the glycerine, and scotch tape only about 13% efficient. The Stanford group found that rubber cement was nearly as efficient as glycerine and was also rain-proof. It was found that the rubber cement was also efficient at high wind speeds, while the efficiency of scotch tape decreased with wind speed. The question was raised if silicone grease would be better than scotch tape and would not dry out in the sun as much as rubber cement. It was said that silicone grease would be tested.

It was thought that the 1-mm-wire-flag sampler was the best but it was not known if this would hold up in outdoor studies. The main objection to most of the pollen counting methods outlined concerned the fact that the volume of the concentration of most pollen samples was still unknown. Dr. Hewson again stated that these studies were wind-tunnel experiments and might be adversely affected by atmospheric conditions, and in addition, that these studies did not invalidate the counts made in the prison cell block at Jackson Prison. He did agree that these results affected the tower pollen counting systems used previously and that these would therefore be modified. It was thought that the millipore filter was good for indoor sampling and that, when it was facing down, it was still adequate. It was pointed out that 6 and 16 mm of mercury vacuum in the millipore filters produced the same flow rate and therefore not as much vacuum was needed as had been used before. It was also mentioned that Sakagami in Japan had devised a simple anemovane using a small glass tube as a bearing. By making a flag of scotch tape on a pin and setting the pin in the glass tube, a simple sampler has been made. Pollen is collected by impaction on the pin. The flag rotates the pin in its holder, keeping the same edge facing into the wind. If the wind speed is known, the volume of pollen can be calculated. This is a highly efficient apparatus in the range of 20μ so it will fit in well with the ragweed study.

In connection with the preseasonal experiments, the meteorological phases for the coming studies were discussed. It was felt that Sutton's equation may not be valid for particulates which are deposited on the ground. When deposition is sizeable and gravitational settling negligible, then, it was postulated, the axis of a plume of particulates will rise downwind from the source. It is planned to test this hypothesis in the coming preseasonal experiment. The work will be done at Willow Run rather than at Jackson because excellent lapse rate and wind data, being used in another research project, are available there.

BOTANICAL PHASE

As far as the botanical phase of the preseasonal experiment was concerned, it was thought that impingers and the scotch-tape-flag samplers should also be placed in the ragweed plot. The botanists will aid in detailed studies this year. All the foregoing discussions of the botanical phase concerned pollen emission and it was pointed out that there should also be some measurements of conditions of pollen production, such as soil moisture and temperature. Planting of female plants at a distance from the plot should also be studied.

Professor Wagner believed that the ragweed plants would be producing by mid-June. Professor Hemphill asked whether placing of the masts would be the same as in previous years. It was thought that the scotch-tape flags would be out for 20 to 30 min and good counts could be obtained in that length of time.

ROADSIDE EXPERIMENT

Professor Hewson then discussed the proposed in-season roadside experiment. He wondered about making measurements and pollen counts along roadsides and comparing them with the pollen counts taken on the hospital roof. It was wondered if any correlation or findings contrasting these two types of counts could be obtained and if it would be feasible to make pollen counts near cars passing on the highway or actually inside cars. This idea was then discussed by various members of the unit and it will be discussed again at a later meeting.

TEST CHAMBER

Professor Akerman spoke on the status of the test chamber. He had a verbal quotation from the plant department concerning the cost of setting up the chamber in the Kresge building. He reported that it had been completely redesigned by the plant department and that their open estimate was $4375. This was considerably over the expected amount and since this was an open estimate, it could be almost certainly concluded that the final cost will be even higher. Professor Akerman had no further information on the subject and did not wish to comment on it further until he has the written statement from the plant department as well as their specifications. Then he will be able to learn what has caused the increase in estimate. The possibility of receiving outside bids on the test chamber was entertained by the group.

RAGWEED BIBLIOGRAPHY

The ragweed bibliography status was then discussed by Professor Wagner. A sample page of the bibliography has already been approved by the editor of the Quarterly Journal of Allergy and Applied Immunology. Professor Wagner said that the job of editing and changing abbreviations to the forms used by Chemical Abstracts was half completed. He also commented that he has already collected ragweed from Louisiana and would like to have some collected from the area of Nova Scotia. He thinks that he can then trace the origin and migration of the common ragweed by contrasting the plants from these two areas.

CLOSING COMMENTS

Dr. Sheldon commented that he and Dr. Hewson had seen Mr. Ernst, the budget director of the Public Health Service, and Dr. Harry Hanson. Mr. Ernst and Dr. Hanson had discussed this project with Drs. Sheldon and Hewson while in Ann Arbor and were pleased with the results obtained to date.

It was decided that the next meeting would be on Tuesday evening, June 17, 1958, at Dr. Sheldon's home.

MEETING OF 17 JUNE 1958

The following were present: Akerman, Baynton, Cook, Dingle, Giever, Gill, Hardy, Harrington, Hemphill, D. L. Jones, Lewis, McLean, Mathews, Remington, Sheldon, Wagner, Warr, and Yang.

TEST CHAMBER

The first order of business was the latest report on the test chamber given by Professor Akerman. The table below summarizes the estimates.

	Old Estimate			New Estimate		
	Material	Labor	Overhead	Material	Labor	Other
Install air conditioner		$ 75				
Unistrut test cell	$ 952	387	$ 134	$ 755	$ 567	
Sheet metal duct work	81	263	34	160	964	
Water supply and plumbing	122	181	31	143	265	
Electrical work	107	223	32	157	315	
Pollen disperser piping				62	88	
Engineering supervision						$ 275
Contingencies						248
Overhead						375
Totals	$1262	$1129	$ 231	$1277	$2199	$ 898
GRAND TOTAL			$2622			$4374

The new estimate for labor consists of paying $24 per day per man. Professor Akerman said that this is on the basis of 20 man-days. He wondered if the cost of labor was a little high; however, the men must work in cramped quarters in the Allergy Laboratory, which may slow down their work. The new estimate includes several items not covered by the original one—the cost of transportation of equipment from the Meteorology Laboratory to the Kresge Building, pollen dispersing equipment, and oxygen piping. It was established that the oxygen piping was not needed.

The overhead includes such payment as workmen's compensation, retirement fees, etc. Although the workers are being paid by the University, this has to be added to their wages over and above their regular salary. This extra money must be paid by the project having the work done.

Mr. Gill thought that it would probably be unwise to get another estimate and although it was agreed that some of the wages were probably high, it was thought that it would be a very complicated matter for an outside firm to estimate the costs. Such estimates would probably come to a higher figure. Dr. Dingle stated that originally the chamber was to have been movable. He asked if it would now be wise to obtain a new estimate on the assumption that it would no longer be movable and therefore less expensive to build. It was thought that it would be best to go ahead with the present plans since they had been submitted on February 20 and May 7 was the first time we had been notified of any estimate. Therefore, if the work was to get underway, it should probably be started now.

Dr. Sheldon said that in the original Public Health grant $5000-$6000 was set aside for the chamber including the air-conditioning unit. Professor Akerman thought that the University estimate was probably fairly accurate within a range of plus or minus $500. It was then discussed whether the Plant Department's plans could be approved now since the Public Health grant will not be renewed before August 31. Sufficient money to accomplish the construction of the chamber will not be available until after that time. It was agreed to inform the Plant Department of our intention to approve their plans but that the order could not be placed before August 31. It was also agreed that the location of the test chamber in the Kresge Building was still the most satisfactory one.

BOTANICAL PHASE

The next item on the agenda was the botanical report by Professor Wagner. He asked those who had been urged to give comments on the introduction of the ragweed bibliography to return those comments to him so that this project could be completed. The botanists are now studying the ragweed species with the knowledge that a new plant, a hybrid between the giant and the short ragweed, has been recognized. The report on the perennial ragweed is now being published. Professor Wagner said that he had learned from correspondents that the northern ragweed in New England and Canada is now becoming extinct and is being overrun by the common ragweed.

A natural plot had been set up at the Botanical Garden and some ragweed began to grow around April 20. This was ragweed which was left over from the previous year. The new plants continued to appear until May 20, 1958. The botanists hope to study ten plants very carefully and also to study weather effects on the plants. Professor Wagner commented that during the rainy week here some of the ragweed plants doubled in size.

The botanists are now attempting to go back and examine some of the original sections from the early specimens, make these transparent with sodium hydroxide, and then to study them in three dimensions. The problem of quantifying pollen production is still being investigated and it is wondered if this can be measured by counting the number of tiny flowers on the ragweed plant discharging pollen rather than by counting the total pollen production. Mr. Beals is setting up an experiment to investigate pollen viability and will use various tissue culture nutrients to study this problem further. He will try to make the pollen germinate on various media and stimulate them with plant hormones and varying physical conditions. One part of the project was to see if the pollen could be made to germinate on mucous membrane.

THE PRESEASONAL EXPERIMENT

Professor Wagner commented that the short day seems quite unnecessary for the germination and pollination of the ragweed plant. The short day is necessary only to start growing the plants during March so they will flower early. Some female plants have been grown in seclusion. They will be brought separately to Willow Run. The ragweed plants have been pollinating for the last three days even though the cold weather held them back somewhat.

Professor Dingle then reported on the preseasonal experiment which was to start the following day. A copy of the plan of the experiment was passed out to each member of the group.

The 1958 preseasonal experiment is designed to answer the following questions:

1. How are emission and transmission of ragweed pollen related?
2. What meteorological variables control emission and what is the lag between change in a variable and emission of pollen?
3. How are pollen concentration and deposition related?
4. What is the rate of attenuation of a pollen plume?
5. How much pollen actually gets into the atmosphere?
6. Does the axis of the pollen plume rise downstream from the source?

CLOSING COMMENTS

Dr. Sheldon said that Dr. Jean Morton would be in town the next day. She is a United States Public Health Service representative interested in aeroallergen pollution of the atmosphere and was coming to talk with the various members of this project. Her itinerary was set up so that she could see the various phases of this project including the preseasonal experiment and talk with various individuals.

MEETING OF 15 JULY 1958

The following were present: Akerman, Barlow, Baynton, Beals, Bianchi, Dingle, Field, Gill, Harrington, Hemphill, Kelley, Lewis, McLean, Nilsson, Remington, Schwemmin, Sheldon, Wagner, Warr, and Willard.

GENERAL DISCUSSION

Dr. Sheldon opened the meeting by announcing that Dr. Karel of the National Institute of Allergy and Infectious Diseases had informed him that in all probability the grant will be renewed and that there probably will also be a supplemental grant. The supplemental grant is contingent upon the President's approval, which appears likely. Therefore, it was felt that the Plant Department could be told to proceed with the construction of the chamber

The next discussion concerned the minutes of the previous meetings. There had been four meetings since the second annual report so it was decided that the minutes should be checked and any corrections would be forwarded to the recorder. Hereafter, the notes will be corrected either before or at the next meeting. Thus the notes will be kept current in anticipation of their use in future annual reports. All members of the group were introduced. A guest, Mr. Robert Patton of the New York Department of Mental Hygiene, was introduced by Prof. Hemphill.

MEDICAL PHASE

The second topic on the agenda consisted of a report on the ionization plans for animal experiments by Dr. Sheldon. He briefly reviewed the concept of ionization and mentioned that scattered reports on the effect of ionization had occurred in the literature for some time. The Wessix Corporation had financed some projects because their founder had been particularly interested in this subject. It has been shown that negative ionization has some unknown therapeutic effect on some patients and positive ionization has some unknown deleterious effect on others. The Whirlpool-Seeger Corporation, a subsidiary of RCA, became interested in the effect of ionization and desired concrete information on it in relation to installation in air-conditioning apparatus. Therefore, Drs. Sheldon, Mello, and McLean made tentative studies last year with 14 ionizer machines in patients' rooms. Seven were set on a positive ionization and seven on negative ionization at the same time. The fourteen patients were checked at weekly intervals during the ragweed season last year. Their ionizer machine was changed each week, i.e., changed to the opposite polarity. The results were inconclusive as to any beneficial effect derived from either ionization. Mr. Schulte carried out some studies specifically on the amount of ionization produced by each machine and found that four did not produce any ionization. He also found that the nose must be within eight inches of the source to denote any ionization effect. The effective ionization range for these ionizer machines is eight inches. The Whirlpool organization has now agreed to finance some basic research on ionization and anaphylaxis.

Professor Akerman is now designing a chamber to hold both sensitized and unsensitized guinea pigs which will be exposed to the ionizer machines and then challenged with the appropriate allergen. If these experiments show any promise, further work will be carried out. Dr. Field will be working on this project. The question of the use of litter mates was raised and whether the ionization affects the allergen or the host. Whether it was a positive or a negative effect on the pollen, on pollen fallout, on pollen absorption, or on the effect of the pollen on the tissues, were other questions raised. The dangers of ionization were also briefly discussed.

PRESEASONAL EXPERIMENT: Botanical Phase

The third main topic on the agenda consisted of the preseasonal experiment report just completed at Willow Run. The first phase, the botanical phase, was discussed by Prof. Wagner. It is now fairly certain, he said, that the photoperiodic method of caring for the ragweed plant is not necessary to make them mature early.

The botanists watched the 3000 plants at Willow Run for a 5-day period. The opening of the flower can be divided into two parts—(1) the swelling of the apex of the flower, then the actual opening of the lobes of the flower and (2) the dehiscence of the anthers or the pollen sacks. Mr. Bianchi and Mr. Schwemmin of the Botany Department are specifically studying this and are also trying to determine the number of flowers opening per unit of time. Professor Dingle said that determination of the actual amount of pollen might help to establish the difference between severe and mild ragweed seasons as far as the patient's symptoms are concerned. If the weather history of the plant could be obtained to see if there is any difference between one pollen plot and another and judge the severity of the ragweed season, it might be possible to determine if the quality of pollen production is actually changed. Professor Wagner said that this would actually require a 5-year study using the same soil, the same seeds, and the same controls over that length of time. A shorter study might not produce significant results. It is thought that there is actually a difference between the out-of-season and the in-season plants. The plants at Willow Run this year were "lush." The drought in the thirties was shown to have caused the pollen peak of ragweed to occur around September 3. Usually it occurs at the end of August. It was wondered if the antigenicity of the pollen varied from year to year with the meteorological changes. It was thought that the pollen may be more antigenic following dry spells as compared to wet spells. The nature of the antigenic substance might depend on whether it was an excretory product or an integral part of the plant cell constituent. Weather conditions might not affect this antigenicity if it were excretory in nature. During the routine pollen collection, pollen must be dehydrated immediately or it will lose some of its antigenicity. Thus the problem of measurement of antigenicity depends on the passive transfer method and the exhaustion of skin test, i.e., an indirect testing method.

Professor Wagner said that the plant physiologists wanted to study the tapetal fluid again. Mr. Harrington said that a change in the position of the patient may be significant as far as symptoms are concerned since a study of poisonous gas escaping into a barracks during the war revealed that subjects who were up had trouble while those who were lying down had no symptoms.

Meteorological Phase

Mr. Harrington then discussed the meteorological phase of the preseasonal experiment. He had analyzed the data of five days and in general the experiment has been carried out as outlined.

Dr. Lewis then asked what volume of air was actually measured by the roto-bar samplers or if it were an analogy. Mr. Harrington then drew a tentative curve showing the efficiency of the various types of pollen collector filters used.

Mr. Harrington briefly described the experiment at Willow Run. Studies were made for 18 days. The various counts done to date show that the maximum concentration of the pollen plume occurred at about 2 ft above the ground at 40 ft from the source, 6 ft above the ground at 60 ft from the source, and had risen to a height of 10 ft at further distances. Significant pollen counts were obtained at 320 ft from the source. The botanists helped considerably in this study in aiding in forecasting the time of emission of the pollen. No success was obtained with the female ragweed plants which were brought out to be planted 600 yards away to see if fertilization occurred.

Medical Phase

The medical phase of the preseasonal experiment was described by Dr. McLean. He said that credit should go to Dr. Lewis who conceived the idea while working on some data relative to the Jackson Prison experiments of previous years. Because of the late start, only two ragweed-sensitive patients were exposed in the ragweed plot for a total of 9 days.

As far as the specific medical objectives are concerned, this phase of the experiment was to determine if the patient would develop symptoms. This can now be answered in the affirmative. Also it was wondered how much pollen concentration was necessary to help design the chamber experiments. It appears that both patients began to have symptoms in a range of 700 to 800 pollen counts. This is only a pollen count and has not been corrected yet to the volumetric figure. The third medical objective was to see how long a patient would require exposure to pollen before developing symptoms. One patient developed symptoms within 10 minutes of exposure and the other patient required longer. The two patients had two different types of exposure curves. In addition, there was a question of an accumulative effect being shown.

Dr. Lewis, in discussing this medical phase, said that this covered actually only one type of experiment, that is, the patient was exposed daily for so long and then was not exposed again that day. It was wondered if a different type of pattern would be seen in patients who were exposed every third day instead of every day. He also questioned the validity of the assumption that the pollen count was the direct cause of the amount of response, i.e., he wondered if there were a direct relationship between the pollen count and the patient's symptoms and if the pollen counts were only an indirect rather than a direct tool in this study.

Professor Hemphill wondered if more in-season experiments similar to this out-of-season experiment could be carried out. The difficulties involved at Jackson Prison and the difficulty with other aeroallergens during that season were mentioned which would make such experiments invalid. It was wondered if old pollen would act as the new fresh pollen. This possibility had already been entertained by those involved in this work. It was hoped that both kinds of pollen could be included in the current wind-tunnel experiments to see if patients developed symptoms with both types. This would help with the chamber experiment since the old type of pollen could be used because of its availability and relatively low cost.

Mr. Baynton briefly summarized his experiences at the pollen plot. While collecting meteorological data, he had inhaled a heavy concentration of ragweed pollen and had noticed tightness of the chest on two occasions and some nasal symptoms. Dr. Barlow then answered some questions asked by the group concerning his personal experience with this experiment.

CLOSING COMMENTS

The next meeting was scheduled for August 13 in the Botany Seminar room of the Natural Science Building because the botany physiologists want to demonstrate some of the experiments they are now carrying out.

MEETING OF 13 AUGUST 1958

The meeting was held in the Botany Seminar Room of the Natural Science Building. The following were present: Barlow, Beals, Baynton, Bianchi, Dingle, Elder, Field, Gill, Harrington, Hemphill, Hewson, K. L. Jones, Kelley, McLean, Mathews, Nilsson, Plymer, Schwemmin, Sheldon, Wagner, and Willard.

OPENING REMARKS

Dr. Hewson opened the meeting by announcing that the supplemental grant for the project had been approved.

MEDICAL PHASE

The report of the 1956 Jackson Prison medical experiment was presented by Dr. McLean. A mimeographed summary of the report was passed out to the group.

The broad purpose of the experiment was to obtain information about the general nature of interrelationships between

(1) pollen concentration and patient reactivity;
(2) meteorological variation (including "time of day") and pollen concentration.

The forthcoming chamber experiments should be designed to answer specifically the following problems, if possible.

a. What is a patient's "reactivity" time for a given pollen threshold?
b. Is there an accumulative effect which modifies this reactivity time?
c. Are there different reactivity times for HF and asthma in a given patient?
d. Is the response and pollen exposure dosage predictable, e.g., straight-line relationship, arithmetic progression, etc.?
e. Can lab and pulmonary function tests detect quantitative changes in the pollen exposures?
f. Will medications (A.H. and broncho dilators) significantly modify these lab and pulmonary function test results and patient's objective and subjective symptomatology?

After carrying out the above, the Jackson Prison experiments should be reviewed to see if any or all of the above findings are corroborated by the collection of information during a "natural" pollen season.

In summary Dr. McLean stated that the exact relationship between dosage and physiologic response and cumulative effect was unknown and that, due to the many other variables, it was difficult to interpret more fully the medical work done at Jackson Prison. It was hoped that, if some of the problems raised by the review could be more satisfactorily answered by the coming chamber experiment, a more comprehensive review of the Jackson Prison medical phases would prove more profitable.

Dr. Hemphill opened the discussion with full agreement on the report, but said that all the final recommendations might not be able to be carried out. Dr. Dingle agreed that it would be important to review the medical work after the chamber experiments. Dr. Mathews mentioned that the work in which intravenous histamine was given to asthmatic patients to produce a drop in their vital capacity showed that the response depended on the time interval since the last attack. Those who had had trouble within three days experienced almost 100% asthmatic response, while no symptoms were produced if the patient had not had an attack within the last three months. Dr. Hemphill thought that many patients would be needed for the chamber experiment or that at least the same patient would have to act as his own control. Dr. Mathews' previous statement had implied that one patient might not be able to be used more than one time a month for the chamber experiment.

GENERAL DISCUSSION

Dr. Hewson reported that he and Prof. Akerman were to meet the following day to finish the paper work on the chamber construction instructions. Mr. Harrington briefly reported that the subject in the wind tunnel using old pollen had experienced some hay fever symptoms. This type of pollen was being considered for the chamber experiments.

BOTANICAL PHASE

A preliminary report on the botanical studies of 1958 was given by Prof. Wagner. He first stated that the article on the perennial ragweed had been published in the botanical journal, Rhodora. He next read portions of two letters. The first was from Dr. E. C. Ogden of New York University who had also found that photoperiodic treatment was not necessary for ragweed flowering. They are now using dye studies and radioactive phosphorus in their study of the ragweed plant. Dr. Ogden wondered if ragweed pollen was changed by being air-borne. The second letter was from the publishers of the Review of Allergy and Applied Immunology concerning the bibliography on ragweed which is to be published in two successive issues starting in March, 1959. The question was raised if further additions should be made to the bibliography before its publication.

Professor Wagner then passed out a sheet containing an outline of the studies on different phases of the floral function and environment in ragweed.

In the discussion period that followed, the question was raised whether there was an effect of combination of humidity and temperature. This was recognized as another variable. It was wondered if the temperature might be influencing the water balance, if this might therefore be a secondary effect, and if these experiments might have to be carried out on whole plants rather than just the spikes or clusters of flowers. It was also wondered whether under certain conditions there might be a secondary pollen release late in the afternoon as noted in past experiments by this group at the Jackson Prison.

Dr. Jones then made some comments on his experience when he had 12,000 ragweed plants over a period of 5 years at the Botanical Garden. He found that the only time he could study the plants was before the dew lifted. When the flowers became dry, the pollen came out and he had symptoms. He suggested that the temperature effects shown by the present experiments might be confined to florets which had been separated from the plant and not be typical of the whole plant itself. In the discussion it was wondered whether both moisture and a cooling effect were important. The possibility of using thermocouple junctions to measure the exact temperature of the plants was mentioned.

Mr. Beals then talked on the biology of the pollen grains. Pollen of Ambrosia has been hard to germinate for experimental study and little success has been reported in the literature. His recent studies now show that a critical time is the main factor in getting pollen to germinate. Fresh pollen, i.e., pollen only a few hours old after release, had to be used. The germination medium used was 15% sucrose in 2% agar.

As far as work in progress goes, the botanists would like to get female plants from culture and try male extracts, too. They also want to use human mucosa, adding antihistaminics to determine whether there would be any effect on the pollen germination.

In the discussion, it was wondered if drying and lypholization might not help preserve the pollen. Professor Wagner thought that the germination should stop entirely instead of slowing down to a steady low rate for a long time. In other words, germination should not continue indefinitely. The curves suggest some very interesting speculations about the reproductive biology of ragweeds. It was also suggested during the discussion that a chemical gradient might control the growth of the pollen tube which grows from the stigma to the ovary.

METEOROLOGICAL PHASE

Professor Dingle discussed the meteorological plan for the present in-season study. Six-head millipore-filter units will be mounted at heights of 6, 25, 50, and 100 ft on the Jackson tower. Sampling will be continuous with each head operating for a 4-hr period. In addition to the samplers, the wind speed at four heights on the tower (measured with four 3-cup anemometers), wind direction at the 50-ft level, and the temperatures differences at 1, 4, 25, and 100 ft on the tower will be continuously recorded. At ground level, the temperature, humidity, and rainfall will be recorded. Data from 1958 will be compared with that taken at the same heights at the same location during the 1956 and 1957 seasons.

A six-head millipore-filter unit will also be installed at the 150-ft level on a tower located on the roof of the East Engineering Building in Ann Arbor.

The third phase is the roadside experiment. Two automobiles will be outfitted with flag samplers mounted on 18-in. aluminum rods extending from the side of the automobile. The first car will sample on the way to and from the Jackson tower. Samples will be taken for five miles over prescribed one-mile intervals. The second car will travel over a prescribed forty-three mile route in and around Ann Arbor. Sampling will be continuous; however, samplers will be changed every mile. It is planned to have an anemometer mounted on the car directly behind the sampler to measure the exact number of miles of air which have passed the pin. In addition, it is planned to place a roto-bar sampler in the car to compare inside and outside concentrations if equipment is delivered soon enough.

Sampling will be carried out three times daily for a period of a week during the height of the ragweed hay fever season.

Professor Wagner said that there was a tremendous patch of giant ragweed just south of Dexter and also one at Pleasant Lake. Both places are close to the proposed route.

Professor Hemphill inquired about the actual objectives of the various phases of the sampling program. Dr. Dingle replied that the aim of the auto sampling was to establish the extent of exposure a person would endure while driving to work and also to compare rural versus urban concentrations. There was some discussion as to whether it would be possible to sample every tenth of a mile in the city.

The question arose as to whether a sampler 18 in. from an automobile is effective. It was suggested that the aerodynamics of the car might repel some of the pollen. The meteorologists conceded that there would be aerodynamic effects but pointed out that at 18 in. from the forward edge of the front door the effect would be small and that the anemometer would give a measure of the volume of air flowing past the sampler.

Mr. Harrington announced that ten roto-rod samplers were available for distribution throughout the city so that an area survey of the variability of pollen concentration could also be made.

CLOSING COMMENTS

Professor Dingle stated that everybody should again check over the notes of the previous meetings to see that they were correct. The next meeting was set for September 17 in the School of Public Health.

MEETING OF 17 SEPTEMBER 1958

The meeting was held in the School of Public Health Building. The following were present: Akerman, Beals, Bianchi, Cook, Dingle, Elder, Field, Giever, Gill, Harrington, Hemphill, Hewson, D. L. Jones, Kelley, McLean, Mathews, Nilsson, Patterson, Remington, Schwemmin, Sheldon, Wagner, Warr, Willard, and Yang.

OPENING REMARKS

Professor Hewson opened the meeting by reporting on his recent trip to England. He and Dr. Sheldon attended the International Symposium on Atmospheric Diffusion and Air Pollution held in Oxford, England, during August of this year. The main part of the meeting dealt with statistical theories of turbulence. One theory tried to correlate positions of particles in the air and their separation with atmospheric turbulence. Drs. Hewson and Sheldon also acquired some new ideas on how to measure the total output of ragweed pollen. Professor O. G. Sutton suggested that a circular band of ragweed be grown around the tower for measurement. Dr. Deacon of Australia suggested a large roto-rod sampler which would sweep a large area and replace the multiple stationary samplers now being used by the local group. Some Swedish workers were testing the sulfur dioxide content of the air by use of airplanes near a commercial firm which produces sulfur dioxide. They found that there was no settling out of these small particles due to the gravitational forces for a 150-mile radius around the factory.

Dr. Hewson described the various meteorological laboratories which he visited in England. It should be noted that Gregory, who originally said that 99.9% of the pollen falls to the surface within a hundred meters of the source, had now corrected this figure to 90%.

Dr. Sheldon remarked that he talked to three physicians at the meeting. One of them, Dr. Tromp of the Netherlands, had collected a large group of data on asthmatics (250 patients) since 1954. He had daily records on the asthmatic symptoms as well as some biological data plus simultaneous weather-recording data. Dr. Tromp thought that symptoms of asthma could be explained at times only by meteorological changes. This was particularly noted when a cold front moved into the area where the patient lived. Dr. Morley of England agreed with Dr. Tromp's observations.

Dr. Wedin, a Swedish physician, who was a consultant in the Air Force, was also interested in certain aspects of contact dermatitis due to poison ivy and sumac.

Dr. Dingle then submitted corrections to previous meeting notes. Also a mimeographed outline on the correct procedure for preparing slides was passed out to the group. Dr. Dingle said this outline had been very helpful to the meteorological group in the preparation of slides for scientific papers and he felt that all project personnel would benefit by having this information.

BOTANICAL PHASE

Dr. Wagner talked on the botanical phases to date. He made the announcement that the botany section was making certain slides of its work. It was decided that in the future any slides made by any particular group working on this project would be made in duplicate or triplicate so that other members could use the same slides for any of their lectures or discussions.

Dr. Wagner talked on the hybrid ragweed, which is a cross between the giant ragweed and the low ragweed. This hybrid has been known since 1915 and has been described several times. Since the plant is sterile, there are no allergic effects from it.

Since ragweed, including the hybrid variety, is present in France, the question arises why there is no ragweed hay fever in France and in Europe generally. The following reasons were suggested: (1) ragweed is not abundant; (2) the plants pollinate at a time when other pollen is in the air. The fact that the plant is not abundant may be due to the threshold population theory, which states that, until the plant reaches a certain proportion, it will not grow abundantly. Once it reaches this proportion, its growth multiplies rapidly. Any control measure, such as used in large cities, would be unsatisfactory at this later stage.

Dr. Wagner said that the Louisiana ragweed plant, which was discussed at the last meeting, was just now beginning to flower. The botany group pointed out that there is an algal parasite of ragweed. Work is being done on it by the Botany Department at this time. However, it has no effect on the host, i.e., the ragweed plant, and the parasite is not specific for ragweed. It was announced that a copy of the bibliography on ragweed has been sent to Dr. Morton, in Washington.

TEST CHAMBER

Professor Akerman reported on the progress of the test chamber. He said that the air-conditioning unit for this chamber had been approved. It appears that special permission is needed for any air-conditioning unit of any type to be used on the campus. There was considerable discussion about the other approvals which had to be obtained now that the go-ahead signal has been given. As yet there has been no date set for starting the construction.

METEOROLOGICAL PHASE

Mr. Harrington gave an account of the 1958 in-season experiment. He demonstrated the large roto-bar sampler with the larger protective covering on top. The sampler would sample 33 cubic feet of air an hour. The grains collected could then be converted to grains per cubic foot. One has been placed on the Kresge Building roof as well as on the roof of the East Engineering Building. It was shown that stain could be applied to a cover slip, thus coloring the ragweed, spores, and other pollens for identification. The sampler on the East Engineering roof was changed every hour from 8:00 a.m. until 5:00 p.m. The sample counts for several days were passed out to the group. It was noted that there is considerable variation during the day with a drop in the pollen count on September 11, when a cold front passed through this area. In general, the count has remained low since then.

It was pointed out that, if this type of pollen counter is to have widespread use, the height of the sampler should be standardized and also some type of standard volumetric measurements should be used. Cubic yards is a rather awkward unit and cubic meters is more generally used. It was also brought out that the roto-rod sampler was being used for detecting gases in certain industries.

As far as this year's in-season experiment is concerned, the full program was carried out at Jackson Prison for one month, that is, the full program as outlined previously. In addition, some tests were being done at Jackson tower to calibrate the millipore filters and the roto-rod counts.

It appeared that the maximum concentration at 72 feet on the roof of the East Engineering Building occurred in the early afternoon, whereas the maximum concentration of the pollen occurred at ground level in the morning. It was speculated that the pollen would be carried aloft by convection currents, which would explain the higher counts in the afternoon on the roof. However, counts at 72 ft would not correspond to the patient having symptoms increase in the afternoon while walking along at ground level.

As far as the route to and from Jackson and the auto-flag sampler were concerned, measurements were taken during seven one-mile segments. For the counts inside Ann Arbor, many complications occurred and no anemometer was used for the car counts. These counts must therefore be corrected for wind speed. Within the city and in the country three traverses were done per day. In general, taking the counts from 8:00 to 10:30 a.m. as being equal to 100%, the counts done from 1:30 to 3:30 p.m. would then average about 25%, and the counts done at 7:00 to 9:00 p.m. would average about 15%. It should be noted that there is a great variability in the pollen counts in the morning. For example, the counts along North Territorial Road were high, due most likely to the abundance of ragweed along the roadside and the closeness of the sampler to the ragweed at the road's edge.

Mr. Harrington reported that no roto-bar samplers had been scattered throughout the city as previously planned because they had not been available. The samples taken within the city were done approximately every two-tenths of a mile. It was noticed that in all of these studies, the counts dropped when the cold front passed by. It was wondered if this meant that some pollen had been coming from the south or if the colder temperature had cut down on pollen production and emission.

Mr. Gill reported on a ragweed pollen counting project carried out under the supervision of Dr. Sussman, of Vanderbilt Hospital in Nashville. Our group furnished assistance to the Nashville group by putting at its disposal one volumetric sampler, twenty-five flag samplers, and two roto-bar samplers. The Nashville group had 113 air-pollution stations for counting spread out through the city of Nashville. The local group was happy that they could lend assistance to Dr. Morton, of Washington and the workers in Nashville, Tennessee.

MEDICAL PHASE

Dr. Patterson reported that the medical group had a fox terrier dog which was ragweed-sensitive. This dog was furnished to the group by a Detroit veterinarian. It was definitely shown that the dog had true hay-fever symptoms during the ragweed season. Therefore the dog was brought to the Allergy Research Laboratory. It was also shown that ragweed pollen had caused pruritis and lacrimation. The dog will now be kept by the group. Any member of the group could have the dog during the winter if he so desired. It was hoped that this animal could be used in future chamber experiments.

As far as the animal chamber is concerned for the ionization study, Dr. Field reported that animals were now being sensitized in preparation for the ionization experiment. The main problem at the present time was getting the proper equipment to measure the ion density for the ionization studies.

CLOSING REMARKS

It was decided to have the next meeting on October 16, 1958.

MEETING OF 16 OCTOBER 1958

The meeting was held in the conference room of the Industrial Medicine Department in the University Hospital. The following were present: Akerman, Beals, Bianchi, Dingle, Field, Giever, Harrington, Hemphill, Hewson, D. L. Jones, Mathews, McLean, Patterson, Plymer, Remington, Sheldon, Schwemmin, Wagner, and Willard.

OPENING REMARKS

Mr. Epstein from Penn. State University was introduced to the group. He has an A.B. (astronomy) from Harvard, an M.B.A. in Business Administration from Columbia, and an M.S. in meteorology from Penn. State. At the present time he is working on a Ph.D. in meteorology at Penn. State. He is being considered to take Harold Baynton's place in coordinating the various disciplines of this project and will work on the statistical phases.

Professor Akerman said that, as of October 24, the plans for the test chamber should have been completed for the second time and were then to be approved.

BOTANY PHASE

The botanists passed out reprints to the group of their recent publication entitled, "Perennial Ragweeds (Ambrosia) in Michigan, with the Description of a New, Intermediate Taxon," published in Rhodora, Volume 60, 1958. This is publication number five under this research grant from the National Institute of Allergy and Infectitious Diseases. Mr. Bianchi discussed the physiology of the ragweed and summarized the 1958 experiments.

It should be noted that this is further extension of the work presented by the botanists at a previous meeting and that these experiments were carried out on excised flowers. Therefore conclusions drawn may not be applicable to natural conditions in the atmosphere and the normal environment. Light, humidity, temperature, and possibly cell turgor were four important factors concerning dehiscence of the pollen.

The results of the preseason experiments in which meteorologists and the botanists combined their efforts were again with the whole plant and not with dissected flowers. Taking June 19 as a typical day, it was shown that there was a simple drying of the pollen with a drop in humidity. The pollen sacs then split open and the pollen was released.

In the discussion that followed, it was stated that the nutrient soil was very important. The opening process of the pollen sacs appears to be related to the humidity and temperature, but the extension-process mechanism is unknown. To extend the present experiments, it would be necessary to purchase additional equipment so that whole plants could be studied rather than just excised portions. Such equipment is available at other institutions but not here. The possibility of using the proposed test chamber was mentioned, but more lighting would be required than was proposed in the present plans. Professor Hemphill wondered about the air pollen counts and whether a plateau of the pollen dehiscence actually occurred. Mr. Harrington said that they were still doing pollen counts; however, there were some data to show that some ragweed counts go up at night, but whether this was due to a fall in the humidity without a rise in temperature is unknown. Actually one day's count did show a pollen peak at about 2:00 a.m. Professor Wagner stated that the Louisiana ragweed plant was now pollenating. Approximately 6000 seeds in the crude state would be necessary for next year's experiment. These must be separated by hand since about 50% are sterile.

Mr. Ted Beals then discussed further the biology of the pollen. He distributed an abstract of his discussion. This talk was also an extension of the previous work reported earlier to the group. Mr. Beals stressed that the important fact brought out by this study was that the pollen ages very rapidly. This has a tremendous effect on pollen-germination studies. The percentage of germination falls off 55% within 1-1/2 hours and drops to a low plateau by 6 hours. However, after 2 hours, there is no further germination in artificial culture media. It was shown that, as far as effect of temperature on the degree of germination was concerned, no germination occurred at 4° and the optimal amount of germination occurred within 2 hours at both 20° and 28°C. Two hypotheses were proposed for the mechanism of the aging effect: (1) that something inside the pollen grains disintegrates and (2) that there is some external factor operating at this time limit. The data at hand suggested that both factors may play a role as growth factors. There is also the possibility that there is some kind of a diurnal timing mechanism. All the pollen does not seem to develop at one time. Ragweed pollen probably develops through July and August. It is also probable that the pollen tube changes its osmotic pressure after it penetrates the plant tissue cells. Other than the 10% of the ragweed left in the culture after several hours, there may be no clinically significant allergenic pollen present in the atmosphere in the afternoon.

In the discussion that followed, it was proposed that one of the first experiments carried out in the test chamber should be to determine if fresh pollen is necessary to produce allergy symptoms or if old pollen may be used.

MEDICAL PHASE

Dr. Patterson reported that the dog which is allergic to ragweed has both positive skin tests and passive transfer tests. This dog's sera also has a high hemagglutination titer. The question was raised whether allergy occurs only in light-skinned animals, that is, whether they will have positive atopic skin tests. The Allergy Group hoped to use the histamine-release method for testing the old and new pollen before the test chamber experiments were carried out. It was thought advisable to test the whole pollen grain versus portions of the pollen-grain cell membranes.

CLOSING COMMENTS

The next meeting of this group will probably be held on November 20. The final data and meeting place will be determined later.

MEETING OF 9 DECEMBER 1958

The meeting on 9 December 1958 was held in the Meteorology Laboratory of the East Engineering Building. The following were present: Akerman, Beals, Bianchi, Bierly, Dingle, Field, Giever, Gill, Harrington, Hemphill, Hewson, D. L. Jones, McLean, Mathews, Mikat, Plymer, Sheldon, Wagner, Willard, and Yang.

MEDICAL PHASE

Mr. Kert Mikat presented the results of his research work on the enzymatic digestion of skin-sensitizing antibody to ragweed. This work was done in conjunction with this project to see if an in-vitro method for studying skin-sensitizing antibody could be devised. The study of skin-sensitizing antibody or allergic reagin has been greatly hampered by its failure to react in the usual test tube reaction as most other types of antibodies will. Therefore the purpose of this experiment was to discover if skin-sensitizing antibody was prevented from participating in the usual in-vitro antibody reaction by some portion of its molecule.

In the discussion period that followed, Mr. Mikat thanked Dr. Mathews for his advice and guidance in this work. Dr. Mathews said that the skin-sensitizing antibody may have a higher molecular weight than does the regular antibody and possibly also an irregular shape. Therefore, if the irregularities or inert particles could be knocked off or digested, then the rest of the antibody could react as do ordinary antibodies. He felt that a by-product of this experiment was the fact that there is rapid inactivation of skin-sensitizing antibody at pH of 4.7. Other enzymes were considered, but passive transfer tests limited their applicability to this experiment. Proteolytic enzymes do not necessarily work on proteins, i.e., are not specific for protein, but only on a specific bond.

TEST CHAMBER

Professor Akerman reported to the group on the status of the test chamber. In August, 1958, the work order was approved, but was then held up by UMRI administration until September 18 trying to get the air-conditioning approval verified. Professor Akerman was then told that the plans would be completed by October 6, but was notified later that Unistrut could not be used in the construction. Therefore the plans had to be changed. December 3 was then set as the date for which the plans were finally readied. On December 4 Prof. Akerman met with the medical group and on the fifth with the meteorological group, so that each could go over the plans specifically.

The following general changes had been made by the Plant Department. (1) An 18-in. bench could be removed from the laboratory, which the Plant Department had apparently overlooked in our original recommendations. Therefore, they changed the chamber so that it would be reduced from a width of 9 to 8 ft, but the depth was increased by 1 ft. However, increasing the depth would block the entrance to the electrical switches in the Allergy Laboratory. (2) Relative to the pollen being blown up through the floor and sucked out through the ceiling, the pegboard flooring was to be supported by sub-structures which could be easily cleaned. The plans submitted by Prof. Akerman originally suggested drilling pegboard supported by a wooden grill work. However, the plans submitted by the Plant Department called for pegboard glued to plywood and then holes drilled through both. This would be a rough opening causing considerable adhesion of pollen to the rough surface with a consequent loss of airborne pollen as well as causing a complicated cleaning problem. It was suggested that expanded metal or metal subway grating be used. It was felt that the 54- x 48-in. panels were too big and too heavy to be taken up and down for the frequent adjusting and cleaning that would be necessary. (3) It was found that the oxygen piping system was still included. This was thought not to be necessary. (4) The height of the chamber had been changed from 7 ft 6 in. to about 1/8 in. less than 7 ft in the present plans.

The cost of these plans is based on a percentage of the cost of the total job. Professor Akerman said it would probably be easier to move the present proposed panels than the Unistrut structure as originally devised. Therefore this change in the chamber plans was approved. It was thought that the panels could be bolted together and the interior plywood placed together at the joints with tape similar to dry-wall construction. The main cost would be the moving of the duct work and utilities.

It was the concensus of the group that, even though more time would be entailed in drawing up new plans, the Plant Department should be instructed that we still wanted the original plans. One of the questions which was not completely answered at this time was that of the exact type of floor gratings for the pollen to pass through. There was a question of steel band supports for the flooring and strips. The main objection to these was that the excess weight would be undesirable since many adjustments and frequent lifting of the floor sections would be necessary. Fingerle Lumber Company could supply wooden gratings 2 x 3 ft which would be lighter, but more costly. Using aluminum stripping was considered. The possibility of having pegboards and supporting plywood units fastened together as a unit was also suggested. Finally it was thought that a grillwork floor board would be preferable rather than both grillwork and flooring. It was decided that Professor Akerman should investigate the flooring possibility further and would then decide on the best arrangement.

PRESEASONAL EXPERIMENT

Mr. Harrington then introduced the subject of the 1959 preseasonal ragweed experiment. He reviewed the work done in the past three years in preseason experiments. The advantages of preseason experiments consisted of having a known source of ragweed and having natural pollen present for medical studies on the patients as well as botanical studies by the botanist. The disadvantage was that such a plot was immobile, therefore requiring many samplers to be spread around for the diffusion studies. In addition, the ragweed was not entirely natural because the root systems were in boxes above ground. However, the botanists stated that this was not too abnormal although it was true that the plants did not receive their normal major water supply from the soil and sub-soil moisture.

The meteorology group would like to continue the preseasonal type of experiment. They would be in favor of doing this on a smaller scale this year in order to make specific diffusion studies. They would like to study the strength of the source, the transfer of pollen from the plot, and the deposition of pollen on the ground.

The botanical group had no particular botanical needs for the preseasonal type of experiment. All their work can be done in the Botany Laboratory. The specific problem they are interested in is studying the separation of the masses of pollen falling from the foilage of other flowers. They are also interested in correlating the meteorological data collected from these preseasonal experiments with their own observations. As far as the coming preseasonal experiment was concerned, it was thought that probably 300 plants would be needed. However, the botanists will probably grow 600 to 1000 plants to make sure that an adequate supply is available. The possibility of using less exacting methods by the botany greenhouse personnel was also considered. It was thought that the ragweed would be grown in the hot house and then transplanted directly to the Willow Run Airport rather than being individually potted and then transported. This would require less labor from Mr. Kleinschmidt.

MEDICAL PHASE

The medical group is still very interested in the preseasonal type of experiment and would like to enlarge on the medical studies done last year. However, there are so many variables in this type of experiment (e.g., the very fixed pollen plot, the wind changes, and getting volunteers to that area) that it was thought the chamber experiment would be much more suitable for their research. The wind-tunnel experiments will be repeated. Fresh pollen (i.e., within 1 to 2 hr old) will be tested versus old pollen to see which type is necessary to produce symptoms in the chamber experiments. The collection of pollen for the chamber experiments will be a tremendous problem if fresh pollen is needed. It was decided that the pollen which was now being grown for Dr. Mathews for his histamine-release studies could also be used for wind-tunnel work to test fresh versus old pollen. The botanists believe that this ragweed pollen will be available in approximately 2-1/2 months. Immediate dehydration of pollen will make more uniform allergic extracts, but the amount of symptoms to be produced by this extract is unknown. Much discussion went into the problems concerned with the growing of ragweed plants and the collecting of pollen, should fresh pollen be necessary for the chamber experiments. Therefore it was decided to make these tests in the wind tunnel as soon as the fresh pollen is available. Dr. Mathews said that preliminary experiments on the histamine-release studies showed that a tenfold increase in pollen was necessary before a twofold increase in symptoms severity was noted. That is with pollen extract rather than with fresh pollen. It was not known if this would be true for the chamber experiment.

Concerning pollen dispersion in the chamber, the possibility of using capillary tubes for spraying of pollen in the room was raised. Mr. Giever wondered if information gathered from animal experiments concerning particulate matter dispersion in the atmosphere could be used. He was to check further on this matter. The fact was brought out that the particulate matter referred to is of such small size compared to ragweed pollen that the information might not be applicable to our experiments.

GENERAL DISCUSSION

During a general discussion of topics introduced from the floor, Dr. McLean said that a mycologist on the campus was interested in mold identification and might be able to help this group with identification, since the quantitative count now available from the roto-bar samplers might make specific identification of the mold spores more important from a clinical standpoint. Much work has been done on identification in the past from the routine gravity-slide samples. Most clinical allergists, at present, think that the major spore groups have been identified from the pollen slides and further identification requires cultural methods which several workers throughout this county have done already. It was wondered if more types of spores could actually be identified from the tape and flag samplers. However, since a quantitative sampling method was now available, it might be possible to do plate and slide counts and compare them with the quantitative counts of the roto samplers. Professor Wehmeyer of the Botany Department could be contacted to see if he could help further along this specific line. Professor Wagner mentioned that Mr. Alan Graham of the Botany Department is also very interested in pollen counting and could help in identifying specific pollens. Dr. Alexander Smith, a mycologist on campus, could also aid in the identification of the molds.

Mr. Harrington said that the roto sampler on the roof of the East Engineering Building revealed ragweed in the air up until approximately two weeks ago. Professor Hemphill wondered if figures were available for comparing roto-bar and flag sampler pollen counts. Mr. Harrington said that such figures were in the process of being compiled and would be made available to the group as a whole as soon as possible.

General announcements from the floor revealed that Professor Wagner gave a very interesting talk on ragweed production and dissemination at the recent Midwest Allergy Forum meeting in Detroit. Professor Hewson and Mr. Giever attended the Air-Pollution Conference in Washington recently, which turned out to be more of a political than a scientific conference. The Surgeon-General and the head of the Department of Public Welfare were present. The main plea of the meeting was for industry to make more funds available for basic research in air pollution. Unfortunately, the problem of air pollution by aeroallergens was not felt to be very important by this group, as their main interest was in smog.

It was decided that the next meeting will be on January 14, Wednesday evening.

MEETING OF 14 JANUARY 1959

The meeting was held in the Research Conference Room of the Kresge Medical Research Building. The following were present: Beals, Bianchi, Bierly, Dingle, Gill, Harrington, Hemphill, Hewson, D. L. Jones, McLean, Mathews, Patterson, Plymer, Sheldon, Schwemmin, Wagner, Warr, Willard, and Yang.

GENERAL DISCUSSION

The botanists introduced Mr. Willard Payne. Mr. Payne is studying the evolution of ragweed and will probably attend some of our meetings in the future.

MEDICAL PHASE

Dr. Patterson was introduced by Dr. Sheldon, who gave a brief background of the acquisition of the ragweed-sensitive dog. The dog had been a great expense to her owner because of the necessary seasonal treatment; the owner had therefore given the dog to a veterinarian, who had in turn contacted the Allergy department to inquire whether the animal might be useful in medical research.

Dr. Patterson reported that, although most of the literature states that spontaneous allergy is rare in animals, it does occur, especially in cows and dogs. The ragweed-sensitive dog is a 2-1/2-year-old oophorectomized fox terrier who began having eye and nasal symptoms and eruption and itching of the skin in 1957. When she came to the University at the end of the 1958 ragweed season, she had profuse lacrimation of the eyes with a secondary infection. There were also eruptions on the back and flexor surfaces of the forelegs. Her white count was 13,000 with 11% eosin. This occurs along with the infection and the allergy. In addition, a conjunctival smear of the eye showed many polys which substantiated the secondary eye infection. The eye symptoms cleared in October, but the skin symptoms persisted until December, which is often the case in both animals and humans so afflicted. Dr. Patterson then briefly discussed the tests that had been performed on the dog. He added that the dog was well treated in the laboratory.

Dr. Wagner asked whether this dog was truly atopic or whether she had developed a sensitivity. Atopy was defined by Dr. Sheldon as a term coined by Coca thirty years ago referring to spontaneous or naturally occurring hay fever, asthma, or eczema in man in relationship to his hereditary background. It was thought that this applied to the dog, as the dog's symptoms were spontaneously induced. Dr. Sheldon said that the dog could be used to test fresh vs. old pollen. The answer to this question will be necessary for the chamber experiments. It would also be important to know if passive-transfer methods of human serum on the dog could be demonstrated. Dr. Hemphill asked if the same human could be placed in the chamber repeatedly, as the dog had been. This question was raised because of the possible accumulative effect. If such an effect is found, a patient could be placed in the chamber only a limited number of times. Dr. Patterson said that the dog's response had not been tested any more frequently than once a week. Dr. Hewson wondered if the amount of pollen used had been quantitated. This had not yet been done. At present, the only method that could be used would be to have a volumetric sampler within the chamber to measure the ragweed pollen concentration.

After Dr. Patterson's talk, the group went to the Montgomery Allergy Research Laboratory in the Kresge Building, where Dr. Patterson demonstrated the production of symptoms in the dog by placing her in the chamber. The group was also shown the area in which the test chamber will be assembled. In the discussion that followed, it was pointed out that the hereditary factors could be better studied if more atopic dogs could be acquired. It would be interesting to take a movie of the phenomenon; the temperature in the chamber would have to be regulated because the lighting necessary for movies would elevate the temperature. It was suggested that fluorescent lighting and fast film be used so that rising temperatures would not be a problem.

BOTANICAL PHASE

Dr. Wagner reported to the group on the recent Northeastern Weed Control Conference. The papers presented at this meeting were published with few exceptions in the proceedings of the Thirteenth Annual Meeting of the Northeastern Weed Control Conference (Rutgers University College of Agriculture, New Brunswick, N. J.). Many of the papers dealt with general weed control, including horticultural crops, agronomic crops, industrial and highway, aquatics, forestry, and conservation. Most of those of interest to the University of Michigan program were presented in the Public Health Section. Of the latter, a number were concerned with public relations and ragweed control programs. Instead of making a general report, only a few have been chosen to bring out points of controversy which seem to have significance to our program.

The overall problem of interest to Public Health Officials is whether or not it is of value to control ragweed in and around congested urban areas. This issue reappears constantly. For example, is a ragweed control program in New York City of no value because pollen will be blown in from New Jersey anyway? Several of the specific problems discussed at the meeting follow.

The problem of photoperiod vs. growth period as determining time of ragweed flowering. In his talk "Ecological Aspects of Allergenic Plants" (Proceedings, pp. 26-32), Wodehouse ascribed flowering of ragweed strictly to photoperiod. "I once watched from day to day a clump of tall ragweed and another of short ragweed under street lamps in New York City. All the plants beyond the influence of the light, about 18 feet, flowered at the appointed time. ... Not so those under the street lamps. All through October the weeds continued to grow ... but they did not flower." Currently this is questioned because it is believed that the growth period at any given locality is the major determiner of flowering. Short days will only speed up flowering in such plants that are late to germinate. These should be watched especially, noting the observations that were made last year on the spread of germination at the Botanical Gardens. Long days or continuous light will probably have no effect on the normal timing, if our hypothesis is correct. This is a problem that needs to be tested from various standpoints.

The problem of how pollen is discharged: Wodehouse, in the paper quoted above, said (p. 28): "Though these flowers are entirely male and produce no seeds, the pistil is retained for its secondary function of forcing the pollen out of the flower, characteristic of the Composite family, even those with fertile pistils." He also showed a picture (unpublished) of the pistillodium (but not the anthers) projecting from the flower, carrying with it a large mass of pollen. He said later that his observations were made on giant ragweed but not on the common ragweed. Our studies showed that it is the presentation of the entire anther mass and their dehiscence which provides the actual discharge mechanism. Up to now, at least, it is believed that the role of the pistillodium is very minimal at best—perhaps as a "clean up" device later in the morning, after the 6:00 to 8:30 critical period. Our demonstration of the stages of the pollen discharge which have been reported upon appeared to be wholly new to this group, and such aspects as the presentation of anthers, the periodicity, and reflotation of pollen were unheard of. Whatever the case, Wodehouse's description of floral function in ragweed seemed to be erroneous. We should, however, attempt to pin down exactly what the pistillodium does in the process and whether giant ragweed differs from the common ragweed in this process.

The problem of how long the toxic effect of ragweed pollen persists: This is a big and very important problem, and one of great significance to our program. Alexander Rihm said in his talk, "Plans for Ragweed Control Program in New York State" (Proceedings, pp. 257-261):

> ... We need to know whether its ability to sensitize is influenced by weathering, by moisture, high temperatures and sunlight in the atmosphere.

Rihm is Executive Secretary of the Air Pollution Control Board of New York, and we would obviously agree with him. It very soon became apparent that various people present already thought we had a good idea. Israel Weinstein (former Commissioner of Health, New York City), in his paper "Administrative Aspects of a Municipal Ragweed Control Program" (Proceedings, pp. 269-273), commented as follows:

> Many people believe that a program for the elimination of ragweed within a local area is futile. ... This is a mistaken idea. It has been shown by Wodehouse and others that ragweed pollen quickly loses its power to irritate as it travels through the air. ....

Thomas J. McMahon in his talk, "Ragweed Control" (Proceedings, pp. 285-292), said:

> Two years ago the tests of a Mr. Samuel F. Potts of the U.S.D.A. from New Haven were reported. Mr. Potts had stated at a meeting in Spring Lake, N. J., that he had been unable to produce an allergic reaction in tests on himself using pollen three days old. "Pollen," said Mr. Potts, "exposed to air and sunlight for three days will not produce an allergic reaction." Dr. R. P. Wodehouse, then of Lederle Laboratories, gave support to Mr. Potts, though he ventured to state that it is necessary only that pollen be exposed to air. He stated "Pollen exposed to air overnight will lose half its potency."

Dr. Wodehouse said later that these statements were too strong and that he felt he had been misquoted, but he does apparently have some evidence to support the idea, and he aims to put it to the test. This surely bears on our work, from two standpoints: (a) As pointed out in 1956, the pollen does adhere in clumps. ... The later work of R. L. Hanline (1957), followed by the work of Bianchi and Schwemmin (1958), only confirms this suggestion that perhaps some surface substances on the pollen grains such as tapetal fluid may play a role in allergy. (b) Beals has strikingly shown (1958), the vast bulk of germination of the pollen itself, under the conditions of his experiments, takes place during the first two to four hours. It seems that we must pursue experiments and studies along these lines.

The problem of pollen counting: This was a subject discussed as much informally as in contributed papers. Of the latter, the statements of Thomas McMahon in his talk on "Ragweed Control" referred to above are cited:

> 1. A pollen count does not afford an accurate index of what the reaction of the sufferer has been. They could not explain this inconsistency; they merely affirmed the fact (in a Public Health meeting of four years ago). And
>
> 2. A pollen count is not a forecast, but a report on what has happened. It is not like a weather forecast, but rather a report on what has been. It confirms a condition; it suggests no way to avoid the condition.

To what extent McMahon actually knew what he was talking about is not known, but it is clear that our chamber experiments, if successful, may bear on it. His first statement may ultimately have to be modified, if, with our new techniques of making pollen counts more accurately, it may turn out that there is an accurate index by pollen count for reaction of the ragweed-sensitive person. In another talk, not published in the Proceedings, Mr. L. B. Hall of the U. S. Public Health described experiments in Savannah, Georgia, and stressed the conclusion that volumetric samplers were preferable to gravimetric.

The problem of ecology of ragweed and its control: There was much discussion of this problem, both publicly and privately. For example, does the spraying of ragweed inhibit rather than promote the natural succession which in due course would eliminate ragweed anyway? Do ragweed roots send out "poisons" that inhibit the other vegetation and prevent its growth (a question brought up in McMahon's talk, pp. 287-288)? Are we destroying the native vegetation and natural values when we promiscuously spray along highways and roadsides? There was a very interesting paper, not presented orally, by R. H. Goodwin and W. A. Niering, "The Management of the Roadside by Selective Herbicide Techniques," that emphasizes selective techniques to eradicate ragweed at the same time to preserve the attractive native plants and prevent unsightly brown swaths. For ragweed, they suggested getting a dense vegetative cover along the road composed of grasses and flowering plants; for the margin of the pavement and shoulder strip itself, they recommended mowing and a light foliage spray for this strip alone. The paper is printed in the Proceedings, pp. 530-532, and from informal discussions with various weed-control people it seems to be quite controversial. The subject does not bear immediately on our problems, but it does point up a need for further work on the ecology of ragweed.

Dr. Wagner said that three prominent botanists were present at this meeting, Drs. A. S. Crafts, Eugene Ogden, and Roger P. Wodehouse. Dr. Wodehouse is in retirement, but at the present time there is a prospect that he will get a grant to continue his research on ragweed. In discussing the problem of photoperiod vs. growth period, Dr. Wagner noted that the conclusion of Wodehouse and others that street lights would inhibit the growth of ragweed is not confirmed by the observations of our group. Concerning the problem of how pollen is discharged, Wodehouse was of the opinion that the pistillodium acted like a plunger and pushed the pollen out. Our studies, however, do not confirm this; in fact, the bulk of pollen discharge in common ragweed appears to be accomplished strictly by the action of the opening of the pollen sacs. The role of the pistillodium is minor at best. The only time we have seen the pistillodium protruding from the flower by itself was under unusual conditions of humidity in the experimental chamber.

The problem of the persistence of the toxic effect of ragweed pollen was discussed at the Weed Control Conference, and it seemed to be more or less accepted that the pollen loses its toxicity in a short time. Dr. Wagner therefore called our attention to the need of continuing our interest in the tapetal fluid. He said that the pollen comes out in clumps and adheres together. The substance that makes the pollen sticky may be the toxic substance.

The problem of pollen counting was studied by Mr. L. B. Hall during the 1956-57-58 seasons in Savannah, Georgia. All ragweed was eliminated in a two-mile circle except for growth in the center. Pollen was collected along many radii during the first year, along three radii the second year, and along only one major radius the third year. The investigators used volumetric samplers, concluding that this method was better than the gravimetric. Five towers were also used for pollen collection. Dr. Dingle wondered if the investigators had taken the idea of isokinetic sampling into account. The lack of isokinetic sampling would raise some doubt about the validity of their studies.

Dr. Wagner said that in a certain area in the state of Washington a telephone survey of the hay fever sufferers would be made next year. Apparently, there is only a very limited ragweed area. The ragweed is planted in orchards for ground cover and there is no other ragweed within hundreds of miles. All ragweed sufferers in this area are to be contacted by telephone, and their symptoms will be recorded. In this way a study of pollen transportation through the air could be made.

It was also mentioned that this meeting of the Northeastern Weed Control Conference was its thirteenth. This association publishes the notes for each of its meetings, and it was thought advisable that our group obtain copies of these notes from all previous meetings.

METEOROLOGICAL PHASE

Mr. Chien-Hsiung Yang talked on the effect of a small ragweed-free area on the concentration of pollen at a point in a large source region. A summary of Mr. Yang's talk was distributed to the group.

This was the first attempt to obtain a reasonable estimate of the effect of eradicating ragweed plants within an area to reduce the pollen concentration at ground level downstream.

The problem is a particularly important one for communities wishing to alleviate the suffering of hay fever and asthma victims by carrying out a ragweed-eradication program. These communities are likely to ask questions such as the following:

> "In how large an area must ragweed be destroyed to reduce pollen concentrations in our community by 50%?"
>
> "Will it be worthwhile eradicating ragweed within a three-mile radius from our city limits?"
>
> "We have attempted to destroy all ragweed plants within our city and have noticed no appreciable change in pollen concentrations. Has the eradication program been incomplete or is the pollen arriving from a distant source?"

The problem was approached in two ways. First, the best diffusion theories were adapted to the exceedingly complex problem of computing pollen concentrations downstream from an area source. Second, the mathematical model is to be tested during the 1959 ragweed season using lakes as ragweed-free areas.

In the discussion which followed Mr. Yang's presentation, it was shown that a lake would be a pollen-free area. By taking counts on both sides of the lake, the theory presented might be put to a practical test. This is a very complex problem that has never been studied before. Previously, meteorologists had been concerned only with the fact that pollen was emitted continuously rather than during a 4-hour period as shown by this group. In addition, the deposition of pollen on the ground had not been taken into consideration, although this is probably not a large factor.

It was also shown that such an experiment could be performed over a grassy area rather than over a lake because there are many different wind currents over a lake. In general, the results presented were thought to be applicable to an area of any size, using the figures in the discussion on a multiple basis. However, the results would not follow exactly because of wind currents.

Dr. Wagner said that the long shoreline south and east of Detroit could be used for a study such as this. He pointed out that the most suitable area would probably be a ranch with grasslands. The grassland bogs in Michigan or in southern Ohio might be suitable for an experiment to test this theory.

TEST CHAMBER

Professor Akerman was to report to the group on the status of the test chamber, but since he is the chairman of another committee which was meeting on the same night, he was unable to attend our meeting. Mr. Gill therefore gave the report. The wooden part of the test chamber is built and assembled, and was to be disassembled and sent to the paint shop on this very day. It is to be moved to the Kresge Building next week, when the actual setting-up procedure is to begin. The metal gratings for the floor have not yet arrived. It was hoped that the assembly of the chamber would be well underway in a few weeks.

CLOSING COMMENTS

Dr. Hewson pointed out that the annual progress report would be due in Washington on April 30. It was therefore suggested that each group of this research project prepare an outline of the material it wishes to place in the report. These outlines are to be forwarded to Mr. Bierly by the end of this month so that they can be put together and presented to the entire group for review at the next meeting.

The next meeting will be held on February 18, in the Botany Seminar Room.

MEETING OF 18 FEBRUARY 1959

The meeting was held in the Botany Seminar Room of the Natural Science Building. The following were present: Akerman, Barlow, Beals, Bianchi, Bierly, Dingle, Elder, Field, Gill, Harrington, Hemphill, Hewson, D. L. Jones, Kelley, McLean, Patterson, Payne, Plymer, Remington, Sheldon, Wagner, Warr, and Willard.

GENERAL DISCUSSION

Mr. Myron Tourin was introduced to the group. He is a new graduate student in meteorology. Dr. Hewson announced that Dr. Mathews had now been appointed to the executive committee of the American Academy of Allergy.

In the discussion of the recording of the minutes of each meeting, it was brought out that several mistakes were being made and the notes were becoming more voluminous. It was decided that the notes would henceforth be much briefer. Any papers or discussions would be written up in the annual report, by the individuals concerned and that all the exact details would be recorded there. The question of omitting the monthly minute notes from the annual report was raised. This has to be decided later.

METEOROLOGICAL PHASE

Mr. Harrington presented a graph to the group and made explanatory remarks on the previous discussion at the last meeting by Mr. Yang on the effect of a ragweed-free area on the concentration of ragweed downstream. He explained how reducing the ragweed in an area would affect the total ragweed count for any particular area studied.

Mr. David Jones then gave an illustrated talk on the characteristics of cold fronts. A summary of the talk he presented to the group follows.

Introduction

The literature on medical meteorology contains a large number of references to the effects of weather on the pathology of the patient. Especially notable is the fact that many physiological changes occur during the few hours attending an air mass discontinuity of the cold front type. This appears to have been well established by much exhaustive research, the large majority of which has been conducted in Germany.

Specific research on allergies and asthma indicate that there may be causative mechanisms in the frontal zone for effecting changes in allergic reactions.

Accordingly, some of the characteristics of cold fronts and phenomena associated with them will be discussed.

Classical Model of the Cold Front

Forty years ago Bjerknes, Bergeron, and Solberg proposed the theory and model of the polar front, although previous work had been done by Shaw and Lempfert near the turn of the century. The juxtaposition of two air masses of different origin is postulated as preceding the development of a cold front (see Fig. 1A). Separating the air masses is a stationary surface of discontinuity known as the frontal surface. A perturbation in the pressure field can result in the initiation of convergent air at low levels which causes the wind to change from flow parallel to the frontal surface to flow in which there is a component directed toward the discontinuity zone. A cyclone has thereby been initiated and the frontal surface is distorted as development continues.

The distinction between a cold front, warm front, and stationary front is made on the basis of the instantaneous motion of the frontal surface at a given location. That is, if the adjacent cold air has a wind component directed toward the front, cold air will replace warm, and a cold front is said to exist (see Fig. 2A). If the cold-air wind has a component away from the front, the receding cold air permits the influx of warm air and the moving frontal surface is classified as the warm type. To specify the front as cold or warm, therefore, only the kinematic distinction is necessary, namely, the direction of motion of the cold air adjacent to the frontal surface.

There are, however, marked differences in other meteorological elements in the several frontal types. Qualitative changes in meteorological properties during the passage of a cold front are outlined below.

Changes in Meteorological Properties During Cold Fronts

One of the most important forces operating at the interface between the atmosphere and the surface is friction. The shape of the frontal surface is markedly altered by friction, retarding the lower portion of the cold front and hence increasing its slope. Figure 3A(c) indicates the average slope of a steep cold-front surface drawn to scale. This fact changes the time scale by an order of magnitude when considering the sequence of events attending warm and cold fronts.

There are two principal types of cold fronts. Figure 3A(a) illustrates conditions when a slow-moving cold front with wind speeds decreasing with height moves into relatively stable air. Stratified clouds and weather similar to that produced in warm-frontal zones results. Figure 3A(b) shows a fast-moving cold front (wind speeds increasing with height) moving into unstable air. This type causes the most rapid change in meteorological variables. Events take place much more rapidly during the cold-front passage and spatial and time gradients are much larger than is the case with warm fronts.

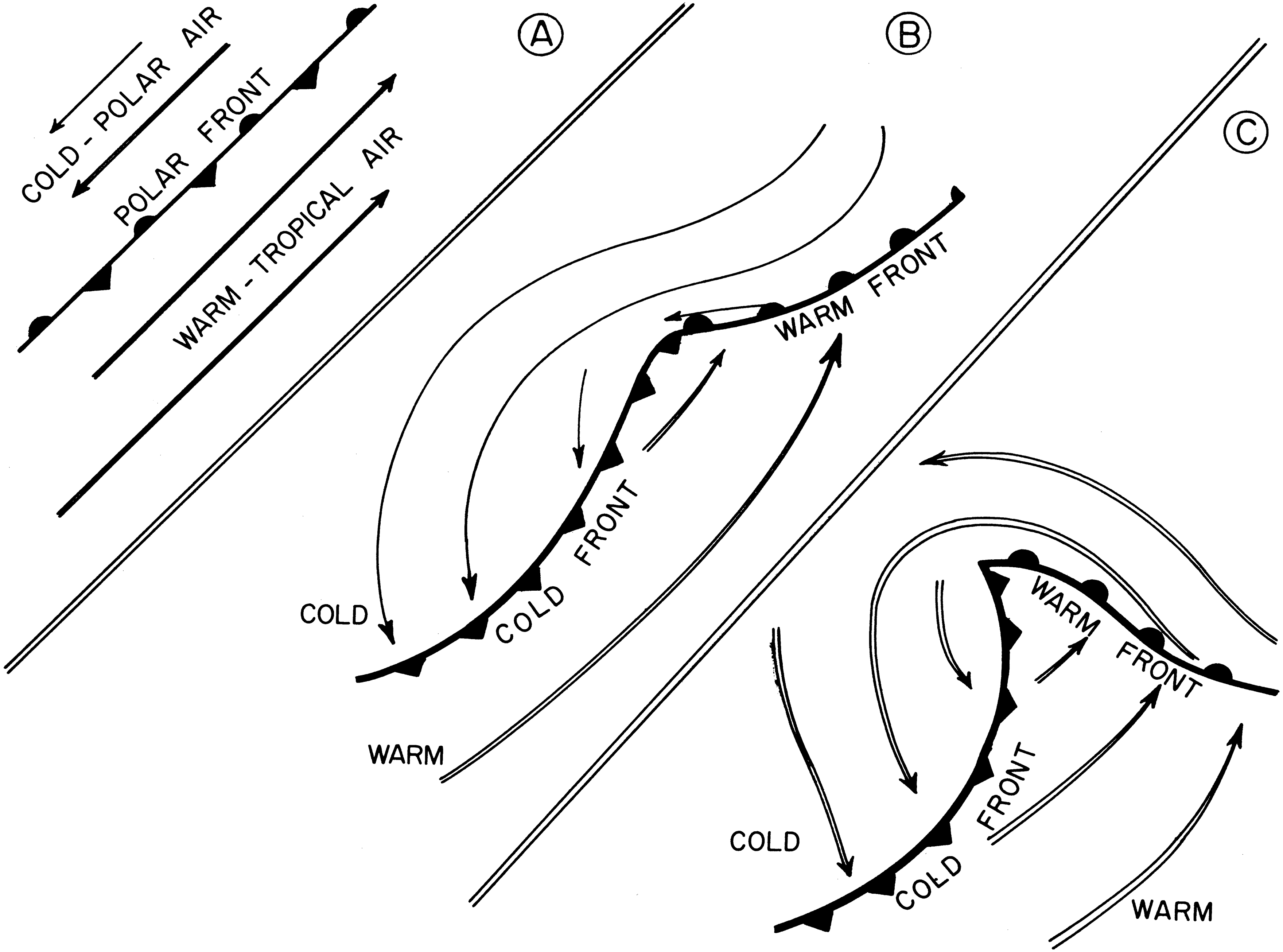

Fig. 1A. Initiation of cyclone and cold front.

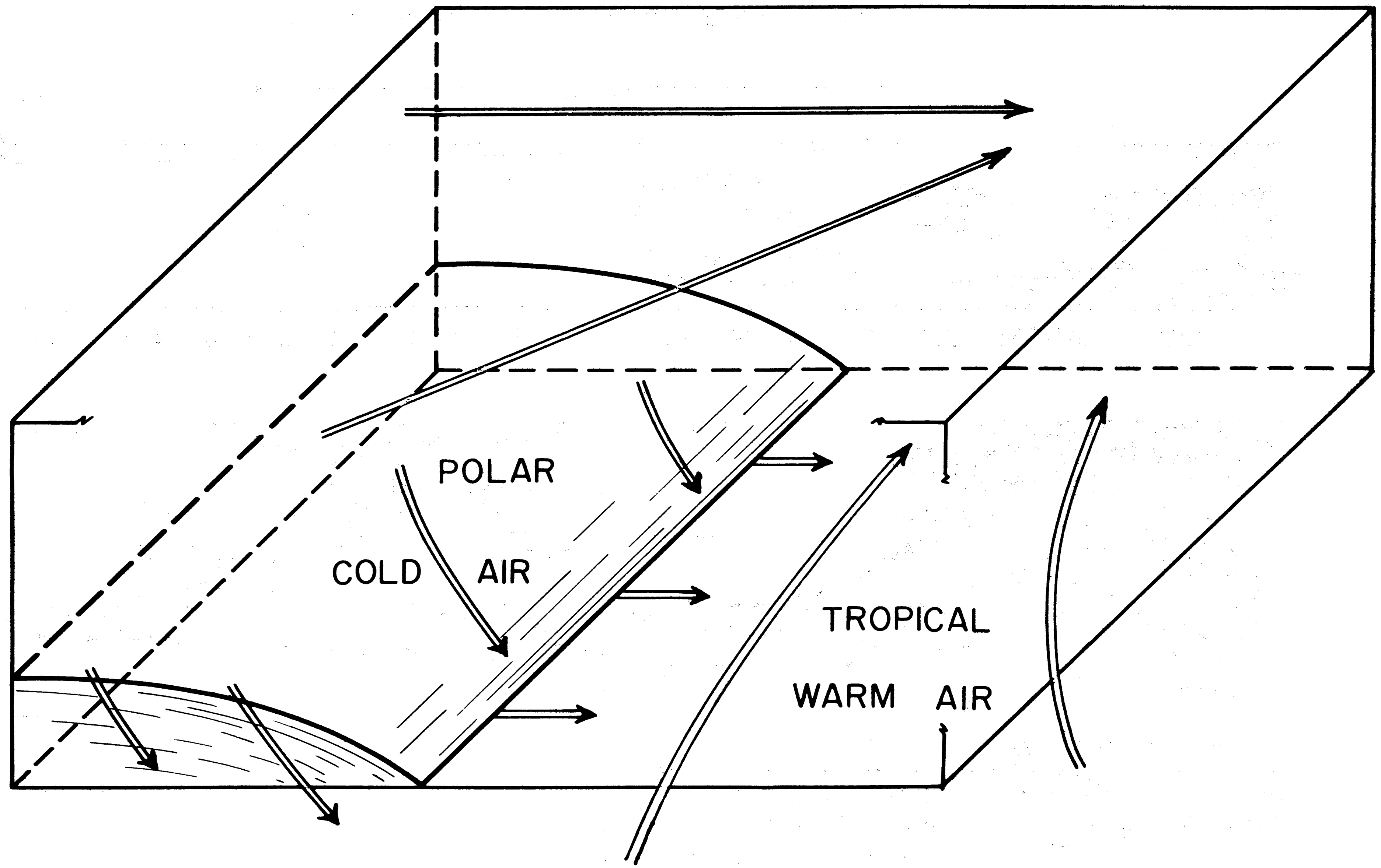

Fig. 2A. Cold-front surface moving into warm air mass.

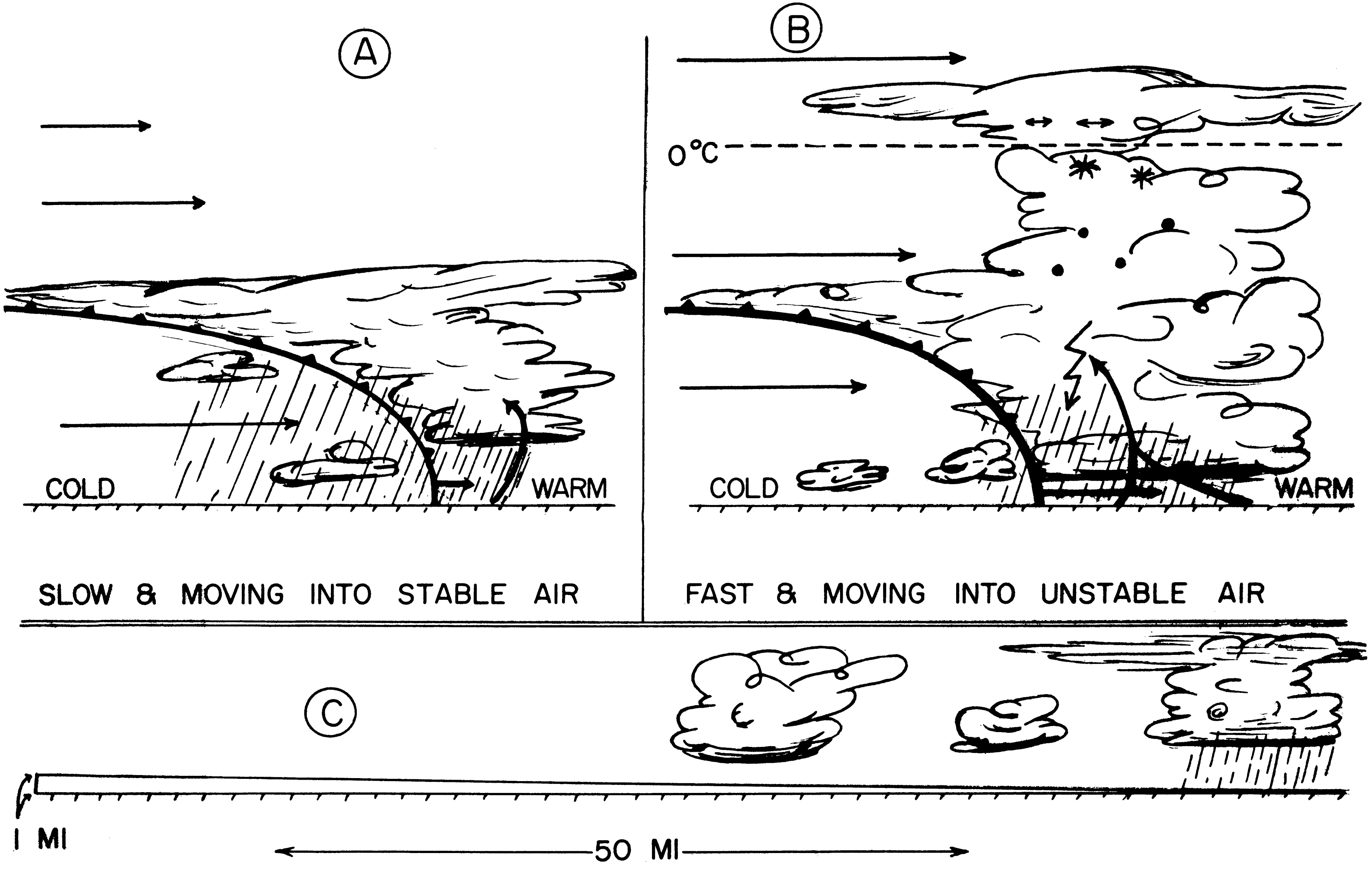

Fig. 3A. Schematic and true cold frontal slopes.

The effect of friction also can produce distortions in the frontal surface (Fig. 4A) resulting in a much greater inflow and consequent upward motion than would otherwise take place in the classical model. A summary of events typically attending cold fronts follows.

Temperature.—In the homogeneous warm air preceding the cold front there is not much temperature change. If any, there is a slight fall during daylight hours just in advance of the front by reduction of insolation from overhanging middle and high clouds. At the front there is a sudden fall in temperature; the gradient increases by an order of magnitude or more (Fig. 5A) and gradually decreases within two to six hours after frontal passage. The autographic trace of temperature during a typical cold-front passage is shown in Fig. 6A.

Pressure.—The barometer falls as the cold front approaches, but usually slowly, since the rapid pressure drop has already taken place in advance of the cyclone center and warm front passage. There is a complete and sudden reversal of the barometric tendency at the front, with brisk pressure rises during the first 6 to 18 hours in the cold air.

The magnitude of the pressure change across a cold front is from 10 to 50 mb per day. The greatest rate of change is within the first 4 hours after frontal passage when barometric rises of 1 to 5 mb per hour can occur (see Fig. 6A). Small-scale, rapid fluctuation of pressure is noticeable also during frontal passages. Gustiness of the wind is caused by small-scale pressure variations of the order of 1 mb per second.

Wind Vector.—Although the speed does change, wind velocity exhibits the most marked change in its direction at frontal passage. There may be slight backing as the front approaches, but as the leading edge of the cold air arrives, the direction veers abruptly often by more than 90° of arc (see Fig. 6A). This event indicates that a discontinuity in the trajectory of the air has taken place; that is, air arriving at station after the cold front passage has a completely difference history from that of air in the trailing portion of the preceding anticyclone.

Wind speed exhibits an increase in both magnitude and gustiness as the front approaches. The speed oscillates about a mean by a factor of two and with a period of one or two seconds; at the front it may vary by a factor of 4 or 5 with similar period as cold air pours outward from individual thunderstorm cells in the frontal zone.

Humidity.—The water vapor content of the air preceding a cold front in the eastern United States is usually high; hence the specific humidity is high. Following the front the specific humidity, being of high-latitude continental origin, drops by a factor of ten (see Fig. 6A). The relative humidity, being a function of air temperature, varies widely even in a homogeneous air mass and hence is not a conservative indicator of the moisture regime. Whether the specific humidity change across a cold frontal zone is positive or negative depends

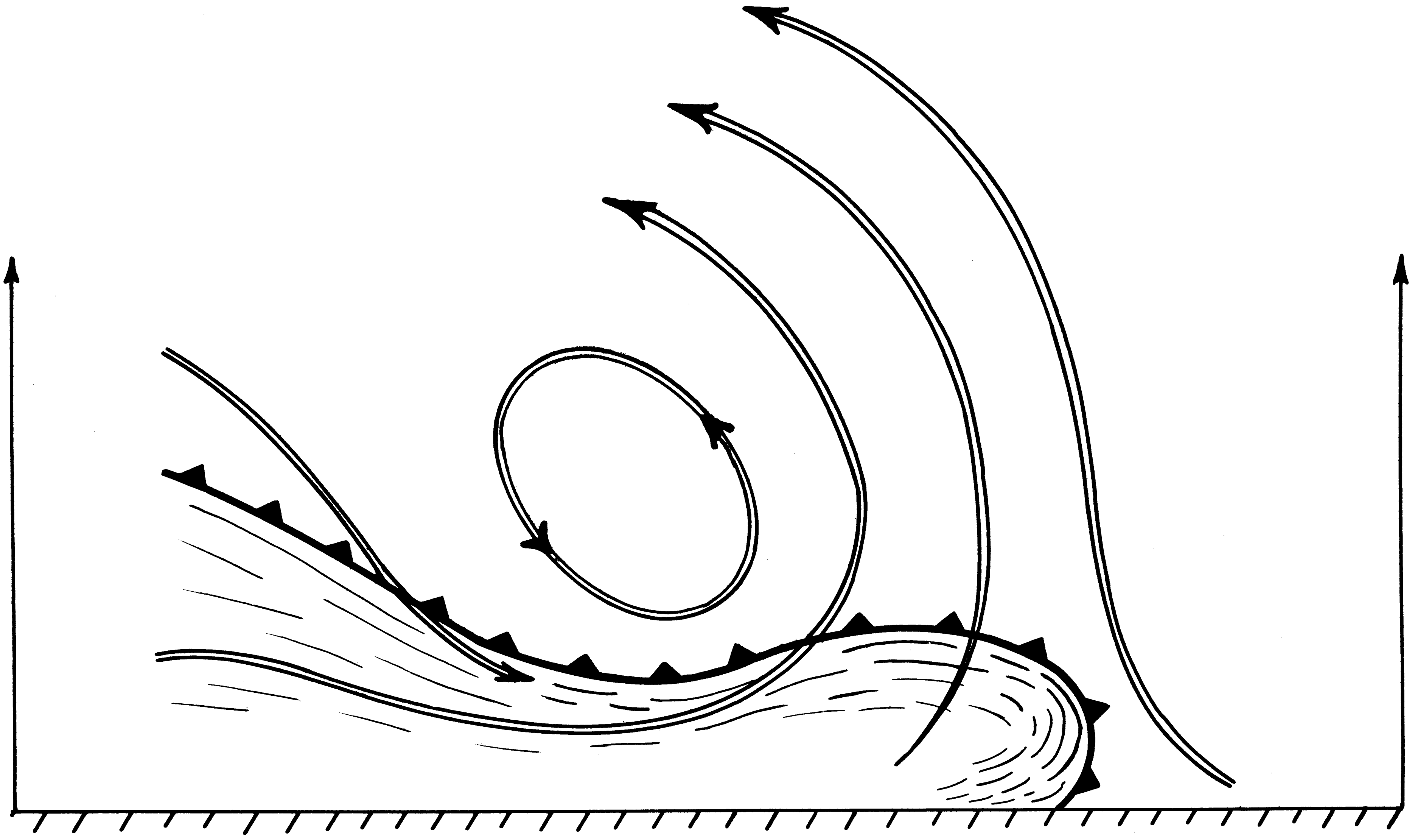

Fig. 4A. Deformation of frontal surface by "friction head." Double-shafted arrows are streamlines of vertical air motion (after Berson).

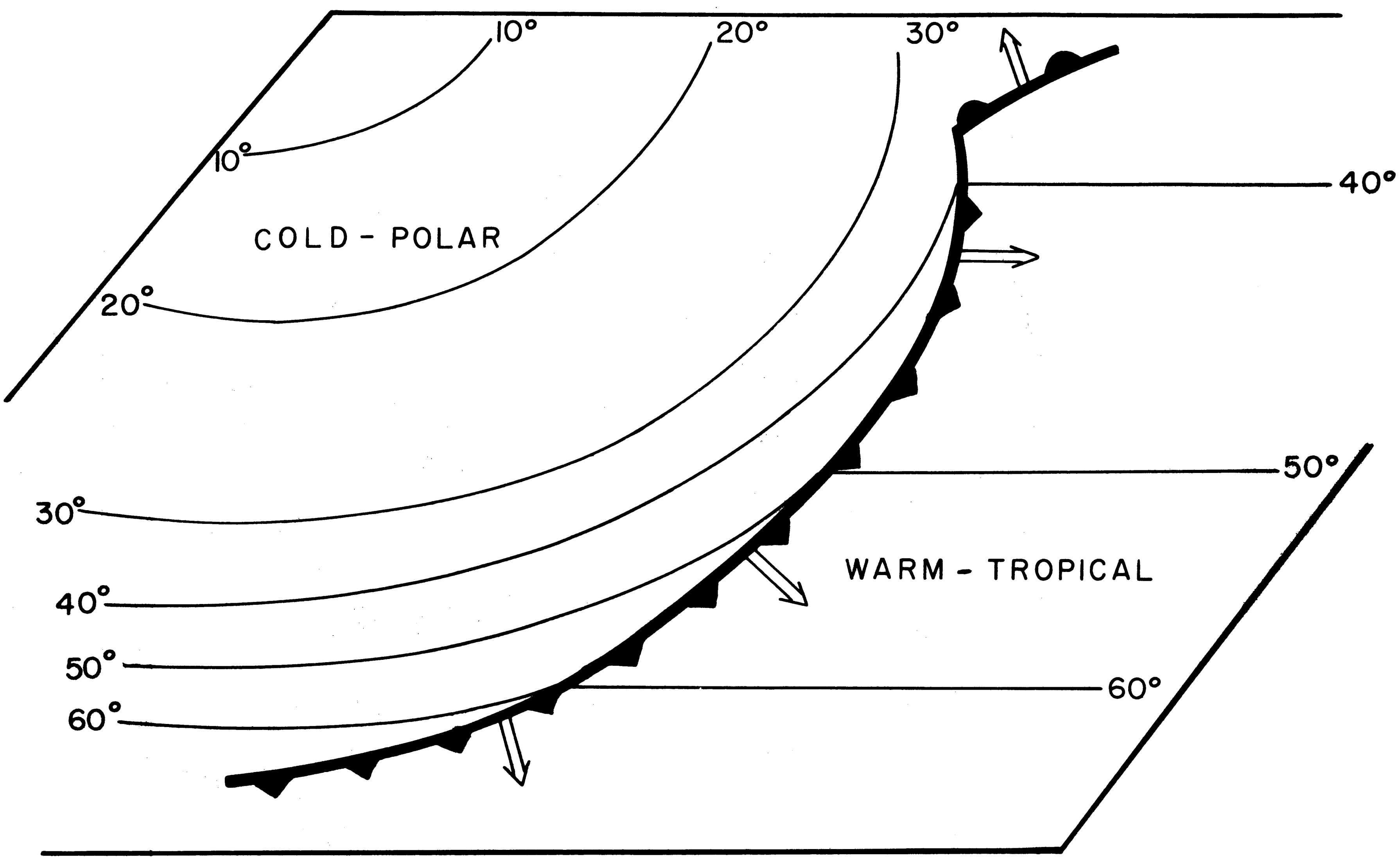

Fig. 5A. Temperature distribution at cold front.

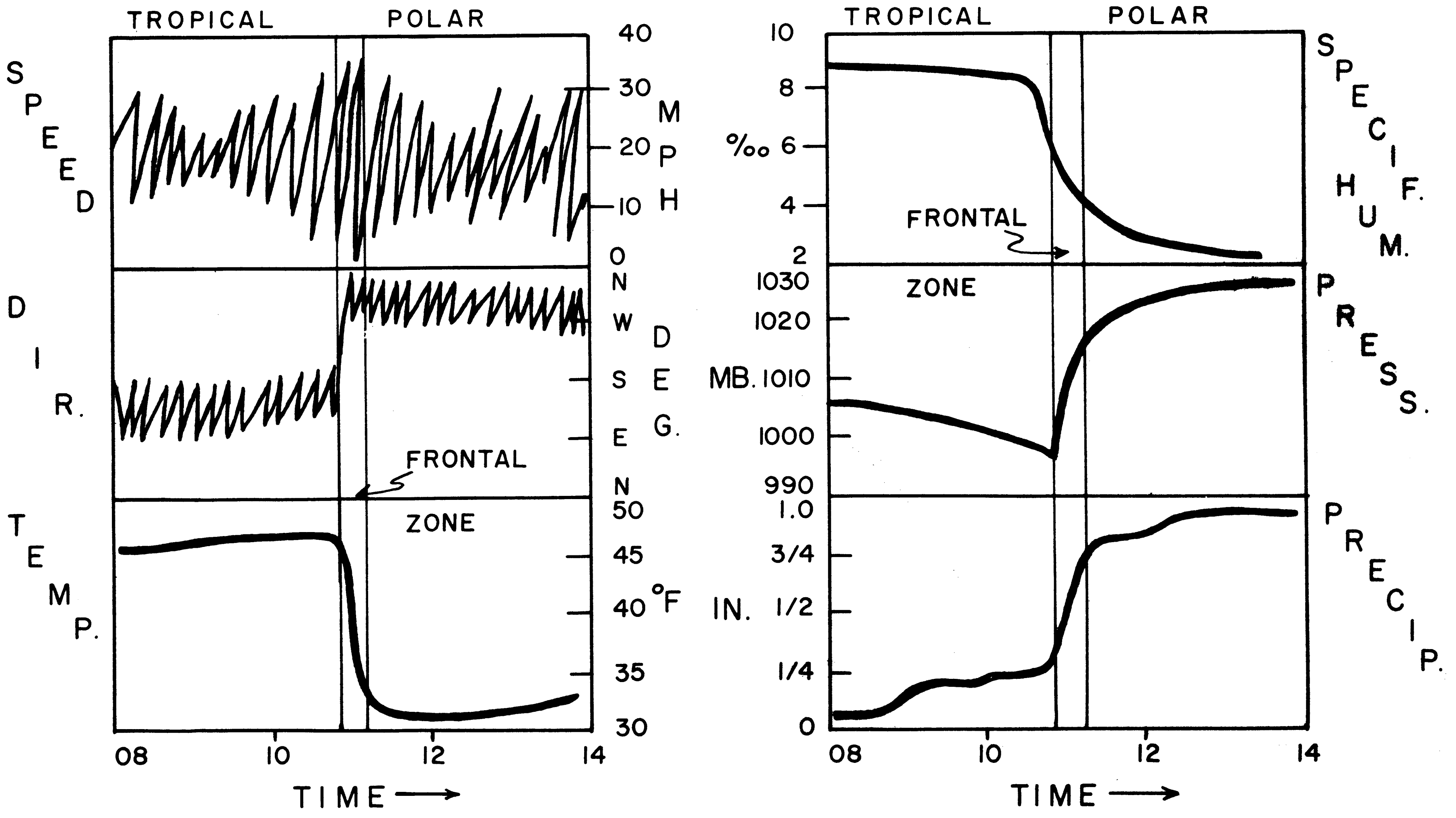

Fig. 6A. Autographic records of cold-front passage.

on the origin and trajectories of the two air masses involved. The change is not quite as abrupt as a kinematic property, say wind direction, but is gradual because of the turbulent mixing brought on by the thermal and pressure differences in the frontal zone.

In the large majority of cases, however, large-scale convergence of air into the frontal zone causes a net increase in moisture in the lowest atmospheric levels and also results in precipitation which, upon evaporation, adds moisture to the air. Therefore the specific humidity nearly always decreases after a cold frontal passage although at a greater rate in following continental air than in following maritime air.

Precipitation.—Precipitation, a result of convergence in the atmosphere, yields its greatest amount in the frontal zone (see Fig. 6A).

Turbidity.—The concentration of condensation nuclei, hydrometeors, lithometeors and other aerosols such as many bacteria and pollen determine the turbidity of the atmosphere. Atmospheric turbidity changes markedly with cold frontal passages. Air in advance of the front has been in contact with the surface of the earth for many hours. The air is relatively stable so that a great deal of particulate and gaseous matter is present in the lower layers. Water vapor, nuclei, and other aerosols are in the concentration of 10^4 per cc in rural areas. Urban concentration is another order of magnitude greater.

As air convergence proceeds in the frontal zone, water vapor is transported upward and condenses around nuclei forming clouds. All these composites make the turbidity the greatest at the frontal boundary. There is a virtual discontinuity in the turbidity at the front. The cold air is much clearer even though obscuring precipitation may still be falling into it. In residual convective showers, of course, scattering and absorption of light can reduce the visibility to near zero; nevertheless, in the same fresh polar air mass the nuclei and meteor count is two orders of magnitude less than in the preceding tropical air. Polar air is unstable and has a strong downward wind component when transported to low latitudes; hence what turbidity exists or is added is thoroughly mixed by turbulence in the fresh air.

Additional Changes in the Atmosphere Attending Cold Fronts

A great deal of research has been made on details of the cold frontal structure and on frontal-zone properties of the atmosphere that are omitted from daily observation. A few of the latter will be discussed first.

Ions.—One of the ion-producing mechanisms in the atmosphere results from the disintegration into radioactive gases of radioactive substances in the earth. These gases emanate into the atmosphere through soil pores and rock fissures and are therefore subject to dispersion and diffusion according to the existing energy state of the atmosphere. Rapid pressure fluctuations, such as those attending the passage of a cold front, cause the soil to "breathe," releasing these gases. Moreover, it has been established that the larger-scale pressure drops occurring with cyclone and frontal passages permit the escape of gases (e.g., methane into mineshafts) that are otherwise confined in the earth. From the foregoing, it may be seen that the greatest rate of ionization from this source accompanies frontal passages.

In the air near the ground, ionization is also produced by lightning and the fracture of raindrops and snow crystals. Since both of these events take place in frontal zones, the ionization caused by them is added to that produced by radioactive gas.

Another significant source of atmospheric ionization stems from the effects of cosmic rays. The ionization from this source, however, is only from 20-40% of the radioactively produced ions; moreover, no evidence is at hand that suggests preferential concentration of cosmic rays in the horizontal. Possibly more ions strike the surface layers of air in the center of cyclones than elsewhere due to the reduced air density there.

The production of ions is intimately related to the turbidity of the air. In unclean air such as precedes a cold front, large ions are produced on atmosphere pollutants. These ions have a life span of about 1 hour; in the clean air following the frontal passage the ions are smaller and have a mean life three orders of magnitude less. From the discussion on turbidity, this is additional evidence that fronts are regions of maximum ionization.

Atmospheric Electricity.—The potential gradient is also affected by turbidity and therefore reflects variation according to frontal passages. The magnitude of the potential gradient at a given station depends on the conductivity of the air near the ground. The greater the air pollution, the lower is the conductivity since large-ion mobility is low. In clean air, small ions predominate, and with their greater mobility produce a small potential gradient. Since the conductivity of the air depends on the rate of ionization, all factors that influence ionization will also influence the potential gradient.

Measurements of the potential gradient may be used to compute the excess in concentration of the number of ions of one sign over those of the opposite sign. Such space charges as these will be large wherever there are large aerosol accumulations.

The normal diurnal variations in the electric field can be disturbed by changes in the state of the atmosphere. Cold-front showers often increase the normal potential gradient more than two orders of magnitude. Moreover, rapid alternations of the direction of the gradient are observed in frontal zones, from +2000 to -2000 volts per meter. The local surface charge of the earth can reverse sign many times per minute during turbulent weather found in cold frontal zones. The deformation in a cold frontal surface caused by attending thunderstorms is illustrated in Fig. 7A.

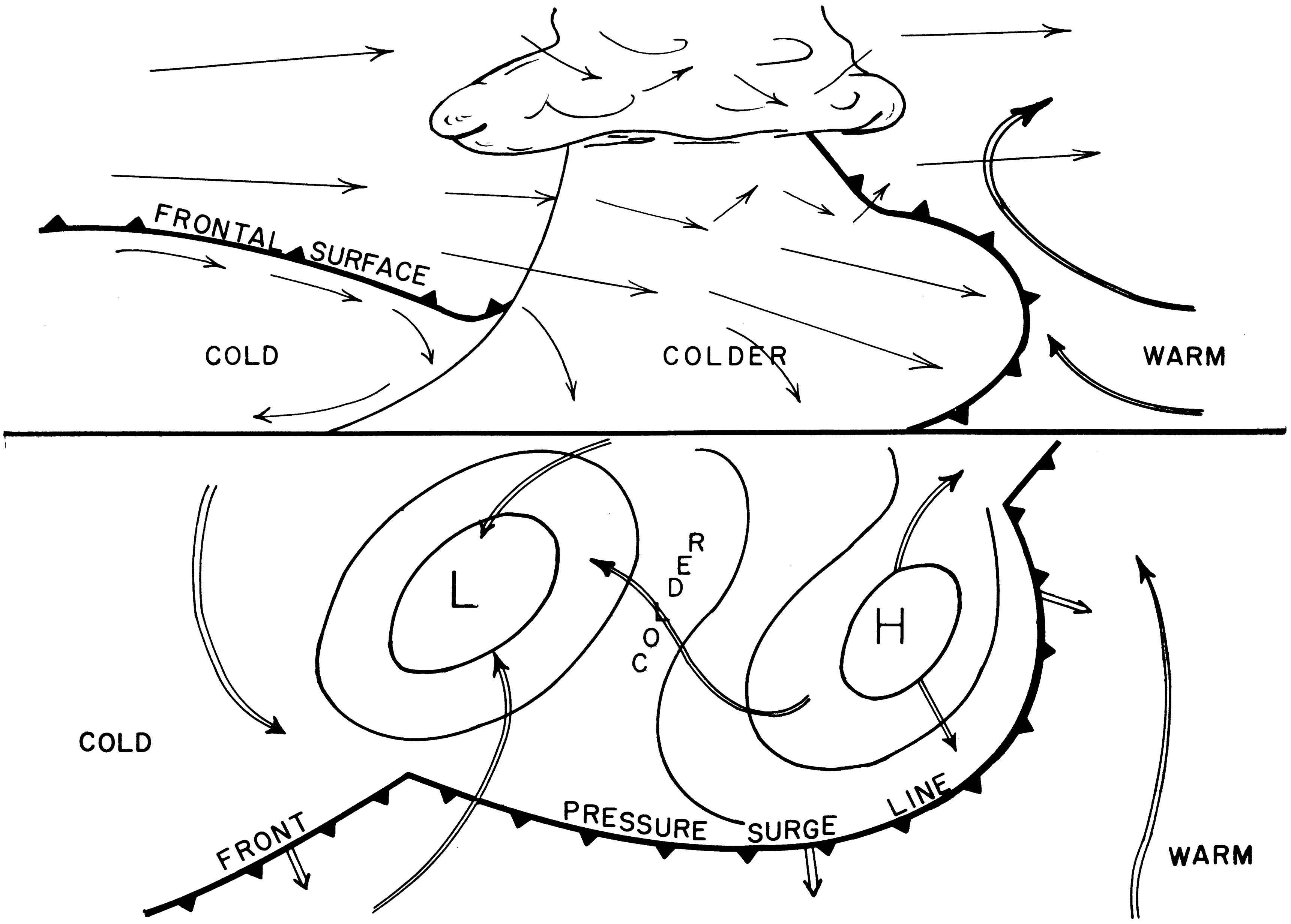

Fig. 7A. Meso-scale analysis of a cold-front thunderstorm.

Triboelectricity (friction-produced space charges) also occurs in precipitation. Since the most intense precipitation occurs in the violent wind currents of the frontal zone, the electric field can be greatly altered by this process.

The most spectacular disturbance in the electric field is, of course, the occurrence of lightning. It is well established that the primary source of thunderstorm energy is the convective activity initiated by thermal and dynamic instability. This activity leads to charge separation amounting to more than a third of a million volts per meter. Although thunderstorms do occur in homogeneous air, the greatest disturbance to the electric field is made during the event of the squall line, where scores of thunderstorms are aligned in advance of and parallel to a cold front for several hundred miles.

Ozone.—Although by far the greatest amount of ozone is produced between 15- and 30-km elevation, a small amount is present at the earth's surface. An additional amount is produced by the action of lightning discharges. The greatest amount at the surface occurs after vigorous cold frontal passages in the forward portion of deep anticyclones (see Fig. 8A). It is postulated that the strong earthward advection of air from the stratosphere which occurs in such anticyclones includes a much higher concentration of ozone than is usually found at low altitudes. The distribution of this property across frontal boundaries is therefore reversed from that of, say, water vapor. It is least before the front (0 to 0.02 ppm), increases during passage, and reaches a maximum several hours behind the front (0.06 ppm).

Rapid Fluctuations of Weather Associated with Pressure Jumps and Föhns.—If a cold front suddenly accelerates into an air mass that already has a low tropospheric inversion in it, the resulting surge of energy is confined beneath the inversion and produces a compression wave. The motion of the wave is propagated at a speed greater than the wind speed and its slope is thereby steepened. When the slope becomes infinite, the condition is attained where a low inversion height in still air is transformed almost instantaneously into a higher inversion of moving air. This event causes a complete discontinuity in the pressure tendency whereby the barograph records a jump from the lower pressure to the higher. Hence the name for the phenomenon, "pressure jump" (see Fig. 9A).

More often than not, violent weather attends the pressure jump. Although it is a cold-front initiated phenomenon, all subsequent events take place in the pre-cold frontal air. The pressure rises 2-6 mb within 5 min; gradients are as steep as 1-2 mb per km corresponding to geostrophic wind speeds of 2500 mph. The latter speeds are never even approached (50 mph being the usual peak gust), but ageostrophic flow does occur with winds often blowing 90° to the isobars in response to the tremendous pressure gradient generated. The wind direction exhibits a near-instantaneous discontinuity veering through 90 to 180° of arc. There is a sudden burst or gush of precipitation lasting for only 10 min and the temperature falls suddenly at the rate of nearly 2°F per min returning slowly to its original level after passage of the jump but before passage of the cold front. This entire meso-scale meteorological event moves over the surface at 45-55 mph.

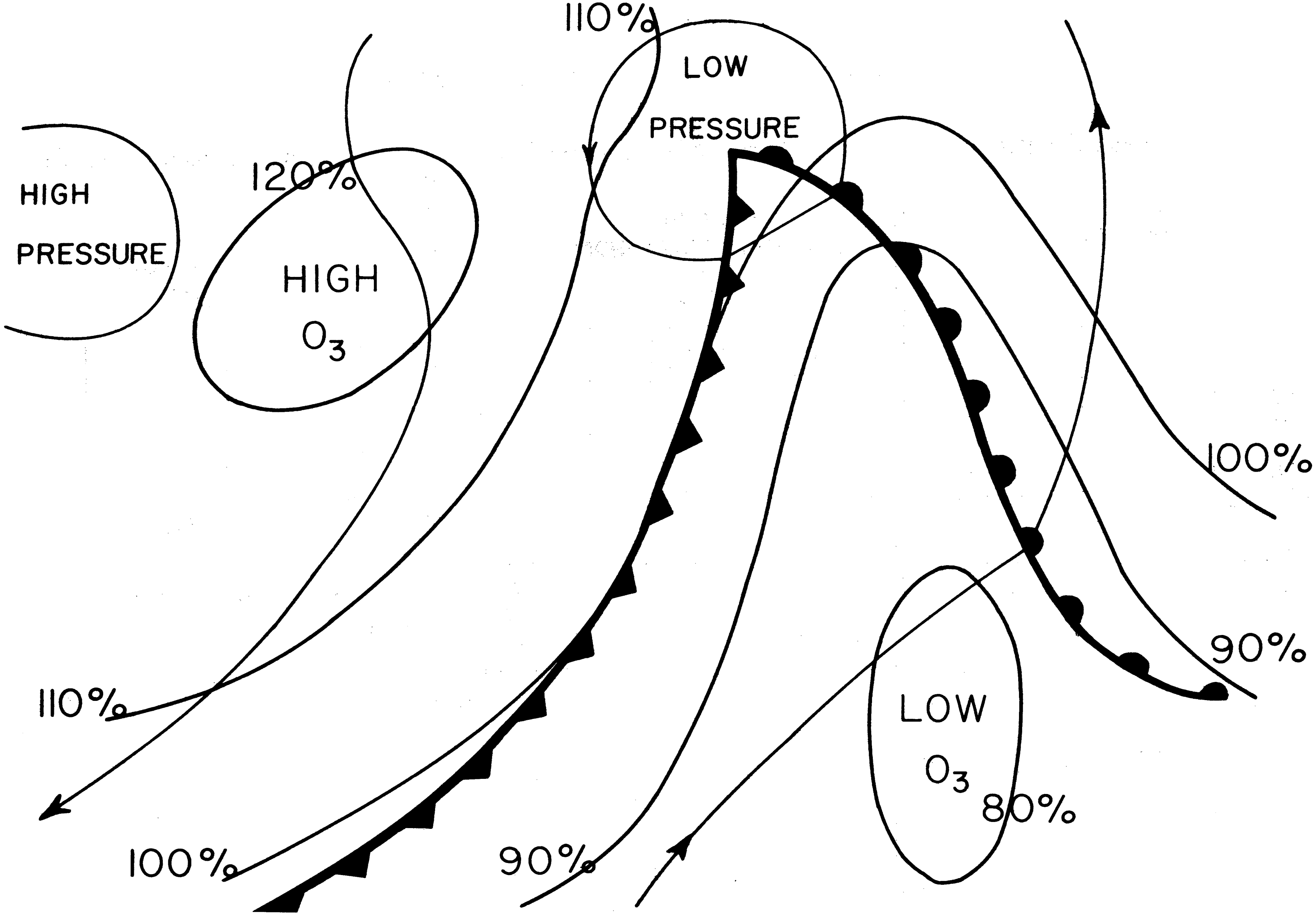

Fig. 8A. Surface distribution of ozone around a cyclone. Arrows indicate typical pressure distribution. Percentages are of normal surface concentrations.

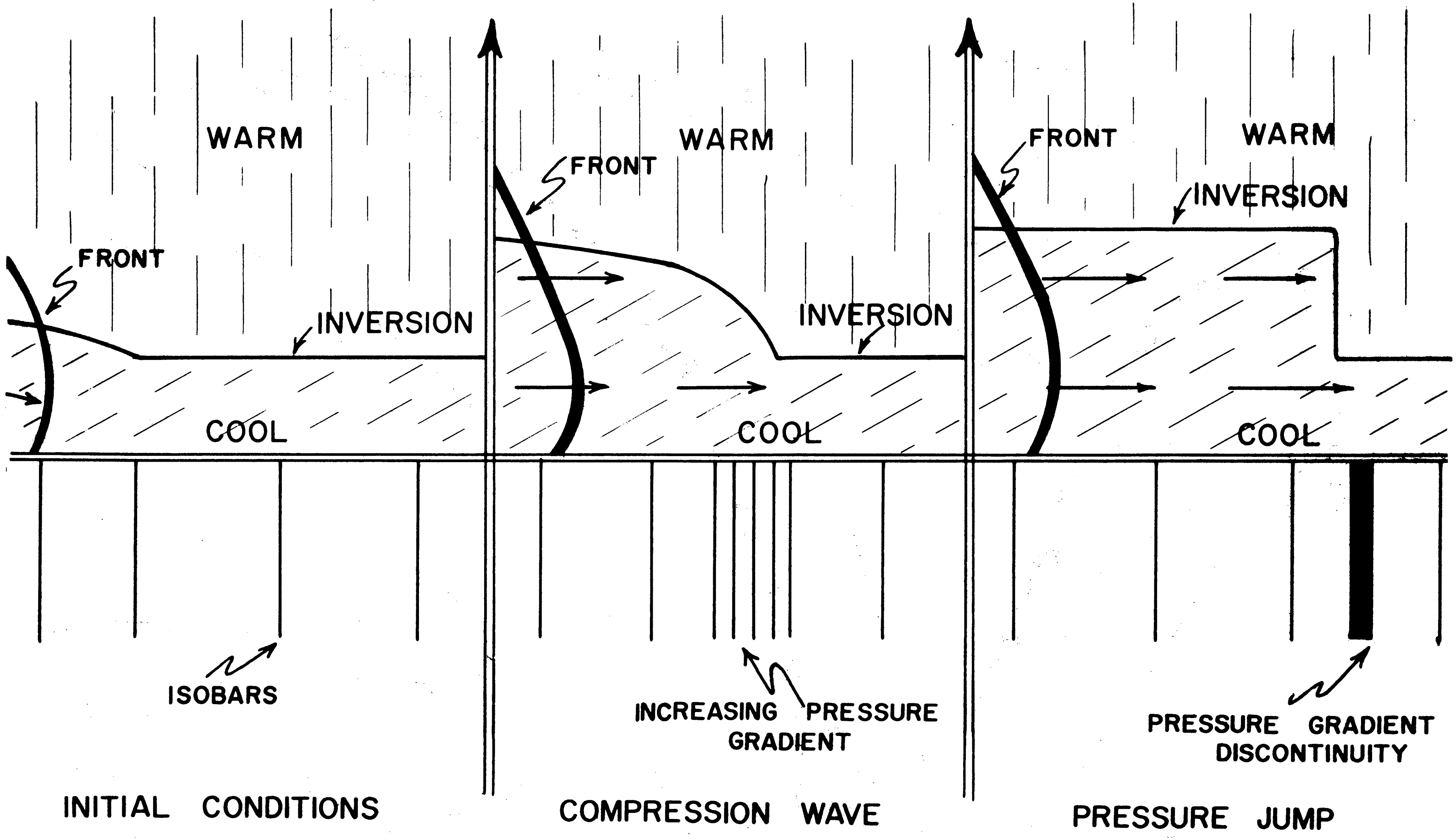

Fig. 9A. Development of a pressure jump.

A mechanism similar to the pressure jump is often associated with the Föhn wind. Although there are large-scale variations produced by the phenomenon, there are also short-period oscillations. For example, variations in pressure of 5 mb in 10 sec have been observed in conjunction with the incidence of "Föhn disease." It has not been proved, however, that the pressure oscillations induce the disease; in fact, the symptoms appear in the absence of the pressure variations. Conversely, greater pressure fluctuations than the Föhn-induced ones, such as occur in elevator and airplane ascents, produce no biological effects of significance.

Fluctuation of Meteorological Variables by Small-Scale Turbulence.—The vertical gradient of temperature is subject to very large variations according to time of day, height above the surface, composition of the surface and the degree of insolation. In warm weather such as precedes cold fronts, the temperature varies by several °C and at such a rapid rate that it is difficult if not impossible for the body to detect it.

Although the mean wind speed near the surface has been studied in detail, the eddy velocities are less well known. The mean wind speed change in the vertical follows a logarithmic power law in the first few meters provided the temperature lapse rate is nearly adiabatic. Turbulent eddies, on the other hand, are made up of fluctuations of widely different periods. Little is known of their magnitude although it has been found that the variations of eddies with height show increasing vertical and lateral components up to 5 meters. It is also known that the amplitude of oscillations decreases as the temperature lapse rate decreases, being least under stable atmospheric conditions. These eddies take place during a period of 1 or 2 sec. Because of the energies involved at the frontal boundary, it is found that a great degree of turbulence on the small scale is found in frontal zones. The eddies are not likely to decrease after cold frontal passage, however, since lapse rates in the polar air approach the adiabatic thereby enhancing mixing and the propagation of turbulence.

The small-scale variations of moisture and pressure are being studied by several investigators, but results are inconclusive at present.

References

Two sets of references are appended. The first contains works on medical meteorology only, not including physiological climatology; and of the large number of medical meteorological works, only references are shown for work done specifically in connection with allergic and/or asthmatic response. The second set represents a sample of the kind of work that is being done in medical meteorology that may well have a direct bearing on allergic response of individuals. Many more works are available in annotated form in the publication Meteorological Abstracts and Bibliography, American Meteorological Society, Boston. A selective annotated bibliography of 148 works on medical meteorology during the last ten years is included in the issue of volume 8, number 11, November, 1957.

References on Medical Meteorology Relevant to Allergies and Asthma

1. Becker, F., 1952: Weather processes and clinical disturbances. Deutscher Wetterdienst in der US-Zone, Berichte, No. 42: 414-418. A refined analysis is made of meteorological phenomena such as fronts for correlation with manifestations of circulatory pathology such as asthma. Asthmatic patients react almost to the same degree to cold fronts, wind turbulence, etc., as do cardiac patients. (In German)

2. Berg, H., 1950: Causation of diseases by meteorological and solar influences. Naturwissenschaftliche Rundschau 3(4): 161-164. The role of cold fronts in the production of asthma in guinea pigs is discussed together with other effects of meteorological and solar phenomena. A mechanism of causation is proposed. (In German)

3. Berg, H., 1953: Statistical results in medical meteorology. Annalen der Meteorologie, Medizin-Meteorologische Hefte, No. 8: 43-51. Since bioclimatic material is often statistically inexact, valid relationships are presented between the events of diseases such as asthma, and fronts. (In German)

4. Dingle, A. N., 1955: Meteorologic approach to the hay fever problem. Journal of Allergy 26(4): 297-304. Atmospheric concentration of pollen depends on the whole complex of "weather." Fronts, wind, and turbulence increase concentration; precipitation decreases it.

5. Dingle, A. N., 1957: Hay fever pollen counts and some weather effects. Bulletin American Meteorological Society 38(8): 465-469. Pollen sampling methods are reviewed in the light of accompanying meteorological events. Gravity slides represent concentrations poorly; the only acceptable standard is the volumetric method.

6. Dingle, A. N., 1957: Meteorological considerations in ragweed hay fever research. Federation of American Societies for Experimental Biology, Federation Proceedings 16(2): 615-627. Relationships of weather to pollen production are discussed. Theory and results of air transport of pollen, measurement of pollen concentration and allergic response of patients are presented.

7. Goodwin, J. E., J. A. McLean, F. M. Hemphill, and J. M. Sheldon, 1957; Air pollution by ragweed: medical aspects. Ibid, p. 628-631. Tests on hay fever and asthmatic patients are reported. Pollen counts followed rather than preceded daily increase in subjective symptoms of hay fever and asthma.

8. Kérdö, I., 1952: Correlation between attacks of asthma and air-mass types. Idöjárás, Budapest, 56(11/12): 338-346. Nearly 500 asthmatic attacks in one patient during six months are correlated with accompanying air masses.

Although cold air masses appeared to promote susceptibility to attacks, the correlation was not statistically significant at a sufficient level. (In Hungarian. Russian and German summaries)

9. Kornblueh, I. H., and J. E. Griffin, 1956: Artificial ionization of the air in physical medicine: preliminary report. Wetter und Leben, Vienna 8(3-4): 57-64. Natural and artificial air ionization and devices available are described. Experiments on 27 asthmatics breathing negatively ionized air showed allergic conditions respond favorably. (In German)

10. Markow, H., and J. Reicher, 1953: The effect of meteorologic conditions on asthmatic patients. New York State Journal of Medicine, 53(22): 2675-2681. Reactions of 90 perennially asthmatic patients were investigated in relation to several meteorological variables. No definite correlation was found between asthmatic symptoms and meteorological factors; however, a trend is suggested in relation to temperature, humidity and precipitation.

11. Myers, W. A., and S. Price, 1954: Kona weather and the incidence of asthma in children in Honolulu. Hawaii Medical Journal, 13: 181-188. No definite relationship is found between the occurrence of Kona weather and asthma attacks in 1259 asthmatic patients.

12. Ordman, D., 1955: Graphical method of comparing climates in the study of perrennial respiratory allergy. South African Journal of Science, 51(6): 187-189. The fact that bronchial asthma and allergic sinusitis and rhinitis is considerably higher in coastal than inland areas of southern Africa is known. It is proposed that the reason lies in the combined effect of high temperatures and high relative humidities.

13. Sargent, F., 1940: Studies on the meteorology of respiratory infections. Bulletin American Meteorological Society 21: 175-178.

14. Ungeheuer, H., 1954: Man and weather. The present position of medical meteorological investigation. Umschau, 54(9): 257-260. Many diseases definitely influenced by weather processes; medical effects of pressure variations and frontal passages described; pathogenic forecasting procedure outlined. (In German)

15. Verzar, F., F. Hügin, and W. Massion, 1954: Retention of atmospheric condensation nuclei in the respiratory tract. Pflügers Archiv für die Gesamte Physiologie, Berlin. Respiratory research described using an automatic condensation-nuclei counter. Size and number of nuclei considered of secondary interest; primary importance is that airborne toxic substances condense on the nuclei. From 60 to 90% of inhaled nuclei are retained. (In German)

Other Exemplary Works on Medical Meteorology
Possibly Relevant to Allergic Response of Individuals

16. Dordick, I., 1958: The influence of variations in atmospheric pressure upon human beings. Weather, 13(11): 359-364. Long- and short-period oscillations in pressure are found to give no conclusive biological reaction.

17. Herrington, L. P., 1954: Biophysical adaptations of man under climatic stress. Meteorological Monographs 2(8): 30-41. Reactions of man to varying climatic regimes.

18. Mugge, R., 1956: Pressure variation and weather: the two weather types. Umschau, 56(21): 656-659. The structure of high-pressure waves is discussed together with the effect of rapid pressure changes on men's nervous systems. (In German)

19. Kanz, E., 1951: Biological methods of measurement in meteorobiology. Annalen der Meteorologie. Medezin-Meteorologische Hefte No. 6: 34-37. Storms and ozone concentration, the effects on patients' senses. (In German)

20. Kérdö, I., 1949: Recent investigations on the effect of the passage of fronts on the occurrence of death. Wetter und Leben, 2(1-2): 16-23. Atmospheric electric charges may influence the nervous system and hence cause death. (In German)

21. Lossnitzer, H., 1951: The importance of the terrestrial electromagnetic field in bioclimatology. Archiv für Meteorologie, Geophysik und Bioklimatologie Ser. B, 3: 297-307. Influences on blood clotting are reviewed; effect of soil humidity on earth current; influence of convection on ionic transport in the atmosphere. (In German; English summary)

22. Reiter, R., 1953: Recent investigations in the problem of the dependence of man upon weather. Archiv für Meteorologie, Geophysik und Bioklimatologie Ser. B, 4(3): 327-377. Behavior of atmospheric electricity during different aerosol conditions in the presence of moving cold air at the surface. (In German; English summary)

23. Schilling, G. F., and R. E. Holzer, 1954: Possible relationships between bioclimatology and atmospheric electricity. Wetter und Leben, 6(1-2): 13-16. Relations between physiological reaction and cosmic and local radiation, nuclei content of atmosphere and ion concentration are discussed. (In German)

In the discussion which followed, it was brought out that the "Föhn" in the Alps was similar to the "Chinook" in Alberta, Canada. The illnesses ascribed to the Föhn, described by Dr. Barlow and Dr. McLean, consisted of ill-defined symptoms and changes in mood and character, and appear to be attributable to a passage of a warm air front down the Alps into the foothill area. It was thought by the group that, in addition to the aeroallergens, other biochemical factors may be operating with weather changes. A study has been done showing that absenteeism among employees with colds in a large firm correlated fairly well with the passage of a cold front when the absenteeism would be increased five to six times. It was thought by the group that evaluation of this problem would open up a new area for research. It was one of Dr. Sheldon's main objectives, originally, in proposing this study to determine why stormy weather would cause an increase in asthmatic symptoms. The work of Dr. Tromp of Holland, who reported at the London meeting that he could correlate some asthmatic episodes in children with passage of a cold front, has stimulated further interest in this problem.

MEDICAL PHASE

Dr. Richard Field then delivered a talk which is reproduced below.

Weather and Asthma

Does the weather affect bronchial asthma? This question among the medical profession is as old as the teachings of Hippocrates. It still remains essentially unanswered, despite numerous efforts over the years to solve the riddle. Almost every doctor has had experience with patients with asthma or rheumatoid arthritis who can sense the approach of damp or inclement weather by changes in symptoms, while their more normal companions are unaware of such changes. In view of our most recent studies in this direction, especially as regards the human chamber experiments, it is felt pertinent to review the state of our knowledge to the present on possible direct and indirect effects of weather conditions on the onset, course, and treatment of bronchial asthma.

Numerous problems immediately make themselves apparent. The bulk of thinking and writing on the subject was folk lore in nature. It has been in much confusion until relatively recently, especially since writers propounded favorite theories of the benefits of high altitudes, low humidities, etc. Most of the studies that were done were poorly organized or merely added confusion. "Asthma" and "weather," by their very nature, are not single entities easily studied, but are complex phenomena to which deceptively simple names have been given. Probably, herein lie the most fundamental and irresolvable difficulties. Certainly subjectivity enters into the diagnosis and measurement of these things, especially allergy, and may, with the other difficulties, produce data whose inadequacy threatens to obscure or distort whatever correlations exist.[1] Much of the literature, both past and present, is in foreign journals, especially German and Spanish, further complicating a comprehensive review of this subject.

In proposing a study of weather and asthma, one must first carefully decide what and how he intends to investigate relative to the problem. For example, he must separate effects on the onset of an asthmatic attack and effects which tend to alter the course of asthma already begun. More specific effects of meteorologic variants on extrinsic factors like pollens, fungi, and dust are being elucidated, as our work here, for example, points up. But are there nonspecific effects on the organism as a whole, or on particular organ-systems, for instance, the respiratory tract? What factors can "weather" be broken down into and how do they interrelate? Should we study "weather complexes" or types, rather than single variables: These are questions which in the past have been approached from various points of view, both in the clinic and in the laboratory.

A review of some of the thoughts and objective results of investigators will form a background against which we can make an intelligent approach to the subject.

It was noted long ago in Europe that the "Föhn" or front passage (with decreased atmospheric pressure or a south wind) was associated with various ill effects, for example, an increased incidence of hemoptysis, etc. Various spas and health resorts were established in areas where altitude was increased or humidity decreased, or temperature warm, etc. Various beneficial effects were described where "ozone" was plentiful or where negatively charged air-ion particles were thought to be in abundance.

In 1929, Storm Van Leeuwen[2] in Holland described "climate asthma" and noted that 80% of his asthmatic patients were helped in a specially constructed sealed "Allergen proof chamber" where he could control air intake, temperature, and other factors. He attributed improvement to avoidance of "climatic allergens" and, of course, he did control pollen and dust concentrations in the chamber. He noted improvement was marked in patients from the low, moist places in Holland (but, interestingly enough, didn't mention mold spores as aeroallergens—probably an important factor there). No mention was made of other specific meteorologic factors and their effects.

Wolfer,[3] in 1934, noted improvement in asthmatics at Davos (mountain country) with improved pulmonary ventilation (better diaphragmatic motion), increased expectoration of secretions, etc. He also mentioned "vegetative equilibrium," however.

Seasonal influences have been recognized in that "in spring and autumn the organism's resistance is lower and the sensitivity increased." Temperature may have a general influence upon races and nations, metabolic stress being greatest in the middle temperate latitude and declining toward the regions of extreme cold and heat.

Barometric changes are said to influence body physiology. The body must adjust to these changes to prevent extravasation into the tissues with a falling barometer, and vice versa. Allergic skin reactions may be stronger when the barometer is low than high, according to an article as late as 1956, by Klotz and Bernstein.[4]

Conflicting results have been obtained while studying anaphylaxis and asthma in guinea pigs. Preuner,[5] in Germany, found that about 46% of his pigs were "weather sensitive," that is, asthma or anaphylaxis could be more easily induced on "disturbed weather days." Almost all who reacted to a cold front also reacted to a warm front. During rapid meteorological changes, severity of the reactions increased by 50%.

Microscopic observations of blood capillaries show that the vascular system undergoes abnormally exaggerated reactions (spasms, paralysis, etc.) following thunderstorms or sudden changes in weather. Mucus membranes and ciliary action of the respiratory tract may be altered. Reflex vasoconstriction and ischemia of the nasal mucosa occur when the body surface is chilled. Damp air and fog are said to exert their detrimental effect because of increased resistance to respiration that the moisture imparts, and, secondarily because of the increase in suspended solid particles.

Asthmatics sometimes feel better in warm, dry climates, particularly those with excessive productive sputum (infection). On the other hand, those with scanty viscid secretions may be worse under these conditions, and improve in moisture sea-shore areas.

High altitudes may be directly beneficial, with decreased barometric pressure and lower humidity, and resultant better ventilation, and may be indirectly helpful due to decrease in pollen, fungi, and bacteria, of course. This latter point is important, obviously, in evaluation of possible good effects in different environments, wherein changes in aeroallergens, both outdoors and indoors, food habits, job and family responsibilities, stress, etc., are of significance.

The type and quantity of air contaminants vary due to geographic and climatic conditions. If air at a high level is cooler than at ground levels, there will be an upward dispersal of air contaminants. High-pressure fronts apparently characteristically move air in a downward direction, tending to keep locally produced contaminants closer to nose level.

Air ionization, particularly of predominately negative polarity, may be significant. (The outdoor air normally has slightly more positive than negative ions.) Negative ionization of pollens, etc., may diminish their allergenic effect by changing their electrical potential, thus rendering them temporarily inactive. On mountain tops, negative ions are said to predominate; with the approach of a thunderstorm, the number of positive air ions is said to increase. Ion generators for room use to develop a supposedly highly negatively charged atmosphere are now available, and clinical studies[6] are in progress to see whether patients with asthma and hay fever will respond favorably to such physical alterations of the environment. Accurate studies in this field have been delayed because satisfactory ion-density meters are not available or are in their infancy. This particular point has made previous clinical and animal studies open to great question. (This includes our earlier ion studies on allergic individuals and has complicated our guinea pig anaphylaxis experiments in the animal chamber.)

Some references are cited from a review of the literature by Rosen[7] in 1950: Yogi,[8] from Formosa, noted increased incidence of asthmatic attacks in the wet season (attributed to increased relative humidity). Young,[9] in Hawaii, reported more patients with infectious asthma had trouble in moist weather; more with "allergic" asthma had trouble in dry and hot weather (? when pollens, etc., were about). Solly[10] found decidedly increased or decreased barometer pressure was detrimental. Parlato[11] thought insufficient air moisture in the home during the winter months was a causation of prolonged attacks. Leopold[12] observed two patients in a dust-free, air-conditioned room. He thought symptoms which were increased by atmospheric conditions preceding a storm or sudden drop in temperature were due to increased wind velocity with increased airborne substances. He did not feel barometric pressure changes and relative humidity had much clinical effect. Petersen and Vaughn[13] reported 13 attacks with falling temperature, and 6 in rising temperatures. Mohr[14] studied 52 children on Fohr Island and noted that half of the attacks took place when there was change of prevailing wind from west to east and found no relation to barometric pressure or relative humidity.

Anglade[15] studied 80 asthmatic children in a preventorium in France and did not believe meteorological factors modified the intensity or frequency of attacks or the interval between attacks. Feige and Rosenbaum[16] in Tel Aviv, Palestine, agreed that there was no meteorological correlation.

The Courtrights,[17] using an inhalant antigen in laboratory animals could find no relation of their results to the weather conditions prevailing at the time (San Francisco). Proetz[18] showed some altered permeability of the mucus membrane (of the nose?) with dryness, possibly allowing a break to occur and antigens or foreign material to gain entrance and establish a foothold locally on cell surfaces. Also, ciliary action may be interfered with in such situations. Suggesting that the viability of certain bacteria is dependent upon relative humidity, Dunklin and Puck[19] found 50% relative humidity was the most lethal to a suspension of pneumococcus organisms, survival being increased on either side of the 50% figure.

Curry[20] mentions an unusual "ozone-like substance" in the air, which he calls "Aran" (O_4), various concentrations of which may "cause attacks" of asthma.

Henry Ogden,[21] in New Orleans, in 1950, stated that his asthmatics were prone to become worse during periods of weather change, especially with decreasing temperature. He felt the added burden of increased concentration of inhalant antigens locally, along with infection, was important.

Some other studies are of interest: Myers and Price,[1] in Hawaii, studied the incidence of onset of asthmatic attacks among 1259 cases from January, 1951, to February, 1953. Apparently there is a type of weather called "Kona Weather," in Hawaii, accepted by many patients and physicians as a "cause" of attacks of asthma. The customary weather in Hawaii is that which accompanies the trade winds, whose usual direction is from slightly ENE, deriving their name from their constancy, always being on course or "trade." Their source is the semi-permanent Pacific high-pressure center, or anticyclone. When cyclonic systems (so-called "Kona storms") come, there are southerly streams of warm, moist, tropical air, with increased humidity and rainfall and increased cloud cover to decrease solar heating. Apparently these "Kona storms" are characterized by smaller diurnal changes, high humidity and moderate temperatures, and are often preceded or followed by longer intervals (called "Kona weather," and of ill repute locally) during which the absence of a general wind induces conditions of sometimes "oppressive stagnancy."

Myers and Price found, in the children studied in Honolulu, that a maximum incidence of asthmatic attacks occurred October to May, with a peak in January to March. Of course, respiratory infections may often be predisposing factors. On the whole, it was found that no definite relationship exists between Kona weather and asthma attacks.

Markow and Reicher[22] in the New York State Journal of Medicine, 1953, reported having studied 90 patients over a two-year period, following outdoor temperatures, relative humidity, barometric pressure, total rainfall, incidence of upper respiratory infections, and pollen counts. They followed the average daily and average monthly incidence of asthmatic attacks. Temperature changes were felt important (incidence January and March). They thought they could see a direct relation between increased humidity, increased rainfall and descreased incidence of asthma and upper respiratory infections. These were, of course, trends. They claimed decreased incidence when barometric pressure fell below and remained below 30.00 inches of mercury.

Rosen[7] followed a smaller group of asthmatics hospitalized in any Army General Hospital in south-central Indiana over a 4-month period (11-44 to 3-45, a nonpollen season). He thought sharp changes in outdoor weather (even though indoor weather remained fairly stable) seemed to cause more attacks. Low indoor humidity and decreased outdoor temperature seemed important. Wisely, he did not try to pick any one factor as of prime importance, noting that weather is a complex of atmospheric forces.

Ordman,[23] in South Africa, describes a "climate group" of asthmatics whose symptoms are aggravated on the coast, where constant high temperatures and high relative humidities are present. But he also found these people to have high degrees of house-dust sensitivity and thought that house dust from coastal areas was more allergenically potent than that from inland areas. Targow[24] also mentions the possibility of meteorological factors affecting house-dust sensitivity and adds "the mechanism must be different from that in play in fluctuating weather conditions, because the response is seen in more or less stable weather conditions."

Schook,[25] in Holland, followed 400 patients for periods from 6 months to 4 years (average 15-month follow-up). He found no correlation with meteorologic factors; however, he failed to find agreement with infectious episodes or so-called "psychical conditions." Also there was no correlation with grass pollen counts, but there was some correlation (of "undetermined significance") with fungus spore counts.

Petersen and Vaughn,[13] in 1944, noted that weather change may be, and frequently is, a factor in tipping the balance toward a fatal outcome in asthma. They reported on several patients collected by Vaughn and Hilding, showing death in asthma associated with sharp drops in temperature and a barometric crest. They previously had pointed out the great "autonomic lability" of the allergic patient and the frequent association of death in asthma with passage of major polar air masses.

In 1933, Nelson, Rappaport, and Welker[26,27] reported studies on the influences of filtered air on asthmatic patients. It was found that the symptoms of pollinosis could be altered and that cooling of the filtered air may aggravate asthma because of increase in relative humidity. In addition to pollen concentrations, it was noted that a sudden drop in barometric pressure, a fall in temperature, an increase in relative humidity would be associated with precipitation of asthmatic attacks, even when pollen concentrations and rate of air change remained about the same. Two possible explanations were offered: (1) that changes in atmospheric conditions may, in themselves, precipitate attacks, or (2) may alter degrees of pollen sensitivity. (An earlier paper by the same authors had reported consistent relief of hay fever in filtered air and not complete, but general, relief of "pollen asthma" under these experimental conditions.)

Petersen, in his book, Man, Weather, Sun,[28] does not specifically mention asthma in the subject index, but refers to various effects of meteorologic variants on diverse physico-chemical, physiological changes in man: he states that the "organic tides of the body, largely reflected in blood, urine, and physiological reactions in general, are largely conditioned by the meteorologic environment of the time." He found a decreased pH of nasal mucus membranes with increasing outdoor temperatures, and stated that anoxia may be associated with cold-front passages. Petersen thought "autonomic imbalance" was important and related to weather changes.

Berg, Mayne, and Petersen[29] suggested in 1940 that increased blood pH is associated with increased barometric pressure, and that at higher temperature (though not as well correlated) a similar increased blood pH can be demonstrated. They noted that changes in BMR (? direction) have been reported, as have seasonal variations in organic iodine content of thyroid gland of domestic animals. It was stated that perhaps the vascular changes mentioned previously (spasm and relaxation) are related to fluctuations in blood chemistry, among them rising and falling pH. Increased barometric pressure is said to increase O_2 content of blood and anoxia is related to greater release of acid metabolites. A further note states that pH changes have been shown to influence oxidation rates of various organs and tissues, with increased rates at lower pH's.

Peterson[30] summarizes organic reactions to weather changes and emphasizes "phase fluctuation" in response. "Change from heat to cold involves contraction of the vascular bed, usually the blood pressure increases, pH is increased, blood sugar increased, tissue oxidation impaired. The ion balance is altered, as is the endocrine balance, even the balance of the vitamins changes, because the excretion is altered." Compensatory changes follow, and may be heightened if warmer temperatures ensue later.

The American Meteorological Society Abstracts and Bibliography,[31] November, 1957, has made available a representative bibliography on medical meteorology, in which are abstracted several papers of interest. Berg[32] considers that bioclimatic material is often statistically inadequate and inexact. Hosemann[33] describes various statistical methods suitable for medical climatologic studies. These methods are simple frequency distributions, correlation analysis, partial regression, and time series.

Becker[34] has shown statistically that asthmatics react (as do cardiacs) to cold fronts, ground layer turbulence, upglide, etc. An analysis of clinical material over one-half year is presented.

Weather effects on noninfectious diseases are discussed by Berg in another publication.[35]

Air mass effects on asthma are noted by Kerdo,[36] although no statistically significant results are available.

The relationship between "sultriness" and vital capacity in asthmatics is not clear-cut, according to an article by Spangenberg,[37] in which vital capacities were done on 30 experimental subjects on days with marked sultriness.

At this time it is impossible to show specific relationships between various physiological alterations and either the onset or continuance of an attack of bronchial asthma. However, it is interesting to speculate upon possible effects of such things as anoxia, vascular spasm, altered pH of nasal mucus membranes, and altered ciliary motility on the respiratory tract of a patient whose immunochemical background makes his reactions "altered" ones, from the usual normal.

It is evident that we have much to learn on this general subject. The human chamber experiments should allow us to study some of these variables under controlled conditions.

Many factors in asthma and allergic disease are labeled "unknown." Perhaps with knowledge gained relative to the above, fewer cases will be put in the "psychological" basket.

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BOTANICAL PHASE

Professor Wagner opened the discussion by reading a note from Dr. Norman who wondered about the change in plans as noted in the previous minutes concerning transportation of the ragweed plants from the greenhouse to the airport. It was decided that the plants could be transported in decomposable pots and an abundant supply would be provided in case the first group that was transplanted did not survive. Professor Wagner announced that Mr. Alan Graham is now working with the group on mold identification; Mr. Graham is a graduate student doing work on plant and mold paleontology.

Professor Wagner announced that fresh pollen would be available for the wind-tunnel and histamine-relief studies within the next two or three weeks. The botany group is also going to study on a trial basis the effect of photosensitivity on ragweed by having some flowers under continuous lights 24 hours a day.

As far as the coming extra-seasonal experiment from the botanical standpoint is concerned, the following factors will be studied: the clumping phenomena of the pollen grains, the chemistry of wall surfaces, and its effect on clumping (an instructor in the Flint branch of The University of Michigan is to help on this), Mr. Beals will continue his studies on the pollen grain counts and the study of the periodicity of giant ragweed, and a Mr. Richard White, a plant anatomist, will study the development of the flower ontogenetically. In addition, Mr. Payne will study the evolution of ragweed.

The people in Oregon wondered if the ragweed now present there had been brought by immigrants or by airborne sources from the midwest. The question of the contamination of soil by viable seeds was also raised but this group in Oregon did not know why their plants were not germinating; Professor Wagner said that this may not occur until another year or two.

In the discussion Dr. Sheldon again emphasized that a tapetal fluid study should be carried on. The phenology of ragweed was again discussed by the group; the botanists emphasized that a large-scale control is necessary because there are many variables when a small study is done (e.g., the natural population of ragweed varies from year to year and from location to location). One of the questions raised was whether the number of plants for a given number of seeds is always the same for a given condition.

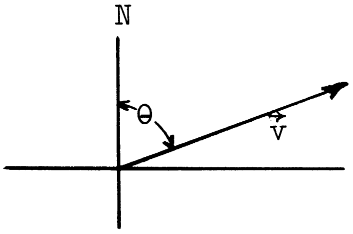
It was decided that the next meeting would be on March 18 in the Public Health School Conference Room. Dr. Sheldon said that Dr. Al Hall, who described sarcoidosis as possibly being due to pine pollen, would probably be invited to the meeting. He has heard of the work being done by this group and would like to meet the participants.

MEETING OF 18 MARCH 1959

The meeting was held in room 2522 of the School of Public Health. The following were present: Akerman, Bianchi, Bierly, Dingle, Elder, Field, Giever, Hemphill, Hewson, Patterson, Payne, Plymer, Sheldon, Willard, Wagner, and Yang.

METEOROLOGICAL PHASE

Professor Dingle discussed pollen dispersion and wind direction. He began by explaining what he termed the Everyman's concept of wind direction and the plume. In this concept a person considers the movement of air in a certain direction measured from north. Here θ is the angle between north and the direction toward which the wind is blowing, and the length of the arrow $\vec{v}$ drawn from the origin gives the speed of the wind.



When speaking of the plume, most people consider the source as continuous and constant in amount. Thus Q, the source strength, is expressed as a certain amount of mass per unit of time. Of course, this simple reasoning does not apply. Actually, the concentration downwind along the direction θ depends upon Q, the wind speed, v, and the distance, x, downstream, so that one may write

$$\chi \propto Q/vx^n$$

where χ is the concentration
Q is the source strength
v is the wind speed
x is the distance downwind to the area under consideration
n is a real number depending upon meteorological conditions, topography, etc.

The facts of the wind in the surface layer were then discussed by Prof. Dingle. He pointed out that the speed is in reality a very "noisy" trace. There is an average wind denoted v with a ± 20% variation on either side of the average. That is, with an average speed of 10 mph, a range from 8-12 mph is to be expected.

The direction trace also is not steady. There may be as much as 150° variation from maximum to minimum in a short interval of time. In any pollen studies, we must consider what scale should be used since there are no simple ways to average the wind speed or the wind direction.

Another complicating factor is that the wind speed varies with height in the surface layer up to approximately 30 ft. There are several different equations that express this variation depending upon the temperature structure of the lower layer.

$$(a)\quad v = a + b \log (z + c)$$

where a, b, and c are constants. This expression shows that the wind varies as the logarithm of the height, z. This variation is true for neutral equilibrium.

$$(b)\quad v_1 - v_2 = a(\sqrt{z_1} - \sqrt{z_2})$$

This expression is used for stable atmospheres or for an inversion condition. Such conditions are evident when fog or thick haze are present.

$$(c)\quad v = v_o (z/z_o)^{1/n}$$

In this expression "n" varies as the stability of the atmosphere.

n = 10 for unstable cases when cumulus clouds and gusty winds are present.
n = 7 for neutral conditions.
n = 0.8 for stable conditions.

As a result of the many variables involved and the difficulty in actually measuring the wind, the behavior of lower layers of the atmosphere is not completely understood. No one has ever studied the relation of wind direction to height in the surface layer. It has been assumed that the wind direction remains constant in the lower layers, yet our data from Jackson Prison do not support this viewpoint.

Professor Dingle then discussed some facts of the plume. A plume of pollen in particular is not continuous but is a function of time and of the wind speed. The plume is not constant at the source, but rather the release is dependent upon the number of blooms that are open, the pollen that is in "open" storage on the leaves and the puffs of the wind. One might write that $Q \propto v^k$ if it is assumed that all biological conditions are constant.

Thus the concentration would be expressed as follows:

$$\chi = \frac{v^{k-1}}{x^n}$$

Using the basic information discussed above, Prof. Dingle proceeded with an interpretation of the field data. He showed how a wind vector for use in our sampling periods must have a suitable scale to be valid. This scale must be right in terms both of space and of time. He pointed out that the gravity slide collection could be thought of as the result of the mean concentration over the slide. It is granted that the gravity slide does not sample much of an area. However, we can write

$$c \alpha \overline{\chi}$$

A physical interpretation of the pollen distribution and wind direction is difficult at best. It is possible that a more complete statistical description would be better.

In 1956 at the North Campus, a sampling network was set up at 10, 20, 40, 80, and 160 ft on rays 40° apart starting from north. This is discussed in Progress Report No. 1. Winds were measured at 8 ft. Several criteria were used in getting the diagram as used in the report. A minimum average wind speed of 5 mph was used, so that only 31 hr of data were used for computing the diagram. The data were superimposed upon each other resulting in Fig. 3.19 of 2421-1-P.

In 1957, during the preseasonal experiment at Jackson Prison, rays were only 20° apart. Sampling was carried out at 20, 40, 80, 160, and 320 ft because the source diameter was 26 ft. Slides were mounted at a height of 2 ft and were changed every 4 hr. Meanwhile a cup anemometer was recording at a height of 12 ft and a wind vane at 8 ft. There were thirteen 4-hr periods which had winds of 5 mph or greater. This gives 52 hr of usable data to combine into a figure similar to Fig. 3.19 of the first report. The new figure shows a very symmetrical pattern about the mean wind direction. There is some shift of concentration to the right in the sampling arcs near the source. The counts are 30% less in number than those of the 1956 data, even though there were 3000 plants in 1957 versus 160 in 1956.

Professor Wagner thought that perhaps the terrain differences and the differences in vegetative cover between the North Campus and Jackson Prison could cause some of the difference in counts. Professor Akerman asked if perhaps there was a masking effect, one plant upon another. He was assured that there probably was but it would have been an effect in both years. Professor Wagner stated that muslin had been used around the 1956 plot but it was not used in 1957. He thought this might be a factor in the pollen pickup.

Professor Hewson said that he did not understand the 1956 figure and thought that perhaps it was distorted in some way. The symmetry of the 1957 data was also difficult to understand; however, the shift to the right in the 1957 figure was readily explainable. He pointed out that the wind gusts in the lower levels are due to momentum being transported down from higher levels. It is well-known that the wind direction at, say, 500 ft is to the right of the wind at the surface due to frictional effect. Therefore a gust from a higher level would be to the right of the surface wind. In this manner it is easy to explain the shift to the right in the figure.

Professor Wagner asked what percent of pollen falls out at different radii. Professor Dingle referred him to the first progress report. Professor Hemphill wondered if there were not other variables that were important in this analysis other than wind. Professor Hewson assured him that, in time, these data would be completely analyzed considering all variables.

Dr. Sheldon then asked Prof. Hewson to make a few comments prior to the review of the proposals. Professor Hewson said that, since we were now in the third year of the program, he thought it was a good idea to look back to see what had been originally proposed. In so doing we could see what we had done and what we planned to do relative to what was proposed. Professor Hewson emphasized that although the Public Health Service is quite lenient in allowing the project personnel to pursue the project in whatever manner they deem most promising, it still would be of benefit to all to know what had been proposed.

Mr. Bierly then passed out abbreviated forms of the original proposal and the supplementary proposal. He asked that each person keep a tally as to whether the aims were accomplished or not. The following is a summary of the comments from the floor.

AIR POLLUTION BY AEROALLERGENS

Review of Proposals - 18 March 1959

I. Specific Aims of Original Proposal	Comments as to Fulfillment
A. First Year (1955-1956)	
1. To observe simultaneously the specific allergic reactions of selected subjects, and the environmental conditions to which they are exposed during the ragweed pollen season.	Dr. Sheldon commented that this was done at Jackson Prison in 1956 and 1957.
2. To observe the details of growth and development of ragweed over the region surrounding the subject area simultaneously with the environmental conditions imposed upon the plants by competition, soil, and weather during the growing season.	Professor Wagner said that there had been more pressing problems and that this problem was too broad to be attacked as such.
3. To predict experimentally, on the basis of meteorological and botanical observations, the relative amounts of airborne pollen in the subject area from day to day during the ragweed pollen season, and to verify these predictions by comparison with observed conditions of air pollution and weather.	Professor Wagner stated that this was impossible to do the first year, but with the background that has been developed it now is possible to accomplish it. Mr. Bierly said that the meteorologists hoped to begin work in this area this year
4. To design and construct a test chamber about 8 by 8 by 8 ft in which temperature, humidity and pollen concentration may be controlled for the purpose of studying subject reactions to known values and changes of temperature, humidity and pollen concentrations.	Professor Akerman reported that the walls of the chamber were up, the air-filtering and air-conditioning equipment had been moved to the Kresge Bldg., and that the chamber should be usable within a few weeks.

B. Second and Succeeding Years (1956-1960)

1. To use the test chamber in various experiments: a. the variation of temperature, humidity and pollen concentration for the purpose of observing in detail the clinical reactions of the individual subjects to such changes. b. the investigation of the effects of aging and exposure of the pollen grains upon their clinical antigenicity. c. the study of the effect of physical exertion and fatigue of the subjects upon their clinical reactions. d. the study of emotional and physiological stresses in relation to allergic reactions of individual subjects.	Mr. Bierly said that these experiments would be carried out in the near future during the chamber experiments. Professor Wagner commented that the dog was not mentioned but should probably be included in these experiments. Dr. Sheldon indicated that the dog would not be used more than once a week in the future.
2. To gather and analyze complete field data	
a. on the growth, development and pollen yield of ragweed in relation to air, soil and biological environments, with a view to the evolution of long-term (up to 30-45 days) forecasting criteria.	Mr. Bierly commented that this area had not been touched as yet but would be worked on in conjunction with IA3.
b. on the release, flotation and transport of ragweed pollen grains with a view to the development of short-term (24 hr and less) prediction of atmospheric pollen concentrations.	Professor Dingle said that during 1958 the first work was done on release and refloatation but nothing had been done on the prediction problem.

c. on the natural cleansing processes of the atmosphere (i.e., sedimentation, impaction and rain-washing) in relation to atmospheric turbulence, rainfall type and amount, and other pertinent variables.	See comments under IIF.
d. on the roles of dew and sunshine in pollen ripening and release.	Professor Wagner stated that some work had been done in the past but more would be done this year.
e. on the methods and results of applying herbicides to wild ragweed.	Professor Wagner commented that this area was largely covered by other groups mainly because of its economic importance. Professor Hewson asked if ragweed were included in this work. He was assured that it was.
3. To conduct chemical investigations for the purpose of	
a. isolating and identifying the antigenetic compounds associated with ragweed pollen.	Dr. Sheldon stated that studies are being carried out on tapetal fluid. Since this is so important it will be pursued further.
b. measuring objectively and quantitatively the antigenicity of various samples of ragweed pollen with and without aging by exposure.	Dr. Sheldon stated that the effect of new vs. old pollen was being tested.
c. studying ragweed eradication and control chemicals.	See comments under IB2e.
4. To determine the relationships between	
a. pollen antigenicity and conditions of growth.	See comments under IB3a and b.
b. deterioration of pollen by aging and conditions of growth and degree, kind and duration of exposure.	Professor Wagner stated that Ted Beals' work was along this line.

5. To continue clinical investigations in the light of the first-year findings on a. a select group of prison inmates. b. larger samples of the prison population.	Mr. Bierly commented that this was done in 1957 at Jackson Prison.
C. Other Ideas Set Forth Under Methods of Procedure.	
1. Complete botanical observations including surveys of the area to evaluate sources.	Professor Wagner stated that a survey was carried out at Jackson Prison in 1956. Professor Dingle indicated that the work was not carried to completion because of many complexities.
2. Airplane sampling.	Mr. Bierly stated that samplers had been made but never used in any of the field experiments. Professor Hewson said that airplane sampling was not applicable to our small pre-seasonal experiments. Balloons were to be used in 1959.
3. Statistical design and control of all experiments.	Mr. Bierly indicated this was being done.
4. Use of lapse time photography for studying the growth and development processes of pollen. 5. Study details of maturation, release, flotation, and high speed microcinematography will be explored and applied as practicable.	Mr. Bierly reviewed the merits of such equipment. Professor Dingle pointed out the difficulty of obtaining such equipment. Professor Wagner stated how useful such equipment would be. Dr. Sheldon suggested we get in touch with a local camera club.
6. Natural cleansing processes of the atmosphere, impaction sedimentation, and rain-washing effects.	See comments under IIF.

7.	Efficacy and economic feasibility of herbicidal procedures to evolve efficient control and/or eradication devices and methods.	See comments under IB2e
8.	Chemical investigations carried out by analytical chemistry and immunochemistry	Dr. Sheldon stated that there had been no analytical chemistry done. Dr. Patterson said that several aspects of immunochemistry have been worked on and reported upon.

II. <u>Specific Aims of Supplementary Proposal</u> (1958-1960)

A. To provide adequate statistical services for the investigation on "Atmospheric Pollution by Aeroallergens."

1. Correlation of signs and symptoms of selected patients
   - with pollen counts.
   - with the following pulmonary function tests:
     - timed vital capacity (one second and total);
     - maximum breathing capacity; and
     - maximum expiratory flow rate.
   - with the following other laboratory studies:
     - quantitative blood eosinophil counts;
     - nasal smears; and
     - white blood counts.
   - with the following meteorological quantities:
     - wind speed;
     - vertical wind profile; and
     - temperature lapse rate.
2. Correlation of individual ventilatory pulmonary function tests with other laboratory tests. This involves comparison of many of the items under (a) with each other.
3. Correlation of subjective and objective signs with pollen counts for each of individual time periods during the day.

Professor Hemphill said that the statisticians were ready to proceed on some statistical correlations.

B. To determine the characteristics of tapetal fluid and to compare these properties with those of whole pollen extracts.	Dr. Sheldon said that the tapetal fluid investigations are now underway.
C. A monographic study of the ragweed genus, Ambrosia, in North America will be a valuable contribution to the understanding of these important allergenic plants. The aims of this research may be divided as follows: 1. To re-examine and compare the diverse types of ragweeds in detail. 2. To interpret the types in terms of their evolution and migration. 3. To express this diversity in modern terms of the categories of species, subspecies, varieties, and hybrids.	Professor Wagner stated that this was a tremendous job and it has taken time to find a person to undertake this task. Mr. Payne is now available to begin this study.
D. To study the atmospheric transport of ragweed pollen by measuring and analyzing the scale and spectrum of pollen concentrations taken in conjunction with measured wind speed, direction, and turbulence.	Mr. Bierly pointed out that WJBK-TV tower was to be used in this phase. Mr. Elder said that a lack of a satisfactory sampler held up this project. Professor Dingle said that the impinger was developed for the work but did not turn out to be satisfactory. Professor Hewson mentioned that Ken Yang's work might be tested using the WJBK-TV tower.
E. To develop and apply improved techniques of sampling the atmosphere and determining pollen concentration levels continuously and volumetrically.	Mr. Bierly enumerated the sampling techniques that had been developed in the past year. Although some testing still must be done, great strides have been taken in this area.

F. To conduct studies of the rain-washing of pollen from the atmosphere employing the raindrop size spectrometer and utilizing radar and other observations of the extent of rainfall.	Mr. Bierly explained that the raindrop spectrometer was to be used in this type of work and asked Professor Dingle to comment on the spectrometer. Professor Dingle explained how the spectrometer would be used and said that the instrument would be operational this year.

Dr. Sheldon summed up the proposals by saying that there were many areas in which work still should be done in all phases of the project.

Dr. Sheldon then called on Prof. Hewson, who presented the idea of beginning to write a new proposal for presentation to the National Institutes of Health by September of this year. Professor Hewson told of a conversation he had had with Profs. Vaughn, Velz, and Miller about renewing the grant. It was their opinion that we should try to renew the grant. They also suggested that several key men on the committee which reviews these proposals be invited here to see exactly what is being done on the project. It was decided that Dr. Sheldon and Prof. Hewson work out the details on these visits.

It was further decided that the various groups should get concrete ideas together by the meeting to be held on 22 April. These ideas could then be discussed so that the proposal can be written before summer and then submitted by September.

Professor Hewson asked if the proposal should contain ideas for branching out into other pollens or whether we should continue on ragweed pollen. Professor Wagner asked if NIH cared. Professor Hewson stated that he did not know but felt that if the medical and botanical groups had good reason for branching out into other fields, this should be considered.

Dr. Sheldon suggested that two meetings be held in April. The first would be to show several guests our facilities and our work in return for which they would report on their work with pine pollen. The second would be our regular meeting to be held on 22 April in the Meteorological Laboratory, 5500 East Engineering Building.

SPECIAL MEETING OF 7 APRIL 1959

The meeting was held in room M7330 of the Medical Science Building. The following were present: Akerman, Beals, Bierly, Elder, Field, Gill, Hewson, McLean, Mathews, Patterson, Payne, Plymer, Sheldon, Wagner, and Yang. Four guests from the sarcoidosis team of the Veterans' Administration were present. They were Dr. Marvin Cummings, Assistant Chief of Research and Experimental Medicine for the VA and head of the team; Dr. Dorothy Heilman, Tissue Culture Laboratory, Washington, D. C.; Dr. J. A. Hall, Forest Products Laboratory, Madison, Wisconsin; and Mr. Paul Hudgins, VA Sarcoidosis Laboratory, Jacksonville, Florida. Many medical students and interns were also present.

Dr. Sheldon gave a brief resume of the work of the Aeroallergen group, emphasizing that it is composed of meteorologists, statisticians, botanists, engineers, and clinicians. Dr. Cummings was then introduced to the group by Dr. Sheldon. After introducing his associates, a review of the historical concepts of sarcoidosis was given by Dr. Cummings. He remarked that no satisfactory etiology has been found. It was noted after World War II that most of the veterans with sarcoidosis originally came from the southeastern portion of the United States. Another fact noted was that there was a 20 to 1 predominance of sarcoidosis in the Negroid race. Two thousand patients with a biopsy confirmation of the diagnosis of sarcoidosis were studied. Epidermiological studies confirmed that the main concentration of Negro veterans with sarcoidosis was in the southeastern U. S. The centers of concentration for whites were in New England and the north-central states. Numerous other epidermiological studies showed that there was no apparent correlation between the disease and soil, humidity, altitude, or other meteorological variables. It was further noted that most of these veterans had occupations classified as outdoor work.

Dr. Hall showed that the major kinds of trees in the regions of interest were pines and other conifers. It has been noted during observations in Florida that pine pollen had been found in the sputum of patients with sarcoidosis. Dr. John Chapman of Dallas had shown that sarcoidosis throughout the world was located in a belt in the north temperate zone, the zone in which pine was the predominant forest tree. The fact that there are no pine trees in Negroid Africa, and that no cases of sarcoidosis have been reported there, is of interest. Studies carried out in Denmark showed that where the pine was the predominant tree, the incidence of sarcoidosis was twice as high as in other areas. Whether it is the tar or resin from the pine trees, the pine pollen, or some mold endemic in the pine pollen, or even if it is anything associated with pine trees, has not yet been established.

Mr. Hudgins stated that no statistically significant correlation had been found between sarcoidosis and pine products. Pine pollen was therefore tested, and an acid-fast material was found which is similar to the lipoidal material in tuberculosis. It was thought that it was part of the fat fraction C in the lipoidal material. It has also been found that certain amino acids found in tubercle bacillus were present in sarcoidosis lesions. Paper chromotographic studies revealed that both pine pollen and the microbacteria of tuberculosis had similar amino acids. This lipoidal material could induce granulomatous tissue in experimental animals within 10 days to 2 months. These tissues had giant cells and granulomatous tissue reactions which were similar to sarcoidosis but the histological picture was not exactly comparable. Also, systematic distributions of lesions in experimental animals could not be obtained.

The group is now carrying out a study on the possibility of immunologic constituents in pine pollen. They are also attempting to obtain purified antigens. If purified antigens can be obtained, they will be used to try to produce sensitivity in animals.

In the discussion that followed, it was pointed out that the true significance of the Kveim reaction was as yet unknown. It was noted that all conifers contain an acid-fast material. Also noted was that a delayed type of skin reaction, similar to the tuberculin reaction, could be obtained in patients with hilar adenopathy. Dr. Cummings stated that in tuberculin-negative, normal patients, a negative skin test to pine pollen was obtained. In tuberculin-positive, normal patients, 20% had a positive delayed reaction to pine pollen. Of patients with sarcoidosis and a positive tuberculin, 25% had a positive reaction. Using guinea pigs, 20% of the guinea pigs with a positive tuberculin also had a positive reaction to the pine pollen. Of guinea pigs having a negative tuberculin, none had a positive delayed skin test.

The question of seasonal incidence was discussed. It was thought that some patients with bilateral adenopathy had the onset of their symptoms according to seasonal variations. It was noted that sarcoidosis admission to VA hospitals also followed a seasonal pattern, but it was the reverse of the usual seasonal pattern of the overall VA admissions.

With respect to the treatment of sarcoidosis, it was thought that steroids were necessary for the acute iritis and acute respiratory and progressive skin lesions. Otherwise, stationary pulmonary lesions and adenopathy would be treated with symptomatic measures.

At the conclusion of the sarcoidosis group's talks, Professor Hewson introduced Dr. Joseph Gast, Professor of Biochemistry, Baylor Medical School, Houston, Texas.

METEOROLOGICAL PHASE

Dr. Sheldon then asked Mr. Bierly to begin a brief discussion of the wind-tunnel studies from the meteorological standpoint. Mr. Bierly stated that the studies could be conveniently divided into groups—that of last year and that of the present year. Mr. Bierly then asked Mr. Gill to discuss the work of the previous year.

Mr. Gill began by discussing the Durham sampler that had been used from the beginning. He described the sampler and how it was used. It was felt that we should be able to measure the concentration in grains/unit volume. Therefore the millipore filter was tried. Mr. Gill showed pictures of the MP filter and explained how it worked. In addition to the volumetric type of sampling, a continuous record of the change of pollen concentration was needed. An impinger was therefore developed to satisfy this need. Field use during 1957 showed that the impinger did not work very well. During the 1957 preseasonal experiment, it was impossible to use MP filters, so plain gravity slides were set in an array 2 ft above the ground. These slides would then give some idea of the pollen concentration.

During the wind-tunnel studies conducted last year, the MP filters, gravity slides, and wires were tested as pollen samplers. It was found that the MP filters performed satisfactorily only at low wind speeds. The orientation of the slides into the wind is quite important to the amount collected by the slides. A slide oriented parallel to the wind collected a great deal more pollen than one facing normal to the wind.

As a result of the wind-tunnel tests, it was decided to use a flag sampler and a modified roto-bar sampler. These two samplers were described and illustrated. Above 3-4 mph, the flag sampler is quite efficient, although the wind speed must be known to compute the concentration. The roto-bar sampler, originally developed by Stanford University Aerosol Laboratory, rotates at approximately 30 mph sampling 1 cubic meter of air per hour. Thus the number of pollen grains on the leading edge is a good indication of the concentration. There is more work to be done as concerns absolute calibration, but Mr. Gill emphasized that the flag sampler and roto-bar are the best field samplers that we have to date.

Mr. Bierly then described the cooperative effort in getting an experiment designed and the wind tunnel ready for exposing ragweed-sensitive patients to new and old pollen. After a conference with Professor Hemphill, Dr. Willard, Mr. Elder, and Mr. Bierly, a simple test was designed. Twelve sensitive people would be used. These would be stratified according to sex, medication, etc., so that two groups could be formed on a strictly random basis. The first group of six would be exposed to old pollen for five days in a row. Then the second group of six would be exposed to new pollen for a week. On the third week, the first group would be exposed to new pollen, and finally on the fourth week the second group would be subjected to the old pollen. New pollen here means pollen no more than 2 hours old. Once a patient began to develop symptoms, he would report to the allergist at the wind tunnel who would then do some tests on the patient. The results should tell if there is a difference between old and new pollen and should also aid in determining if there is an accumulative effect of the pollen on the patient.

Mr. Bierly then asked Mr. Elder to discuss the present meteorological tests and the future tests to be run in the wind tunnel. Mr. Elder described the comparison tests to be done on the samplers since the samplers are not 100% efficient. There is no standard sampler; however, an MP filter using isokinetic sampling will serve as our standard in the wind tunnel. The roto-bar and the flag samplers will be calibrated against this standard so the collection efficiency of both samplers can be computed for various wind speeds. Various adhesive surfaces will also be evaluated.

MEDICAL PHASE

Dr. McLean then described the Willow Run experience in 1958 when two ragweed-sensitive patients sat downwind from the pollen plot. Symptoms were made to develop prior to the regular ragweed season. These men were then exposed to dry ragweed pollen in the wind tunnel late in July and early August, 1958, before there was a significant amount of natural ragweed in the air. It appears that fresh pollen will cause symptoms for the test chamber experiments no matter what time of year the study is carried out, and that the old pollen might do the same.

In March, 1959, two ragweed-sensitive subjects were placed in the wind tunnel for one hour and subjected to fresh pollen. The wind speed was 8-10 mph. Neither subject had any symptoms after an hour's exposure. One of the subjects was exposed for a second consecutive day to similar conditions and again noted no symptoms.

Dr. McLean then reiterated the objectives of the present series of wind-tunnel experiments:

(1) to determine the approximate amount of pollen necessary to produce allergic symptoms;
(2) to ascertain whether a 2-hour interval is the determining factor between fresh and old pollen;
(3) to determine if the same patient has the same kind of response to similar test conditions;
(4) to determine if an accumulative effect occurs after repeated exposures; and
(5) if possible, to see if an objective means of measuring the clinical effect of exposure and development of symptoms is available.

TEST CHAMBER

Dr Sheldon asked Professor Akerman to give a report on the status of the test chamber. Professor Akerman reported that the sheet metal shop had started on the duct work. The plumbing work is being done and the electrical work has been completed. Professor Akerman and some of his graduate students are going to check on the flow rate, temperature, and humidity when they get an opportunity.

CLOSING REMARKS

Professor Wagner noted that a senior in the College of Literature, Science, and the Arts is compiling the literature on the phenology of ragweed for a term paper in a botany course. Professor Wagner also mentioned that Mr. Egler has collected a group of references on the control of ragweed which will be made available to us soon.

Dr. Sheldon announced that the next meeting would be on 24 April so that Mr. Irving Gerring of the Division of Research Grants, NIH, would be able to attend.

MEETING OF 24 APRIL 1959

The meeting was held in room 5500 East Engineering Building. The following were present: Akerman, Beals, Bierly, Dingle, Elder, Field, Gill, Hemphill, Hewson, D. Jones, Mathews, McLean, Plymer, Sheldon, Wagner, and White. Mr. Irving Gerring of the National Institutes of Health was present.

Professor Hewson introduced Mr. Gerring who had been influential in helping this project get started. Professor Hewson said that the group was very happy that Mr. Gerring was present and to have an opportunity to speak with him about the project.

MEDICAL PHASE

Dr. McLean was then asked to speak on the wind-tunnel studies. Dr. McLean's talk centered around the two following objectives of these studies:

(1) to determine the approximate amount of pollen necessary to produce allergic symptoms, and
(2) to ascertain whether a 2-hour interval is the determining factor between fresh and old pollen.

The two subjects used during the Willow Run preseasonal experiment in 1958 received an average concentration of 1000-3000 grains/m^3 or 83-100 grains/m^3/minute of exposure. Once these patients developed symptoms, after about 5 days, then symptoms could be produced again with very low concentrations. In the wind-tunnel tests held later in 1958, symptoms were obtained with concentrations varying between 1000-8000 grains/m^3. The average concentration during the ragweed season is 800 grains/m^3 with a peak concentration of 3000 grains/m^3. Thus the indication from this work is that a concentration of about 1000 grains/m^3 is needed to produce symptoms.

In the wind tunnel, symptoms were produced using both old and new pollen. The present wind-tunnel tests should indicate whether the old or the new pollen is more potent in producing symptoms. Dr. McLean also briefly explained the wind-tunnel tests to be held in May.

Mr. Gill mentioned that UMRI might assume control of the wind tunnel. If so, then the drive mechanism might be changed so the tunnel could be better controlled at low speeds. This would suit our needs much better.

METEOROLOGICAL PHASE

Professor Hewson then asked Mr. Elder to speak about the Jackson Roadside Experiment results. Mr. Elder began by briefly explaining how the flag sampler is constructed and operated. The main reason for conducting the Jackson Roadside Experiment was to determine how much pollen a person going to work in the morning might encounter. Mr. Elder showed a map of the route from Ann Arbor to Jackson. He described the vegetation and the topography of the area involved so that an idea of the amount of ragweed that might be available would be obtained. Wind speed measurements were obtained from the meteorological tower at Jackson Prison. All counts on the flag sampler, which was located on a moving automobile, were reduced to volumetric counts. Runs were made during the morning on the way to the prison and about noon on the return trip to Ann Arbor.

Mr. Elder showed plots of the average count in g/m^3 from the seven measured miles that were used as measuring stations. These data embraced the period 17 August to 14 September 1958. It was noted that the station nearest to Ann Arbor was out of phase with the other stations on a diurnal basis and that station no. 4 gave low readings all the time. Mr. Elder felt that the Ann Arbor station was receiving its peak concentration later in the day than the other stations due to pollen being transported in from adjacent sources. He also indicated that station no. 4 was located in an area of low density of ragweed plants, thus accounting for the consistently lower counts.

A second graph showed that the morning counts were always higher than the afternoon counts except for Ann Arbor. The effect of rainshowers and gusty winds could also be seen on this diagram.

The data are to be grouped using wind directions so the pollen sources will be a constant factor. Data from rainy days will be removed in an attempt to make a more statistical analysis of the data.

The immediate conclusion is that it seems that the pollen is coming from local sources rather than from a great distance.

POLLEN CONCENTRATION PREDICTION

Professor Hewson asked Prof. Wagner to start the discussion of pollen-concentration prediction. Professor Wagner said that the problem of forecasting pollen counts should be broadened to include the annual output of pollen. Thus it has been planned to study the ragweed phenology from year to year in association with the meteorological variations. Mountings of the ragweed plant from its beginning to 25 May 1958 were shown by Prof. Wagner in an attempt to show how the plant begins to develop.

A review of the literature of ragweed has been made by Mr. Thomas Detwyler, an undergraduate in the College of Literature, Science, and the Arts. Professor

Wagner passed out a list of 10 important questions concerning the life history of the common ragweed which were compiled by Mr. Detwyler while reviewing the literature. This list is included for continuity.

Important Unknown Factors in the Life History of Common Ragweed (Ambrosia Artemisiifolia)

1. What are the requirements for seed germination?

2. How does the successional stage which immediately follows common ragweed dominance inhibit, and later prohibit, ragweed growth? Is the changed environment unsuitable for the young ragweed seedlings or does germination not even occur?

3. Does common ragweed reproduce vegetatively? If so, how, and to what extent in nature?

4. What are the nutritional requirements and tolerances of low ragweed?

5. How does the nature of the upper leaf surface affect the adherence of pollen clumps?

6. Is frost the primary natural cause of death? When do plants which are maintained under late summer climatic conditions eventually die of old age?

7. How effective is wind dispersal of seeds? To what extent are the spines on common ragweed seeds advantageous for seed buoyancy?

8. Common ragweed seeds are known to be eaten in large quantities by quail. What other animals consume the seeds, and in what amounts? Are the seeds viable after passing through the digestive tracts of quail or other animals?

9. What are the after-ripening requirements of common ragweed seeds? How do these requirements differ for seeds of Ambrosia artemisiifolia from different geographic localities (especially distribution range extremes)?

10. To what extent do seeds which have lain dormant in the soil help populate a newly exposed area? (From various plots with cover of known ages observe the appearance of common ragweed plants after the cover is removed. Be sure to protect the denuded area against seed invasion from nearby stands.)

The forecast of pollen counts must be a joint cooperation of both the botanical and meteorological group. The plant behavior is important, but the meteorological phenomena important to the plant's production of pollen must also be determined. The botanical phase involves the maturation of the plant and the formation of the flower. The flower sequence and their meteorological conditions can and have been studied in the laboratory.

Professor Wagner briefly explained the study to be begun at Willow Run Field Station. The plan is to measure meteorological and botanical variables during the growing season of the plants for a period of several years. The number of flowers per spike will be counted plus the number of spikes on selected plants. In this manner, it is hoped that the production of pollen availability based upon past weather conditions may be made.

Mr. David Jones continued the discussion emphasizing the role of the meteorological variables. Mr. Jones stated that it was proposed to measure the standard meteorological parameters and stay away from the micrometeorological problems, at least for the present. Therefore such parameters as wind fields, radiation, temperature, relative humidity, soil temperature, and soil moisture would be measured. A visual demonstration of the use of a regular synoptic pressure chart and a meso-scale wind-field chart was given.

Mr. Jones also mentioned the problem of reflotation and its importance to the prediction problem. It was his idea to insert a platform onto which the pollen would fall and then could be observed.

Mr. Gerring asked if perhaps smog had an effect on ragweed. Professor Wagner said that a study was done in Detroit on the blighted areas but he had not looked into it completely. Mr. Gerring then asked if a test of smog on plants could be performed. Professor Wagner said that he didn't want to push into chemical control of the plant, but would rather study the botany of the plant.

GENERAL DISCUSSION

Professor Hewson asked whether the group should plan to continue the study of ragweed or should shift to other aeroallergens. The group was reminded that Mr. Harry Hanson of NIH had indicated that new ideas would help any proposal. Professor Hewson thought that perhaps Mr. Gerring could help answer this question.

Mr. Gerring stated that NIH does not propose to tell people what to do or how to do it. There is a revolving review group which looks over each proposal. Mr. Gerring felt that the interdisciplinary approach was excellent, especially in an area of few answers and large problems. In a sense this present project is from seed to consumer. The Public Health Service thinks that the type of research involved in this project is important and well within its scope of activity. The research could continue on the present basis or be divided into perhaps six separate aspects. The prevalent thought in Washington is that this project should not be divided but should be kept together using the present large-scale, interdisciplinary approach. This project can be better controlled on a large basis. Mr. Gerring added that each proposal is studied on an individual basis, assessing the approach to the problem, individuals doing the work, the University at which the work is being done, etc.

Professor Hewson asked if perhaps the project should branch out. Mr. Gerring said that the answer would depend upon our opinion of the importance of ragweed. Techniques could be developed for ragweed that could then be used in the study of other aeroallergens. There is no doubt that ragweed is significant. The tunnel tests and the test-chamber work are important facets and they are still to be done in the future.

Professor Wagner said that we are beginning to assume a certain stature in this field which we do not want to lose. The botanists want to put pressure on the ragweed problem. They are just beginning to know something about it and want to continue along these lines.

Dr. Sheldon agreed that we should continue to work on the ragweed problem and obtain definite results. He visualized the project as a long-range study of ragweed, added that the project was going to obtain answers one way or the other and said that he would hate to see it stop now.

Mr. Gerring suggested that we should ask for at least five years in our new proposal. Some grants last for 7-10 years. Naturally such a project would depend on many factors, but the present project is a long range one and should be dealt with as such. Mr. Gerring pointed out that stabilization of a project of this size requires a long-term basis. A long-range program is necessary to retain project personnel.

Professor Hewson said that we all wanted to continue on the project. Mr. Gerring suggested submitting a new proposal by 1 July 1959. This would mean that members of the reviewing panel would probably visit the group during the next several months. Mr. Gerring emphasized the importance of getting the proposal in early to maintain continuity.

The new proposal would need 40 copies of a special progress report containing 8-10 pages of summary, work done, and definite plans for the future. The special progress report should also include as appendices the first three regular progress reports. In addition, 20 copies of each publication resulting from project work should be included.

It was decided to hold the next meeting on 13 May 1959 in the Botany Seminar Room of the Natural Sciences Building.

MEETING OF 18 MAY 1959

The meeting was held in the Botany Seminar Room, 1139 Natural Sciences Building. The following were present: Akerman, Beals, Bierly, Detwyler, Dingle, Elder, Field, Giever, Gill, Hemphill, Hewson, D. Jones, Kelley, D. McLean, J. McLean, Mathews, Patterson, Payne Schwemmin, Tourin, Wagner, Willard, and Yang. Dr. Noel H. Gross of the National Institute of Allergy and Infectious Diseases was our guest at the meeting.

MEDICAL PHASE

Professor Hewson introduced Dr. Roy Patterson, who discussed the ragweed-sensitive dog. Dr. Patterson first reviewed the objectives of the investigation:

1. To investigate the animal with all available measures used to study human allergy. A corollary would be to establish the differences between the spontaneous sensitivity in the dog and in the human.
2. To investigate the value of the spontaneously sensitive dog as a research tool. Several questions must be answered to find the degree of usefulness. Will the reaction be reproducible? Will there be a definite measurable response? Will suitable controls be negative?
3. To perform actual experiments such as dose-response relationship between antigen and symptoms, comparison of antigens, and the effect of various medications.

The question of the choice of an end point in the reaction was then discussed. The dog has pruritus of the skin, eczematorial reaction of the skin, conjunctiviti, and some nasal discharge during the regular ragweed season. Most of these symptoms are unsuitable for any quantitative measurements. It was found that definite asthma could be produced in an experimental chamber, so this was chosen as the end point of the reaction.

Dr. Patterson then reviewed what had been done to fulfill the objectives of the investigation. Most of the usual tests of clinical evaluation have been completed. These indicate that the dog is quite similar to a human who is spontaneously sensitive to ragweed.

The dog's asthma is reproducible, demonstrating a definite end point both chemically and graphically. This indicates that the dog is in fact a suitable experimental tool for research in asthma.

As concerns the experiments, the following observations have been made.

1. No conditioning effect appears to be demonstrated in the animal.
2. Asthmatic attacks are relieved spontaneously or by epinephrine. An attack subsides spontaneously in approximately 80 minutes. With the use of epinephrine in a dosage of 0.3 ml of 1:1000, the attack subsides in 25-30 minutes.
3. A certain threshold of concentration of antigen, either in the form of the water-soluble antigen or pollen in a water suspension, is necessary before an asthmatic attack occurs.
4. This threshold value changes with a higher concentration required to produce an attack on the next exposure.

Dr. Patterson showed a movie of the dog from the time she was placed in the experimental chamber until her attack had subsided and she was removed from the chamber.

BOTANICAL PHASE

Professor Hewson called on Professor Wagner, who discussed several letters that he had received from other groups which are studying similar problems. All the letters indicated that an exchange of information would be helpful.

Professor Wagner then announced that the paper written by Mr. Thomas R. Detwyler entitled "The Ecological Life History of Ambrosia Artemisiifolia L. (Compositae)" was completed and available for distribution.

The group then went to one of the botanical laboratories where a display of the botanical aspects of the project were shown by Messrs. Beals, Payne, and Schwemmin.

METEOROLOGICAL PHASE

Upon reconvening, Professor Hewson introduced Mr. Elder, who outlined the plans for the 1959 preseasonal field experiment.

A. Objectives

1. To determine the rate of pollen production by ragweed plants as a function of meteorological parameters to document the preliminary results obtained during the 1958 experiment.

2. To determine the total source strength of pollen emitted from a small area of ragweed, and to employ this value together with measurements of pollen concentration at an array of points downwind from the source to determine the characteristics of pollen diffusion in a natural wind.

3. To employ the measurements obtained in B-2 below for formulation of a diffusion model that will include rate of deposition of pollen with distance downwind.

B. Description of planned experiment

1. Ragweed source plot.
A group of about 800 ragweed plants will be grown in the Botanical Gardens to mature on or about June 20. About 450 will be transplanted into the soil at the Willow Run Field Station. The plants will be arranged within a 7-ft-diameter circle prior to the maturity of any flowers so that recovery from shock of transplanting will be complete prior to flowering. An adequate supply of soil moisture will be furnished in the event natural rainfall is inadequate. Through this method of planting and supply of moisture, it is believed that natural growth and development of the ragweed flowers will be achieved.

2. Measurement program.

a. Source strength.
Analysis of the 1958 preseasonal array data indicates that measurements of pollen concentration within the ragweed plot fail to give adequate measure of the total amount of pollen actually leaving the plot. Since this value is necessary for a full evaluation of measurement made on the array, effort has been devoted to development of a device to accomplish a measure of source strength. This device, called a whirling arm sampler, will consist of a vertical arm rotating about the perimeter of the plot at a rate of about 450 meters per minute on which flag samplers will be mounted at 1-, 2-, 3-, and 4-ft elevations. Each flag will sample at the rate of about 0.89 m^3/hr and a vertical profile of pollen leaving the plot will be obtained over the period of sampling. Concentrations thus measured will be multiplied by the mean wind speed at each level and the results integrated over the total height to give the total amount of pollen emitted in the sample period.

It is anticipated that samples of only about 10 minutes' duration will be possible during peak periods of emission; during other periods, one-hour samples may be feasible. Such short-period sampling of the source strength will also provide a more accurate measure of the time variation of pollen removal from the plants than has been available in the past.

b. Array measurements.
The 1958 experiment array measurements indicate that for most wind conditions, the 40° array is not broad enough to permit an accurate computation of the character of the pollen "plume." An analysis

of the wind record indicates that a 60°, or greater, arc will be required to permit computation of the deviation of pollen concentration about the maximum value. This will require a number of samplers greater than that employed in the 1958 experiment. To reduce manpower requirements to an acceptable limit, it appears that the more intense effort must be limited to fewer days of observation. This point must be decided after all factors are considered.

Array measurements will be made employing the same type of mast and flag samplers as employed in 1958. Tests of the flag sampler have shown that it is capable of providing consistent measurements, although its exact efficiency of collection as a function of wind speed is still in doubt. The sampling period will again be two hours to permit a sufficient amount of averaging of the short-period, high-concentration bursts of pollen emission.

c. Meteorological measurements.

In an effort to achieve objective A-1, a complete program of meteorological measurements will again be carried on simultaneously with the ragweed observations and pollen concentration measurements. These will include continuous measure of temperature and relative humidity in close proximity to the flowering plants. A record of solar radiation will be maintained at a nearby point, while a complete log of the general weather conditions such as cloudiness, visibility, and rainfall will be accomplished.

The precise relationship between pollen removal from the plants and speed or gustiness of the wind remains in doubt. Instrumentation is being developed to measure this effect. The operation of two or more roto-bar samplers will be controlled by an anemometer in such a way that one sampler will operate at a certain chosen wind-speed increment, while another will sample only at a higher or lower wind speed. In this way pollen concentration will be determined as a function of wind speed to a certain approximation.

A record of wind-direction fluctuation will be maintained near the plot through use of a fast response bivane. These data will be employed in estimating the angular spread of the pollen plume and in evaluating the gustiness of the wind movements. A measure of the vertical profile of wind speed will again be obtained through use of fast-response anemometers mounted at logarithmic intervals on a 4-meter mast.

d. Botanical observations.

Throughout past experiments, measurements of pollen concentration in the air near the ragweed plants has been employed as an index

> of the rate of production of pollen by the plants. Attempts to find correlations between this value and meteorological parameters have shown only limited success. It is possible that the factors governing pollen removal from the plants have obscured the actual rate of pollen productions in these studies.
>
> It is therefore proposed, during the 1959 experiment, to observe the number of dehisced flowers carefully and record them at frequent intervals. In this way the rate of dehiscence, and therefore the rate of potential pollen emission, will be known independently of actual release of pollen into the air. This latter factor may, to a large extent, be dependent on the character of the wind and therefore independent of the factors influencing pollen production by the plant.

Upon the completion of the discussion of the preseasonal experiment, Prof. Hewson asked Mr. Bierly to discuss the new phenological experiment. Mr. Bierly listed the aims of the experiment:

1. To study pollen yield in relation to the climatic history of the plants.
2. To study the allergenic effect of the pollen as a function of climatic history.

A diagram of the proposed site at Willow Run Field Station was sketched, showing the plots that would be used and the location of the meteorological measuring instruments. A Stevenson screen will be placed in the center of the field. A hygrothermograph and maximum and minimum thermometers will be used to measure the relative humidity and the maximum and minimum temperature. Wind direction and speed will be measured by an aerovane on top of Building No. 3 at WRL. Data from the pyrheliometer on top of 5500 East Engineering Building will be used for solar radiation measurements. Soil-water availability will be estimated by observing the water depth in several test holes at WRL. It was emphasized that this experiment was to use standard meteorological field data rather than micrometeorological measurements so that the techniques developed might be adopted to any meteorological station without the need for special instrumentation.

The botanical measurements were then discussed. Mr. Bierly showed several log sheets that would follow the course of the ragweed development. Since this subject poses a great many questions and has yielded few, if any, answers, the measurements that are taken may not be adequate or may be too dense; only time will tell.

It was emphasized that this was a pilot study, not to be considered as statistical at all. The results of this experiment will be used to design a long-term statistical study of the ragweed phenology.

CLOSING COMMENTS

It was decided to hold the next meeting on 8 June 1959 in the School of Public Health.

MEETING OF 8 JUNE 1959

The meeting was held in Room 2522 of the School of Public Health. The following were present: Beals, Bierly, Elder, Field, Gebben, Giever, Gill, Harrington, Hewson, McLain, McLean, Mathews, Patterson, Payne, Remington, Sheldon, Wagner, Willard, White, and Yang.

BOTANICAL PHASE

Dr. Sheldon asked Professor Wagner to begin the discussion of the botanical plans for this summer season. Professor Wagner introduced Mr. Douglas McLain, who will serve as the botanical utility man; Mr. Richard White, who will study the development of the ragweed flowers; Mr. Willard Payne, who will study the migration and evolution of ragweed; and Mr. Alan Gebben who will study the ecology of ragweed.

Before beginning a discussion of the forthcoming work, Professor Wagner asked that everyone please continue to send references on ragweed to him so that the bibliography could be kept current. He pointed out that this bibliography was of great usefulness to anyone desiring a current list of references on any particular aspect of ragweed.

Professor Wagner mentioned that, when Mr. Gerring of NIH was in Ann Arbor, he had asked Professor Wagner about the variability of soils as a factor in pollen toxcity. Professor Wagner had asked Professor Norman of the Department of Botany whether he would be interested in such research should it be included in the new five-year proposal, and Professor Norman had said yes. Professor Wagner said that he would therefore include the aspect in the new proposal.

Mr. White was then introduced by Professor Wagner. Mr. White reiterated that he would study the flowers of the ragweed. He outlined his aims as follows:

(1) To determine the effect of climatic factors on the development of the flowers.
(2) To study in detail the flower itself from primordia to maturity, including the duration of each step in the development.
(3) To determine the structure of the mature flower, including both male and female flowers and the pistillodium.
(4) To describe the mature seed.

Mr. White said that most of his work would be done using slides that have already been prepared; however, some field work would be involved, too.

Professor Hewson asked exactly what would be the value of such a study relative to the present status of the project. Professor Wagner stated that the phenological study would be helped through such study. He also pointed out that the study of flower formation, relation of tapetal fluid to the various phases, steps in the development of the flowers, the nature of mature flowers, and the description of mature seeds were all botanical fundamentals of ragweed which were not well defined or known as yet. Professor Wagner recalled that Wodehouse has a different description of the mature flower from ours. Professor Hewson noted that the Public Health Service has hopes that a break in the life cycle of the ragweed may be found as was in the life cycle of mosquitoes. If this could be done, great studies could be made in controlling the ragweed population.

Mr. Elder asked if Mr. White's research would be with "forced" or natural growing plants. Professor Wagner assured Mr. Elder that only natural plants would be used in the study.

Mr. Payne then described his summer program. He divided his work into several sections:

(1) Continuation of the bibliography on ragweed.
(2) Review of all the collections of ragweed that exist around the country.
(3) Collection of new specimens (six trips of about 5000 miles) from all over the United States.
(4) Determination of the chromosome number of the various ragweeds collected on the field trips.
(5) Growing in the greenhouse of as many different kinds of ragweed as possible.

Mr. Payne hopes to answer the following questions:

(1) What is the origin of ragweed and how has it migrated, including the effect the Indians had on the migration?
(2) Why is ragweed so aggressive?
(3) How many kinds of ragweed are there? (Although supposedly there are 32 species, Mr. Payne feels that there are more probably only 7-10 distinct species.)
(4) What is the evolution of ragweed?
(5) What is the plant?
(6) What is the dispersal mechanism?

The final result would be a monograph on ragweed.

Professor Wagner emphasized that Mr. Payne's problem is basically the broad geographical distribution of ragweed. Professor Wagner feels that ragweed will begin to increase in the Upper Peninsula of Michigan due to the opening of the Mackinac Bridge. Mr. Payne noted that Dr. Pearlman of Oregon was keeping his eye on the increase of ragweed that has been noted in Oregon. Mr. Beals suggested that the dispersal mechanism might be associated with the whole plant rather than just with the seeds alone.

Professor Wagner next outlined briefly the work that Mr. Schwemmin will be doing this summer. He will study the clumping phenomenon and will continue Mr. Beals' work on the pollen grain, including the growth and development of the pollen tube. These studies will emphasize the chemistry of the plant. Mr. McLain and Dr. Ray Holton will be helping with some of the chemical problems.

METEOROLOGICAL PHASE

Dr. Sheldon introduced Mr. Elder, who outlined the final plans for the preseasonal experiment which will start shortly at the Willow Run Field Station.

General Program

Plans for the 1959 preseasonal experiment have been revised to provide for a more intensive study of the relationship between the rate of dehiscence of the ragweed florets and several meteorological parameters of the plant environment. To facilitate intensification of this phase of the study, two plots of ragweed, each containing approximately 400 plants, will be employed. One plot will be termed the "Diffusion" plot and the second the "Phenological" plot.

The experiment will be located at the Willow Run Field Station of the Meteorological Laboratories, an area of uniform level terrain west of the east parking apron of the Willow Run Airport. The "Phenological" plot will be a "C"-shaped plot 10 ft in diameter having a 4-ft-diameter open-center area with the opening toward the northeast. The "Diffusion" plot will consist of a solid planting of ragweed 7 ft in diameter and located 260 ft south-southwest of the other plot. Since north-northwest winds are only rarely experienced, a minimum of sampling time will be lost due to interference of pollen from the "Phenological" plot entering the diffusion measurements. Pollen from the "Diffusion" plot will not significantly affect the phenology study.

Observational Program

1. Diffusion Study.—Four hundred ragweed plants will be planted in the soil to form a 7-ft-diameter circle about 10 days prior to the expected date of flowering. After planting, sufficient water will be added each day so that availability of moisture to the plant will not be a factor in pollen production.

Sampling Program.—Measurements of the spatial distribution of pollen as transport from the plants occurs will be accomplished by sampling at fixed points on an array of masts located downwind and by a "Whirling Arm" sampler at the source. The "Whirling Arm" will consist of a vertical member rotated about the perimeter of the plot at a speed of about 17 mph. Bar samplers will be located at 1, 2, 3, and 4 ft on the rotating member and will thus give an average height distribution of pollen concentration leaving the plot during the period of sample.

A measure of the average wind speed over the same period at each level will permit computation of the total amount of pollen leaving the plot and therefore provide a measure of source strength. Sample duration will vary with concentration as dictated by ability to count the collected pollen. Downwind concentrations will be measured by flag samplers supported on mast levels of 1, 2, 4, 6, 8, 12, 16, and 20 ft. The masts will be located on arcs at 40 and 160 ft distant from the ragweed plot. Spacing of the masts will be at 10° intervals on the 160-ft arc and at 20° intervals on the 40-ft arc. The total angular extent of the arc will be 80°. Duration of the samples will be for a 2-hr period.

Meteorological measurements necessary for evaluation of the diffusion properties of the atmosphere in the vicinity of the study will be accomplished. Wind speed at levels of 0.5, 1, 2, and 4 meters will be measured by low-inertia cup anemometers. Temperature gradient over the 0.5- to 4-meter layer above the surface will be sensed by use of the thermocouples having a low thermal mass shielded from radiation. Wind direction and fluctuations in direction both in the horizontal and vertical directions will be measured by use of a fast-response bidirectional vane.

2. Phenological Study.—Four hundred ragweed plants will be planted in the manner described above. The arrangement of plants will be in the form of a "C" of 10-ft diameter with a 4-ft-diameter center.

Sampling Program.—The rate of pollen removal from the plants will be measured by exposure of roto-bar samplers in the center of the plot. Three samplers, the operation of which is determined by wind speed measured by an aneometer located near the plot, will give a measure of pollen removal as a function of wind speed. Samples will be in intervals of 0-3, 3-11 and 11+ mph. A continuous record of wind speed and a total time of operation of each sampler will be maintained.

Measurements of the wet and dry bulb temperature in the vicinity of the plants will be obtained through exposure of a thermocouple psychrometer within the plot. Approximate leaf temperature of a ragweed plant will be obtained through exposure of a small thermocouple in thermal contact with the leaf surface. Intensity of solar radiation will be measured by exposure of an Epply pyrheliometer at a site near the ragweed plots.

Botanical Observations.—Evidence from the analysis of 1958 preseasonal measurements indicate that the rate of pollen production by the plants may differ from actual pollen emission because of the variability in the effectiveness of pollen removal from the plants by the wind. Thus efforts to find significant correlations between meteorological parameters and observed concentration of pollen some distance from the plants have met with only limited success because of this factor intervening between pollen production and pollen removal from the plants.

The feasibility of observing the rate of opening of the florets of the ragweed plant was demonstrated during the 1958 study. It is believed that this observation is a valid measure of the rate of "potential" pollen emission by the plants, and as such should result in a more meaningful correlation with meteorological parameters effective in determination of plant behavior. Accordingly, observations of the number of dehisced florets on a number of selected spikes will be made at intervals that will vary with the rate of dehiscence. Thus, observations at 15-min intervals may be made over the period of rapid development of the flowers with 1-hr intervals throughout the remainder of the day.

3. General Observations.—A general record of weather conditions will be obtained from the U. S. Weather Bureau office located about one mile from the field test site. In addition, a detailed record of special phenomena such as deposit or evaporation of dew will be maintained at the station.

In the discussion that followed, Messrs. Beals and Gebben suggested that soil moisture be measured in the plots and outside the plots. Mr. Elder stated that he hoped to get rid of this variable by giving the plants all the water they needed.

MEDICAL PHASE

Dr. Sheldon then asked Dr. Willard to give a preliminary report on the wind-tunnel studies. Dr. Willard stated that by exposing ragweed-sensitive people to pollen, it was hoped to find:

(1) The amount of pollen needed to create symptoms.
(2) The time of onset of symptoms.
(3) Differences in new and old pollen.

The return duct of the wind tunnel in the basement of the East Engineering Building was used as explained during an earlier meeting. A total of nine patients was used, divided into one group of five and another of four. The two groups were balanced as to medication, allergenicity, etc.

The patients' vital capacities were checked before each test run began using 1 sec, 2 sec, and total vital capacities. The maximum expiration flow rate of the first 200 cm of air expelled was also figured.

Dr. Willard then discussed the five weeks of experiments which are summarized in the table on the following page.

Patients were seated in the south return duct of the wind tunnel facing into the air flow. Pollen was continuously emitted into the air stream at a nearly constant rate upwind from the fan. Thus a relatively uniform distribution of pollen was attained in the environment of the patients due to diffusion and mixing of the pollen by the fan.

Measurements of pollen concentration in the vicinity of the patients were accomplished by exposure of two roto-bar samplers, one near the front and one

	1957 Commercial Pollen									1-Mo.-Old Greenhouse Pollen			New Pollen		
	1a	2a	3a	1b	2b	3b	1c	2c	3c	1d	2d	3d	1e	2e	3e
P^F	2780	1875	2510	2480	1435	4150	1400	1920	3050	51	2590	--	2450	1700	2580
P_R	2240	1470	2600	1170	1960	2140	2620	1700	2200	56	2020	--	3120	1715	2005
Te				81	79	71	78	79	77	73	75	--	77	75	75
RH				57	47	48	61	65	48	46	65	--	69	41	48
Ti	45	45	45	45	45	45	40	45	45	43	45	--	60	60	60
Sx:															
DS	0	0	0				--	--	--						
JW	0	0	0				4+	4+	4+						
KB	±	0	±				±	0	±						
EJ	0	0	0				0	0	0						
MH	0	0	0				0	0	1				0	0	0
LY				1+	1+	2+				0	2+	--	1-2+	1+	--
CS				1+	±	2-3+				0	1+	--	1+	1+	1-2+
RS				±	2+	1+				±	3+	--			
HL				3+	--	--				--	--	--			

P = pollen conc. gr/m^3 (F = front, R = rear roto-bar sampler)
Te = temperature
RH = percent relative humidity
Ti - time of exposure in minutes
Sx = symptoms — graded 0 to 4+ (4+ = patients most severe symptoms during ragweed season)

near the rear of the tunnel return, at about the height of the patient's head. Sample duration was for the period of exposure of the patient, so that a representative value of the accumulated dose of pollen was obtained. Measurements of temperature, relative humidity, and wind speed were made in the environment of the patients.

The results of the pulmonary function tests showed no correlation of the tests with pollen concentrations or with patient symptoms. However, the question whether symptoms could be obtained with a longer exposure remains unanswered.

The conclusions of the test may be summarized as follows:

(1) Old pollen is as potent as the new pollen.
(2) Repeated exposure seems to cause symptoms or an increase of symptoms.
(3) There seems to be a certain threshold concentration necessary to cause symptoms. This concentration is 2000-2500 grains/m^3.

Mr. Beals pointed out that the 2-hr germination period that had been used as a criterion in determining "new" pollen might have nothing to do with the antigenicity of the pollen. He also suggested that the vacuum method of collection might make a change in the pollen.

Dr. Richard Field was introduced by Dr. Sheldon. Dr. Field gave a brief resume of the work that had been done on altering the ion concentration of the air and the effect these changes in concentration might have on hay fever. Dr. Field showed the Wessix machine that had been used in the guinea pig chamber.

The experiments were designed to make changes in some well-known immunological tests. Ionization levels were varied while the temperature and flow of air into and out of the chamber were controlled so as to keep the environment stable.

The result can be stated briefly. There was no difference in the first observable symptoms or of death in the guinea pigs as was usually noted. Both positive and negative ions were used. Dr. Field pointed out that more tests are needed to verify the result of the previous tests.

CLOSING COMMENTS

Mr. Elder reported briefly on the phenological experiment which is underway at Willow Run. Mr. Elder and Professor Wagner decided to use the field east of Building No. 3 for the experiment with the same design as reported previously. Meteorological measurements are being taken regularly, but a good measure of plant growth is still lacking. Mr. Gebben and Professor Wagner are looking into this problem.

Dr. Sheldon suggested that meetings be called during July and August only if needed.