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ATMOSPHERIC POLLUTION BY AEROALLERGENS

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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, airborne 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.

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 statisticians working together in close cooperation. As in past years, this team effort has proven very useful in gaining new knowledge of the several areas under investigation by project personnel.

Botanical research has been concerned with proper maintenance of ragweed cultures, method of bettering ragweed classification, comparisons of chromosomes and pollens of ragweeds, estimations of the abundance of ragweeds, reactions of the plants to introduction into various types of habitats, and field and laboratory investigations on ragweed germination.

In medical research, work has been undertaken to isolate allergens from ragweed pollen extracts by combining a physical method ion-exchange column chromatography, with separation on the basis of immunologic specificity. Ragweed pollen extract and human serum globulins containing skin-sensitizing antibody (SSA) were fractionated separately on diethylaminoethyl cellulose columns. Reaginic globulins adsorbed to the cellulose column were then used to absorb ragweed allergens, after which the allergens were eluted by manipulation of pH and salt concentration. Although minute quantities of allergen were obtained in this way, they were contaminated with serum proteins, including reagin, and probably also with nonallergenic constituents, since normal serum globulins absorbed and eluted allergen as well as reaginic serum globulins.

Preliminary experiments showed that washed ragweed pollen grains may be a useful immunosorbent for SSA. Attempts to demonstrate SSA on the leukocytes of atopic ragweed-sensitive subjects by layering with ragweed extract and then staining with fluorescein conjugated rabbit anti-ragweed globulin yielded negative results. Both washed normal and atopic leukocytes fluoresced after exposure to fluorescein conjugated rabbit anti-human globulin.

Guinea pig intradermal tests have been found useful in evaluating the components of allergenic emulsions. Mouse intraperitoneal toxicity tests appear helpful in evaluating irritative properties of allergenic emulsions themselves. Since the institution of routine testing of emulsions in laboratory animals, no significant irritative reactions have been noted in a large number of patients treated with emulsions.

The difficult problem of properly controlling pollen dispersion into the human pollinosis test chamber has largely been solved by employing a modified

Wright Dust Feed Mechanism. Progress has been made toward an objective evaluation of subjects with hay fever by simultaneously recording pressure drop and flow through the nasal passages with the aid of a catheter in the oropharynx.

In an epidemiological study of a total community (Tecumseh) of about 8900 persons in southeastern Michigan, data on the cumulative prevalence rates for asthma and hay fever showed that the former more frequently began at an earlier age than the latter. In 75% of persons having a history of both asthma and hay fever, asthma developed first or the two conditions appeared within the same year of age. Of all persons with hay fever who were at risk to develop asthma, the condition subsequently developed in only 5 to 10%. The data imply that allergists are exposed to a segment of the hay fever population in which the prevalence of asthma is disproportionately high.

Meteorological research has consisted primarily of two studies. In a study of rural and urban air pollution by ragweed pollen, low annual ragweed was found to grow chiefly in cereal grain fields. During the period of emission in the morning, rural pollen concentrations exceeded those in urban areas. During the afternoon and evening the pollen concentration steadily decreased everywhere, with very little difference evident between urban and rural areas. In a study of washout of ragweed pollen by rainfall, data gathered in two rains were analyzed, with emphasis on some of the restrictions imposed by present theoretical work on the nature of washout of particulates from the atmosphere by rainfall.

During the past year the biostatistics group provided statistical consultation to the investigators in botany, meteorology, and allergy concerning experimental and study design, data collection, data processing, statistical analysis, and the writing of reports.

1. BOTANICAL PHASE

by

A. I. Gebben, W. W. Payne, and W. H. Wagner, Jr.

1.1 INTRODUCTION

The botanists have continued to receive the cooperation of their fellow researchers in other disciplines involved in this study of ragweed hayfever. During the past year the botanists have carried out studies of proper maintenance of ragweed cultures, research on bettering ragweed classification, comparisons of chromosomes and pollens of ragweeds, estimations of abundance of ragweeds, reactions of the plants to introduction into various types of habitats, and field and laboratory investigations on ragweed germination. Two of the botanists, A. I. Gebben and W. W. Payne, have used or will use their research in connection with this project as a basis for theses to be submitted in fulfillment of the requirements for their Doctor of Philosophy degrees.

1.2 THE MAINTENANCE OF RAGWEED CULTURES

In connection with studies of ragweed hayfever and related botanical problems, it is necessary to maintain viable seeds that can be used in growing plants for experiments. Willard Payne, in collaboration with W. F. Kleinschmidt, Superintendent of The University of Michigan Botanical Gardens, has worked out techniques for overwintering seeds of northern races of ragweeds, which they recently published.[1] The results of this work are briefly summarized below.

Fruits (i.e., the fruiting involucres) of the most important ragweeds found in the eastern and midwestern United States are shown in Fig. 1 (D-H). The fruits of the species illustrated are dormant when first matured. This dormancy is broken under natural conditions by the external factors associated with the winter season. They fall to the soil, are wetted by the late autumn rains, and are subjected during the succeeding months to low temperatures. They germinate in the spring after a period of fluctuating, diurnal temperatures.

For artificial storage and germination of ragweed seeds, it was decided that treatments that most nearly simulate natural conditions would be the most effective. Accordingly the following procedure was developed:

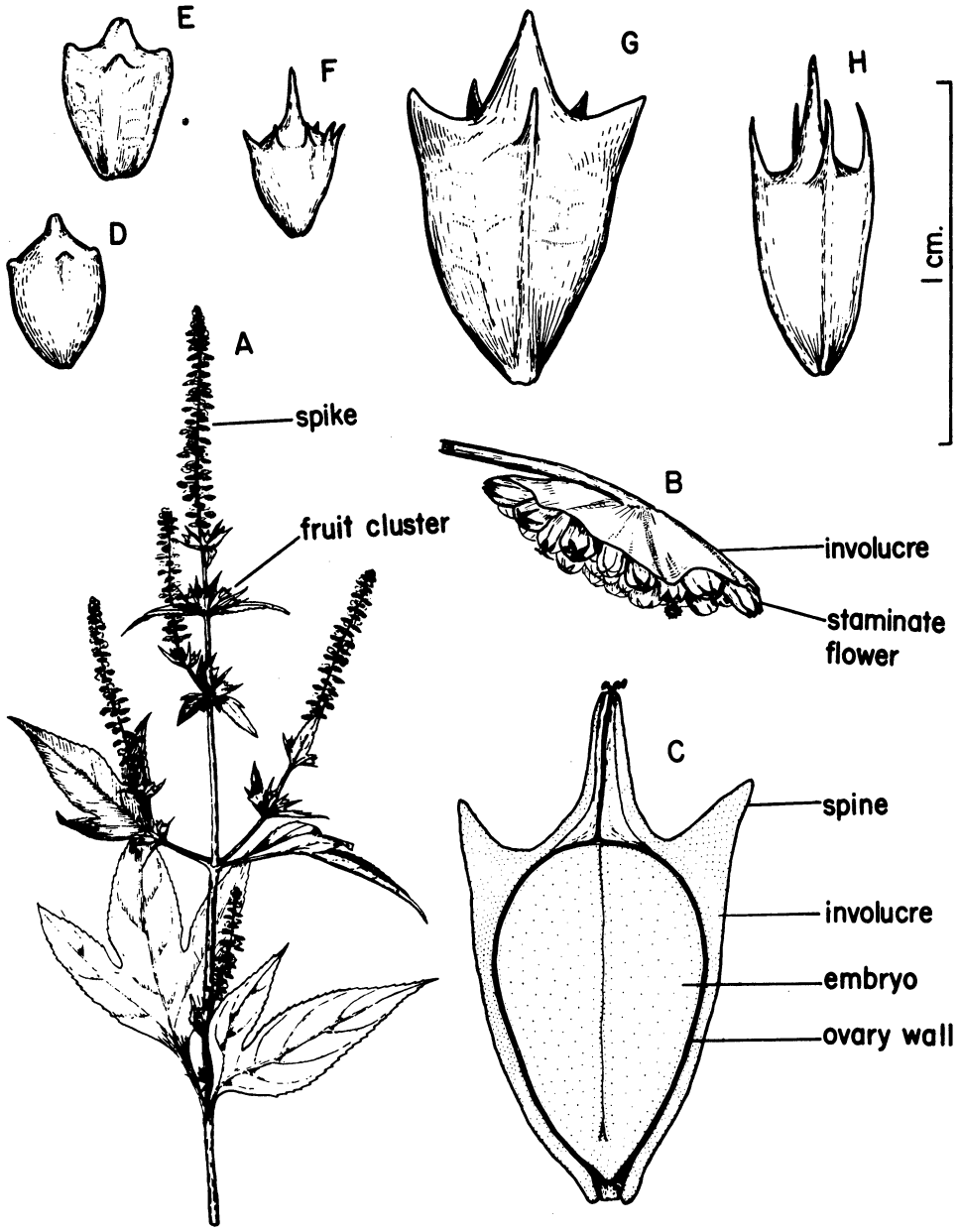

Fig. 1. Ragweed inflorescence and fruit types. A, Flowering branch of gaint ragweed, Ambrosia trifida, showing the position of male spikes and fruit clusters. B, Enlargement of male head. C, Longitudinal section of a mature fruit. D, Fruit of common eastern expression of perennial ragweed, A. psilostachya DC. (A. coronopifolia T. & G.). E, Fruit of western expression of perennial ragweed, A. psilostachya DC. (typical). F, Fruit of common or short ragweed, A. artemisiifolia L. G, Fruit of giant ragweed, A. trifida L. H, Fruit of southern ragweed, A. bidentata Mich.

(a) In the fall, the fruits are stripped from the plants and dried.

(b) The material thus collected is winnowed to remove the fruits from the accompanying debris.

(c) The cleaned fruits are placed in small, cotton bags suitably labeled for identification, and are soaked in warm water for an hour.

(d) The bags are then placed in flats containing an inch of clean sand, and covered with another inch of sand.

(e) The flats are watered well, and placed in open cold frames where they are exposed to full winter conditions.

(f) In the early spring, the flats are brought indoors with their contents still frozen. The bags are removed and either they are stored in a freezer or the fruits are dried and stored at ca. 20°C in loosely covered containers.

Seeds treated in this manner are from 60 to 90% germinable, and they maintain a high degree of germinability for a year or more. Seeds of all of the species illustrated in Fig. 1 were found to respond well to this treatment, and a number of other species have been found to react just as favorably.

1.3 FIELD AND GREENHOUSE STUDIES OF RAGWEED TAXONOMY

Since Progress Report No. 4 was written, W. W. Payne conducted a field trip to the southwestern United States. The trip yielded valuable field observations and materials for laboratory and herbarium investigations that bear on the inter-relationships of western ragweed species with those of the eastern United States. Among the results of these studies is the conclusion that two groups of plants previously considered by most workers to comprise the distinct genera Ambrosia (ragweeds) and Franseria (false ragweeds) are actually all members of a single genus. The false ragweeds should thus be classified with the "true" ragweeds in a single genus, Ambrosia. This conclusion is based upon the following observations:

(a) The single criterion that has been used to separate these plants into two genera is whether the spines of the mature fruiting involucres are scattered over the entire surface (Franseria) or are localized more or less in a single whorl just below the terminal beak (Ambrosia). However, several species are intermediate with respect to this character. For example, Franseria confertiflora tends to produce many spines, but they are inserted on the upper half only of the fruiting involucre. Specimens of another "false ragweed," Franseria acanthicarpa, often possess two fruit types on the same plant—some which have many, scattered spines, and some which are

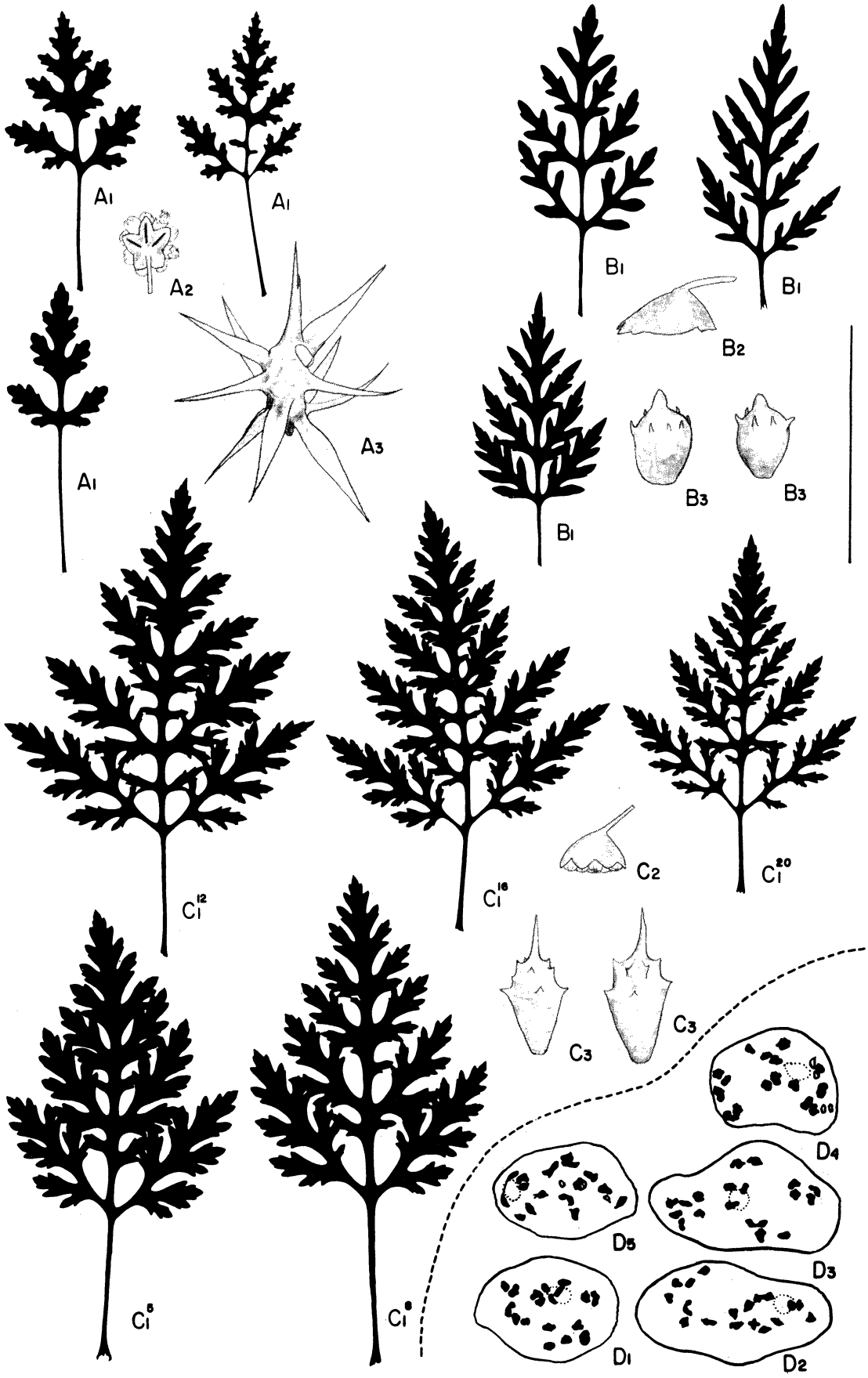
A1
A1
A2
A3
A1
B1
B1
B2
B1
B3
B3
C1 12
C1 16
C1 20
C2
C3
C3
C1 8
C1 6
D4
D5
D3
D1
D2

Fig. 2. Ambrosia artemisiifolia x Franseria acanthicarpa. A, Franseria acanthicarpa, the pollen parent. A_1 Representative leaves; A_2 representative staminate involucre; A_3 representative fruiting involucre. B, Ambrosia artemisiifolia, the seed parent. B_1 Representative leaves; B_2 representative staminate involucre; B_3 representative fruiting involucres. C, Characters of the hybrid. C_1 Leaves of hybrid (superscripts denote nodes from which leaves were taken); C_2 representative staminate involucre; C_3 representative fruiting involucres. D, Meiotic figures drawn with the camera lucida. D_1 to D_3 Diakinesis showing 18 bivalents; D_4 to D_5 diakinesis showing mixture of many bivalents (black and stippled chromosomes) and few univalents (white chromosomes). Scale at right equals 10 cm for leaf silhouettes and 10 mm for involucre drawings.

entirely devoid of spines. The fruiting involucres of giant ragweed, Ambrosia trifida, typically have spines inserted just below the beak, but in two or more poorly defined whorls. Thus, separation of the different species of "ragweeds" and "false ragweeds" on the basis of the arrangement of spines on the fruiting involucre is by no means clear-cut, and strongly indicates that the criterion is probably not a valid one at the generic level.

(b) If the members of the ragweed-false ragweed complex are compared on the basis of all their other characteristics, it can be seen that there are several species traditionally placed in the genus Ambrosia which are apparently more closely related to certain species of Franseria than they are to other species of Ambrosia. For example, Ambrosia pumila, a somewhat weedy plant restricted to San Diego County, California, appears to be closely related to Franseria confertiflora. Ambrosia hispida, a strand species found throughout the Caribbean region, is also probably most closely related to Franseria confertiflora. Ambrosia polystachya, a species of central Brazil, can hardly be distinguished on most of its characteristics from Franseria artemisioides, a plant of Ecuador and Bolivia. Ambrosia artemisiifolia and A. trifida are probably closely related to Franseria acanthicarpa.

The result of separation of the species into two genera thus makes Ambrosia, as currently interpreted by botanists, an unnatural assemblage of only distantly related species-groups, which are themselves most closely related to species found in another genus. Ambrosia is therefore a polyphyletic and artificial genus. The goals of taxonomic expression would be better served by combining the species heretofore separated into two genera, Ambrosia and Franseria, into one genus. Such a treatment would more accurately indicate the evolutionary patterns of the species.

(c) A hybrid (see Fig. 2) has been created in the laboratory between Ambrosia artemisiifolia, the common ragweed, and Franseria acanthicarpa, one of the so-called "false ragweeds." Studies of the cytology of this hybrid gave strong evidence supporting the above conclusions. During the formation of pollen cells by the hybrid, it was discovered that nearly perfect pairing of homologous chromosomes occurred, indicating that the relationships of its parents are much closer than would be expected if they belonged to separate botanical genera. Further evidence of their close relationship was found in observations of the pollen of the hybrid: Approximately 30% of the pollen grains appeared to be normal and viable (as indicated by staining techniques), and 41% of the seeds were viable (11 seeds germinating out of the 27 seeds tested).

For these reasons—i.e., the ill-defined morphological basis for generic segregation, the criss-crossing of relationships between members of the allegedly distinct genera, and the nature of an experimentally produced "intrageneric" hybrid—some important taxonomic changes will have to be made. This will involve the transfer of a large number of specific names now in the genus Franseria to the genus Ambrosia. Some of these species are Franseria

acanthicarpa, F. confertiflora, and F. tomentosa, plants which in the western United States are becoming widespread and locally abundant. These as well as a number of others now placed in Franseria should henceforth be treated in the literature of allergy as species of Ambrosia. The necessary name transfers will be made in the near future, as soon as the appropriate bibliographic and nomenclatural research has been completed. In the meantime, it is expected that further research on the evolutionary relationships of ragweeds will provide additional information bearing on the validity of the traditional separation of "ragweeds" and "false ragweeds."

1.4 CHROMOSOMES OF RAGWEEDS

Of immediate interest to our current investigations are several previously unreported chromosome counts made by W. Payne. These are presented in Table 1, and drawings of these chromosomes (as shown in acetocarmine preparations), as well as other drawings that support previously reported numbers, are shown in Fig. 3.

With the additions shown in Table 1, data now exist which show that all

TABLE 1

New Chromosome Counts In the Genus Ambrosia (Including Franseria spp) Which Have Not Appeared In Previous Literature

Species	Haploid Chromosome Number
Ambrosia acanthicarpa	n = 18
Franseria ambrosioides	n = 18
Ambrosia confertiflora	n = 36
Franseria ilicifolia	n = 18
Ambrosia peruviana	n = 36
Ambrosia psilostachya	n = 72
Ambrosia pumila	n = 72
Franseria tomentosa	n = 18

but two of the species of Ambrosia found in the United States have chromosome compliments of n = 18 or multiples of 18 (i.e., 36, 54, and 72). The remaining two species have fewer than 18 chromosomes (viz. A. bidentata with n = 17; and A. trifida with n = 12). Both of the latter species are in other respects specialized plants in which the lower chromosome numbers are probably aneuploid derivative of the primitive condition of n = 18. Thus their chromosome compliments do not represent primitively lower numbers for the genus. The basic chromosome compliment of the ragweed ancestor is, therefore, probably n = 18.

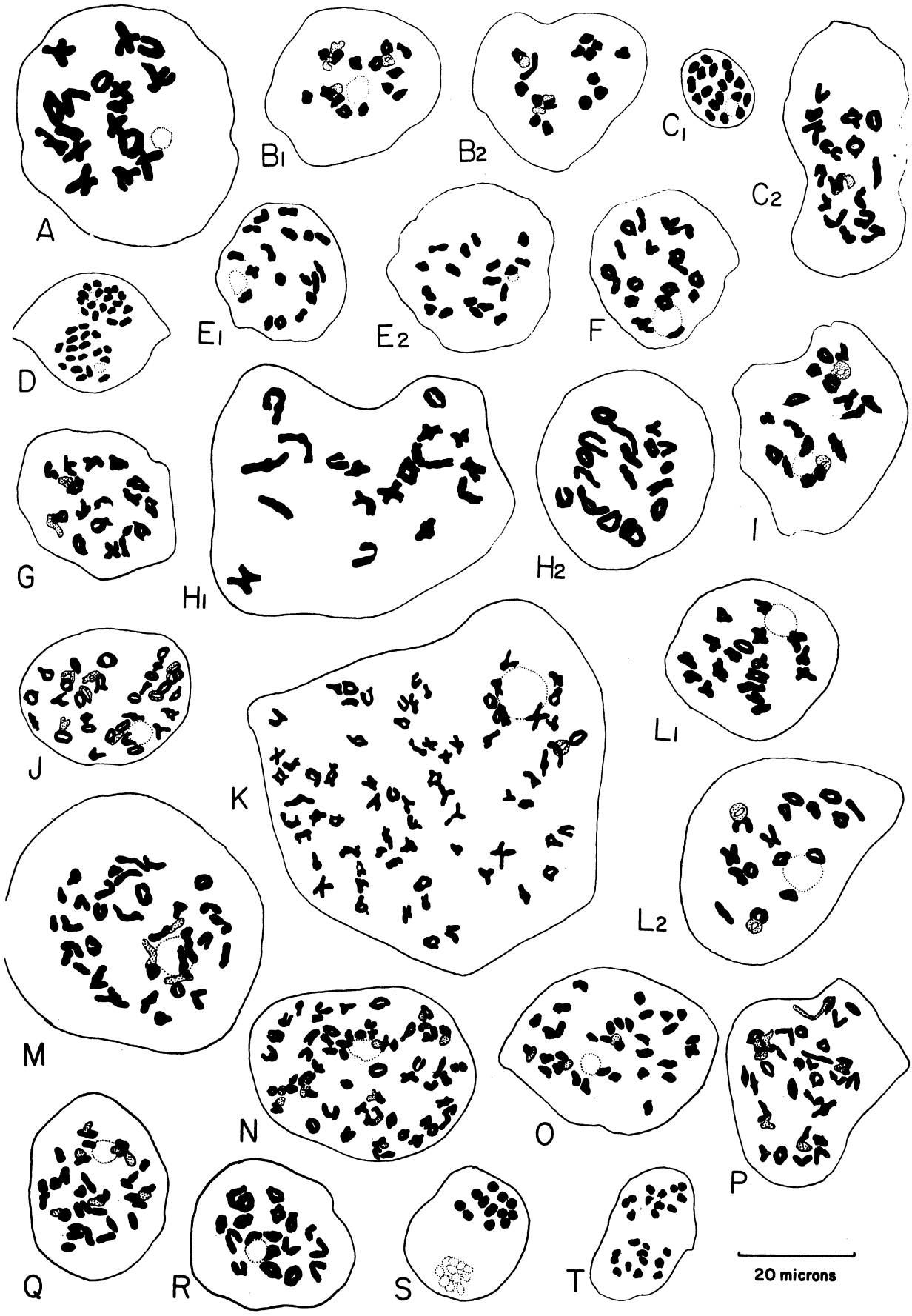
A
B1
B2
C1
C2
D
E1
E2
F
G
H1
H2
I
J
K
L1
L2
M
N
O
P
Q
R
S
T
20 microns

Fig. 3. Meiotic chromosome figures in the Ambrosieae drawn with the aid of the camera lucida from male floral material. Vouchers for these figures (cited in parentheses) are filed in The University of Michigan Herbarium.

(A) Iva annua; n = 17 (Payne, 1944, Athens County, Ohio). (B) Ambrosia acanthicarpa; n = 18 (Payne, AHI-2323; Madera County, California). (C) Franseria ambrosioides; n = 18 (Payne, 2699, Maricopa County, Arizona). (D) Ambrosia artemisiifolia; n = 18 (Payne, AOT-b-3034, Laramie County, Wyoming). (E) A. artemisiifolia "var. maritima"; n = 18 (Payne, AIR-2324, Nancy, France). (F) F. bipinnatifida; n = 18 (Payne, 2733, Marin County, California). (G) F. bipinnatifida "x chamissonis"; n = 18 (Payne, 2727, Marin County, California). (H) A. bryantii; n = 18 (Porter, 456, Baja California, Mexico). (I) F. chamissonis; n = 18 (Payne, 2735, Marin County, California). (J) A. confertiflora "var. tenuifolia"; n = 36 (Payne, AIG-2984, Los Angeles County, California). (K) A. hispida; n = 72 (Payne, AHK-a-3142, Monroe County, Florida). (L) F. ilicifolia; n = 18 (Payne, AJO-3089, Yuma County, Arizona). (M) A. peruviana; n = 36 (Payne, AIU-b-3143, Jamaica, West Indies). (N) A. psilostachya; n = 72 (Payne, ALE-2975, Davis County, California). (O) A. psilostachya; n = 36 (Payne, ALC-3347, Tulsa County, Oklahoma). (P) A. psilostachya (var. coronopifolia); n = 36 (Payne, 1619, Bay County, Michigan). (Q) A. pumila; n = 36 (Payne, 2718, San Diego County, California). (R) F. tomentosa; n = 18 (Payne, 2621, McPherson County, Kansas). (S) A. trifida; n = 12 (Payne, AHU-2525, Lenawee County, Michigan). (T) A. trifida; n = 12 (Payne, AHN-2319, Leiden, Netherlands).

1.5 STUDIES OF RAGWEED POLLENS

To facilitate our study of ragweed pollens, more than 300 slides of acetolyzed grains have been prepared for investigation. These include not only species of Ambrosia and its relatives (Iva, Hymenoclea, and Xanthium), but species of other genera of possibly related tribes of the Compositae as well. Two sets of these slides have been retained by workers in this project. Other sets have been filed in the permanent collections of pollen and spores at The University of Michigan, and individual slides have also been attached to the herbarium sheets from which the pollen samples were removed. The slides are available through loan to any investigators in other institutions concerned with similar studies.

Although our work on the pollen preparations is not yet complete, the preliminary investigations indicate that (1) The ragweeds and their relatives comprise a distinct tribe of the Compositae, and should be recognized as the Ambrosieae; and (2) Fine structure of the pollen wall indicates that the Ambrosieae may be more closely related to the Anthemidieae (the Compositae tribe containing the tansies, chrysanthemums, et alii) than they are to the Heliantheae (the sunflower tribe) in which they are commonly included. It is hoped that our work will establish criteria for identifying species and species-groups of ragweeds by their pollen.

1.6 A TOWNSHIP SURVEY OF RAGWEED OCCURRENCE AND ABUNDANCE

To evaluate the behavior of ragweed plants in experimental studies, we attempted to estimate the presence and abundance of common ragweed in the Ann Arbor area during the 1960 field season. A previous survey performed as part of a Mobile Sampling Experiment by a team of meteorologists connected with this project in 1958[2] demonstrated the greater abundance of ragweed plants and pollen in rural areas than in urban areas. Their survey also showed that the abundance of ragweed plants and pollen in rural tilled areas exceeded that in rural untilled land. Our present survey was conducted within parts of Ann Arbor and Superior townships of Washtenaw County, the same county in which the Mobile Sampling Experiment had been conducted. Ragweed abundance was scaled in density classes from "0" through "4," as indicated in Table 2.

Our sampling method involved assigning land use classification along selected roads and highways according to a number of categories, as listed in Table 3.

TABLE 2

Abundance Scale Used In a Township Survey

Index	Criterion
0	No ragweeds observed
1	Ragweeds present, but averaging less than 0.5 plants per square meter
2	Ragweed density averaging from 0.5 to 1 plant per square meter
3	Ragweed density averaging from 1 to 10 plants per square meter
4	Ragweed density averaging over 10 plants per square meter

TABLE 3

Abundance of Ragweeds According to Land Use Categories

Land Use Category	Percent of Areas Represented in Each Abundance Scale Rating (Table 2)				
	0	1	2	3	4
Cropland—Corn	8.5%	50%	33%	8.5%	0%
Cropland—Wheat	0	0	0	33	67
Cropland—Oats	0	0	33	33	33
Alfalfa Meadow	75	25	0	0	0
Pasture	81	0	9.5	0	9.5*
Grass Meadow	91	0	0	9	0
Parklands	90	10	0	0	0
Woods	100	0	0	0	0
Marshes	100	0	0	0	0
Roadsides	87	10	1	2	0
Residence Property	100	0	0	0	0
Soybeans	0	50	50	0	0
Clover and Clover Mixtures	40	0	40	20	0
Timothy and Timothy Mixtures	100	0	0	0	0
One to Three-Year Abandonment	0	44	14	28	14
Summer-Fallowed Fields	50	50	0	0	0

*This value is based on observations of pastures used exclusively by swine at the time the study was made.

The results of this survey corroborate those of the Mobile Sampling Experiment, and of a similar investigation in Lenawee County, Michigan, by Payne and Gebben in 1958. The greatest density of ragweed occurred in the cereal grain crops. Densities were especially high in fields of winter wheat and oats. Because of the common crop rotation pattern of plowing under the oat stubble for winter wheat planting, the ragweed population in oats often produces a light pollen yield. The oats is harvested prior to the heavy pollen season in mid-August and, as oats is a short-stemmed grain (especially on light soils), it is often cut close to the ground. Primarily vegetative parts of the plant are left after harvesting. Shorter plants unharmed by harvesting and lateral branch inflorescences on cutoff plants can bear pollen. However, even the latter plants may be destroyed prior to anthesis by early fall plowing as farmers generally plow for wheat as soon as the surface soil moisture is high enough. In the crop rotation scheme, wheat is often seeded to a cover crop which will serve as a hay crop in the year subsequent to wheat harvest. As such, the wheat stubble is often left unplowed or untilled in the autumn. As wheat is a long-stemmed grain, it is usually cut a few inches higher than oats. This leaves more ragweed inflorescences per plant for pollen production. For these reasons, the pollen production from wheat fields is often very great, and we propose that wheat fields produce more pollen than any other source in the Ann Arbor area. Cultivated row crops such as corn and soybeans are not densely populated with ragweeds, although the plants which are present are often large, many-branched individuals with a high total pollen potential. Some farmers use "2,4-D" weed control sprays, which decreases the pollen potential in corn fields.

The widespread opinion that common ragweed is primarily an occupant of tilled sites is substantiated by the survey. First-year clover fields and clover-timothy hayfields usually support ragweed densities from 0.5 to 10 plants per square meter. Second- and third-year clover fields exhibit much lower densities of ragweed as well as lower densities of other annual weeds. Pastures, lawns, meadows, woodlands, and marshes are generally free of common ragweed. Roadsides, both those sprayed for weed control and those left unsprayed, exhibit low ragweed densities. Out of 122 (i.e., 87%) of the roadside plots surveyed, 104 were of the "0" density class.

1.7 GROWTH AND PROPAGATION OF RAGWEEDS TRANSPLANTED INTO VARIOUS VEGETATION TYPES

During the spring of 1960, ragweeds were transplanted into a variety of vegetation types at The University of Michigan Botanical Gardens. The sites were tilled in 1959 and 1960: an oak-hickory woodland, a floodplain woodland, an herbaceous marsh, a clover meadow, and a grass meadow adjacent to a forb meadow on a dry site. The plants were started in the greenhouse from seed in April and transplanted to the sites during the second week in May. The plants were established in meter-square plots with 25 plants per plot. Three

replicate plots were planted at each site. Periodic records were kept of growth in height and of phenological stages in development. The plants used in the original transplanting were selected for uniformity in height and the number of nodes visible at planting time. The difference in average final height at maturity is given in Table 4. The tallest plants were produced on the 1959 tilled site.

TABLE 4

Height, Node Number, Percent Survival, and Seedling Number of Ragweeds Transplanted Into Various Habitats

Site	Average Final Height (cm)	Average Number of Nodes on Plant Axis	Percent Survival	Average Number of Seedlings Produced Per Square Meter
1959 Tilled	58.9	18.6	100.0	406.0
1960 Tilled	49.6	14.7	98.7	--
Oak-Hickory Woodland	37.0	13.9	76.0	0.0
Floodplain Woodland	37.5	15.7	73.3	0.0
Herbaceous Marsh	55.3	13.1	93.3	0.3
Clover Meadow	46.6	17.0	98.7	122.7
Grass Meadow (dry site)	23.7	12.5	94.7	6.0
Forb Meadow (dry site)	27.3	12.1	96.0	30.3

The plants on the 1960 tilled site were also among the taller plants recorded (49.6 cm av). Ragweeds transplanted into nontilled types of vegetation, such as meadow, marsh, and woodland, varied in height with the height of the surrounding ground cover plants and the nature of the substrate. Ragweeds in the herbaceous marsh (av height 55.3 cm) were taller when they grew interspersed with Joe-pye weed (Eupatorium maculatum) than when they grow interspersed with members of a clone of sensitive fern (Onoclea sensibilis). A comparable contrast was noted among the plants growing in the clover meadow; the taller ragweeds grew among the tall cover, and the shorter ones grew among the short clover. The over-all survival of ragweeds in the various habitats was high (see Table 4). All plants survived the original transplanting, with survival at maturity varying from 73.3% in the floodplain woodland to 100% in the 1959 tilled area. It is interesting to note that the two sites on which survival was lower than 90% at maturity are both woodland sites.

Another parameter measured to assess the vigor of ragweeds on various sites was the number of nodes on the plant axis at maturity (cf. Table 4). The plants which exhibited the greatest average number of nodes were those from the 1959 tilled site. These plants were also taller than those from other sites. Those with the fewest nodes (12.5 av) were also the shortest plants (23.7 cm av). Within these two extremes the correlation between height and number of nodes was not complete. The plants growing in the marsh and the woodland habitats tended to develop a tall and "spindly" (i.e., etiolated) habit with widely spaced nodes throughout. The plants which grew in the open (i.e., tilled) areas tended to have closely spaced nodes; hence, the taller the plants, the greater the number of nodes it possessed. Plants grown in the meadow sites tended to exhibit widely spaced nodes near the base, and closely spaced nodes near the apex of the plant. Although the number of nodes on the plant axis is not directly related to the seed potential of the ragweed plant, the number of nodes does indicate the possible number of lateral branches and hence is indirectly related to total seed potential and total pollen potential of the plant. We feel that an index based upon the total number of nodes of a plant (i.e., axis nodes plus nodes of the lateral branches) would be more accurately predictive of the seed and fruit potential of the plant than one based on the number of nodes of the central axis alone. It is planned to explore such an index in our future work.

Although data on the actual seed production per plant was obtained, these data are not yet ready for publication. However, actual counts of seedlings produced the following growing season are given in Table 4; in each instance they are the averages from counts in three replicate plots. Ecologically, the actual or realized seedling crop produced in the subsequent growing season is much more significant than is the potential seedling crop expressed as seed crop during the year of study. The results in Table 4 show that the number of seedlings produced in the 1959 tilled site far exceeded those produced on any other site, averaging 406 seedlings per sq. meter. As 25 plants per sq. meter were planted on this site, the observed number of seedlings represents a high potential for ragweeds in the succeeding season. It is interesting to note that the marsh and woodland sites showed few or no seedlings for the reproduction of ragweeds in the succeeding season. Ragweeds grown in these sites did produce seed during the 1959 season, and this seed appears in all respects to be normal and viable. Actual viability tests of this seed material have not been completed. The observed results of the growth, survival, and seedling production of plants grown in the various habitat sites seems to indicate that the persistence of ragweeds on tilled sites and their lack of occurrence on other sites is related to both the increased vigor of plants grown on tilled sites and to the far superior method of propagation through seed germination on tilled sites. Supporting evidence for the favored germination of ragweeds on tilled sites is given in the following section.

1.8 LABORATORY AND FIELD STUDIES OF RAGWEED SEED GERMINATION

Many researchers have considered the longevity of the seed in explaining the presence of annuals on tilled sites. The fact that ragweed seeds are viable over long periods has been well established by experiments initiated by Beal at East Lansing and by Duvel at Arlington. E. Brown, reporting in 1946 on the Duvel experiments, mentions 84% germination for common ragweed after 21 years, 57% after 30 years, and 22% after 39 years. We have collected soil samples in 1-inch increments in Wayne Co., Michigan, at the Willow Run Airport site, in which it is known that the land has not been tilled for at least 14 years. Samples to the depth of the plow zone at 7 inches produced ragweed germination in excess of one seedling per sq meter.

Our experimentation with the depth at which ragweed seeds germinate leads us to believe that most ragweed seedlings arise from seeds germinating at or slightly below the surface of the soil. In both laboratory and field experiments initiated in April, 1961, we observed the highest percentage of germination in surface plantings. Emergence at the 1-inch depth was significantly higher than controls, although numbers of seedlings emerging from the 2-inch depth were not significantly different from the controls.

The temperature requirements for ragweed germination also tend to indicate that ragweed is adapted to surface germination. Although some ragweed germination occurs at temperatures of 3-5°C over a prolonged time interval, germination below the "threshold" temperatures of 11-16.5°C was less then 5% over a 5-day period. Above this seeming "threshold" temperature, ragweed seed germination increased with increase in constant temperature up to 60% germination at 25°C. Seeds placed in an alternating temperature of 16.5° and 25°C showed only 44% germination, whereas those placed in alternating temperatures of 3.5, 7, or 11°C and at 25°C gave from 71-80%. We conclude from this evidence that ragweeds collected at this latitude are adapted to an alternating temperature regime that closely approximates the normal diurnal fluctuations of the early spring months. These diurnal fluctuations would occur at the soil surface, and at most only a few centimeters below the surface.

The data shown in Table 5 indicate that disturbance of the soil surface influences ragweed germination. The average number of ragweeds emerging from seeds planted in a loamy sand covered mainly with perennial forbs and grasses is shown in this table. The emergence in the control plots averaged 26.9%; in plots in which the surface was only scarified it averaged 42.8%; and in tilled plots it averaged 69.5%. These results indicate that factors inherent in the nature of the tilling process itself influence ragweed germination and emergence. These factors probably include changes in the physical properties of the soil (e.g., aeration and drainage) and perhaps alterations in the availability of nutrients and the production of toxins by associated species which inhibit germination.

TABLE 5

Number of Ragweeds Emerging From 21 Seeds
Planted in Each Replication

Replicate		Treatment		
Column	Row	Control	Scarified	Tilled
A	1	9	15	13
	2	4	9	17
	3	3	6	17
	4	4	10	13
B	1	10	12	--
	2	3	5	--
	3	7	8	11
	4	6	7	16
Average Emergence		5.75 (26.9%)	9.0 (42.8%)	14.5 (69.5%)

1.9 RESEARCH PLANNED FOR 1962-63

Gebben plans to investigate the effects of light intensity on the vegetative growth of the ragweed plant in both laboratory and field conditions. Different levels of light intensity will be used throughout the entire life history of the plant to determine the effect of light on the growth rate and growth form of the ragweed. W. W. Payne will elaborate his work on the four widespread weedy species of ragweed in the form of a reference book, and will make a field trip to Mexico and the southwestern United States to find primitive ragweeds. He will continue to bring together biological data on the ragweeds bearing on their evolution, migration, and general botany. R. W. White will complete necessary anatomical slides and prepare the final manuscript on the development of the flowers of ragweed.

2. MEDICAL PHASE

2.1 INTRODUCTION

During the period of this progress report, work has been continued on four projects reported upon in previous progress reports: (1) studies of repository antigen preparations; (2) observations on artificial unipolar air ionozation; (3) epidemiological studies of asthma and hay fever; and (4) the preparation of a chamber for studies of pollinosis. In addition, three new projects have been inaugurated: (1) immunochemical fractionation of ragweed pollen extracts; (2) studies of skin-sensitizing antibodies by absorption onto washed pollen grains; and (3) attempts to detect skin-sensitizing antibodies in atopic patients' leukocytes by means of a fluorescent antibody technique.

As is evident, this medical approach to aeroallergens encompasses studies dealing with the chemical and physical nature of the aeroallergens, the immunologic reaction of the atopic subject, secondary factors affecting hypersensitivity and immunity to aeroallergens in humans, quantitative aspects of the clinical reaction in humans, and the ultimate occurrence of atopic disease in the community. An attempt has been made to select problems of critical importance for either theoretical or practical reasons. Some of these problems will require prolonged study, whereas work on others has been completed during the period of this progress report.

2.2 IMMUNOCHEMICAL STUDIES OF RAGWEED POLLEN EXTRACTS (J. L. Bentz and Y. M. Kong)*

2.2.1 Introduction

Immunologic and clinical studies of most spontaneously occurring hypersensitive states have been hampered by the lack of the purified antigens and

*The titles of all separate studies in the Medical Phase are followed by the authors' names in parentheses.

antibodies which are involved. This is particularly true of atopic hypersensitivity. In the past, the use of chemical and physical methods of isolating the allergens in ragweed pollen has met with limited success. It was our hope that by combining a physical method, ion-exchange column chromatography, with separation on the basis of immunologic specificity, these allergens could be isolated and characterized.

Ragweed pollen extract and serum globulins containing skin-sensitizing antibody (reagin) to ragweed were fractionated separately by column chromatography on diethylaminoethyl cellulose. Reaginic globulins absorbed to the cellulose column were then used in an attempt to absorb specifically the ragweed allergens, which were subsequently eluted by manipulation of pH and salt concentration.

2.2.2 Preparation of Ragweed Pollen Extract

In an effort to control variability due to pollen source, low ragweed pollen was obtained through the Committee on Standardization of Allergens from the Division of Biologic Standards, National Institutes of Health. We depleted this supply from the 1959 crop early in 1961 and applied for pollen from the 1960 crop. During the waiting period we used defatted low ragweed pollen obtained from the Greer Drug and Chemical Corp.

Before extraction, the pollen was defatted with diethyl ether in a Soxhlet extractor—2 hours for previously defatted pollen and 6-8 hours for undefatted pollen. The ether was evaporated off, and the pollen dried in a desicator and then weighed and extracted with phosphate-buffered saline, pH 8, without phenol. A 15% extract (w/v) was usually prepared. The extraction was carried out for 24 hours at 4°C with constant stirring on a magnetic stirrer. The slurry was then centrifuged to remove the pollen grains, and the supernatant was filtered through Whatman No. 1 paper to remove any traces of particulate material. The 15% extract contained an average of 2.2 mg of total N(Kjeldahl)/ml and 1.2 mg of phosphotungstic acid-precipitable N/ml. Extraction of small batches of pollen yielded higher N content than that of large batches.

During extraction, the pH dropped to 6.5. When the pH was adjusted to 7.0 or 7.5 prior to freezing at -20°C or lyophilization, a precipitate usually developed upon thawing or reconstitution. The extract was more stable if stored at the slightly acid pH and was adjusted with 0.1 N NaOH immediately before use.

Immediately after extraction, the extract was dispensed into 1-oz and 2-oz serum bottles, lyophilized at the acid pH, and stored at -20°C. Some extracts were also stored in the frozen state at -20°C. For intradermal skin testing, the extract was sterilized by Seitz or Millipore filtration.

2.2.3 Immunization of Rabbits Against Ragweed Pollen Extract

Because human reagins and blocking antibodies are nonprecipitating, they cannot be used to identify ragweed antigens by the usual precipitation techniques used in immunology. However, the rabbit does produce precipitating antibodies to antigens in ragweed pollen extract (RWE). It is probable that some of these antigens are also allergenic in the human. Rabbit antisera against RWE may be used to detect ragweed antigens in various fractions and also in immunoelectrophoresis.

Accordingly, 12 Dutch Belt rabbits and 20 New Zealand (albino) rabbits were immunized against RWE. Since RWE is a poor antigen, adjuvants were employed. Thirteen of the rabbits received alum-precipitated RWE by several routes (IV, IP, SC) three times weekly for three weeks. The other rabbits received three weekly injections of (a) 1 ml of 15% RWE intravenously, and (b) 1 ml of 15% RWE emulsified with Freund's adjuvant (1:1) injected into multiple subcutaneous sites. Blood was obtained from the marginal ear vein 7-9 days after the last injection, and the serum was tested for precipitating activity against 5% RWE by the agar gel double-diffusion method of Ouchterlony.

Sera from the rabbits which had received RWE in Freund's adjuvant gave one to three lines in Ouchterlony plates after the first series of injections. Sera from rabbits receiving alum-precipitated RWE gave no lines. The latter group was then given RWE in Freund's adjuvant and subsequently developed detectable precipitating antibodies. The New Zealand rabbits proved to be better antibody producers to RWE than the Dutch Belts.

The rabbits were restimulated every three months with three weekly injections of 0.5 ml of 15% RWE in Freund's adjuvant The number of precipitating lines generally increased after each series of booster shots. The maximum number of distinct lines obtained with any rabbit was seven. There was great variability among rabbits in the number and identity of precipitin lines. Figure 4 illustrates some of the patterns obtained with antisera from different rabbits. All sera possessed at least one common antibody, but the presence of other antibodies varied greatly.

2.2.4 Column Chromatography of Ragweed Pollen Extract on DEAE-Cellulose

Five column chromatography experiments (E1-E5) were carried out with whole or dialyzed ragweed pollen extracts. The adsorbent used was the cation exchanger N,N-Diethylaminoethyl cellulose (DEAE-cellulose) obtained from Eastman Organic Chemicals. The DEAE-cellulose was prepared according to Peterson and Sober[3]; 2.5-3 gm was packed in 1.1 cm-diameter columns with air pressure (5 lb/in.^{2}) until a constant column height of about 25 cm was obtained. The column was equilibrated with the starting buffer (0.01M phosphate, pH 7.5) before the sample was applied. Two gradient elutions of decreasing pH and increasing ionic strength were employed: (a) 0.01M phosphate, pH 7.5 to

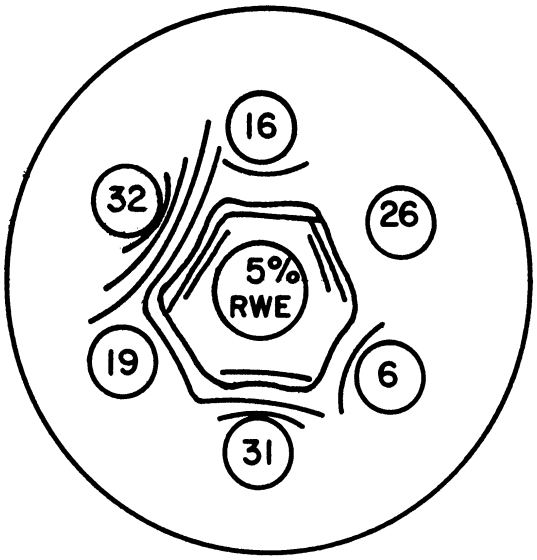

Fig. 4. Drawing of an Ouchterlony Plate. Ragweed antisera from rabbits No. 6, 16, 19, 26, 31, and 32 were placed in the peripheral wells. Ragweed pollen extract (5%) was placed in the center well.

0.02M phosphate, pH 6.3; and (b) 0.02M phosphate, pH 6.3 to 0.40M phosphate, pH 5.0. A high-ionic-strength buffer (0.40M phosphate in 2 M NaCl, pH 5.0) was used to elute the remaining proteins from the column. The cellulose could then be regenerated with 0.5 N NaOH. All buffer solutions were saturated with toluene before use, and the elutions were carried out at 4°C. An automatic fraction collector with a drop-counting device was used; each tube contained 4-5 ml.

The effluent tubes were examined for protein by measuring the absorbence at 280 mμ in a Beckman DU spectrophotometer. Allergenicity of the fractions was determined by direct prick tests on allergic subjects. Fractions which were negative by the prick test were usually tested intradermally after sterilization through Millipore filters (Swinny type).

Figure 5 shows the elution pattern when 1.5 ml of 15% RWE is subjected to one gradient elution from DEAE-cellulose. The RWE had been dialyzed 2.5 hours against the starting buffer, in this case 0.02M phosphate, pH 6.3. The gradient 0.02M phosphate, pH 6.3, to 0.4M phosphate, pH 5.0 was followed by a one-step elution with the 2 M NaCl buffer. Approximately every fifth tube was scratch tested on subject P.B. Only the initial tubes and those in the range of the initial gradient change (No. 71-90) were scratch-positive. Those tubes which were scratch-negative were tested intradermally on two subjects,

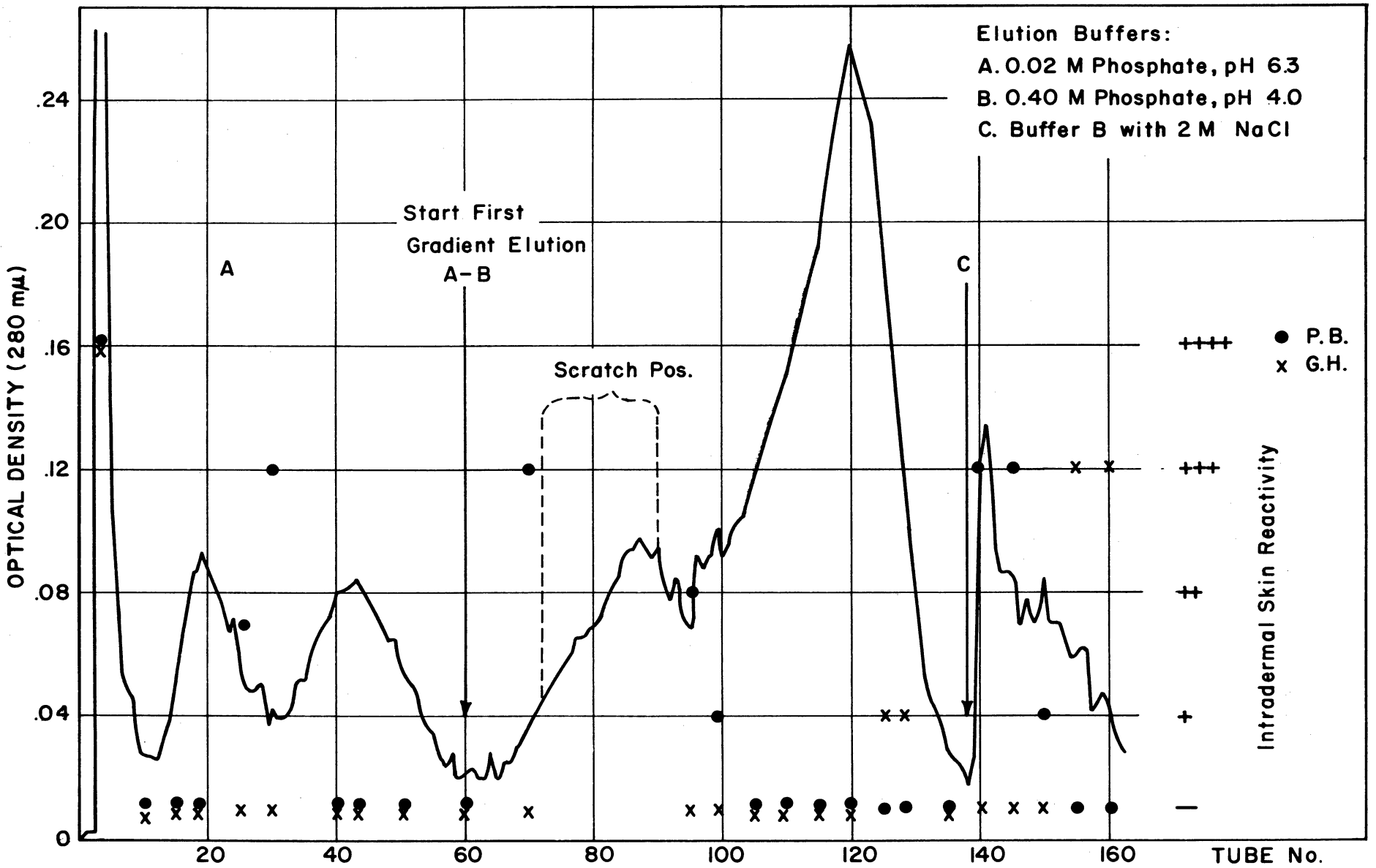

Fig. 5. Column chromatography of 1.5 ml of 15% ragweed pollen extract on DEAE-cellulose (Exp. E-2).

P.B. and G.H., and differences in reactivity between the two subjects were observed. Although some reactions were identical, P.B. seemed to be more reactive to the early and mid-fractions, whereas G.H. was more reactive to the last fractions. Except for the initial unadsorbed protein peak, there appears to be no correlation between protein concentration and skin reactivity.

In Column E-4 (Fig. 6) two gradient elutions were employed and the tubes were scratch tested. Although the same amount of RWE was applied to this column as to E-3, more tubes were scratch-positive. The tubes from E-4 were scratch tested immediately after collection without being stored by freezing, whereas the tubes from E-3 had been stored ten days in the deep freeze before skin testing. The lability of the ragweed allergens in dilute solutions accounted for the difference in reactivity, since retesting of the tubes from E-4 on the same subject two weeks later (the tubes being frozen during the interval) showed a marked decrease in allergenic activity. This continued to be our experience when working with very dilute solutions of ragweed extract, and complete loss of activity was frequently observed.

Both columns, E-3 and E-4, indicated either that clear-cut separation of allergens by this method was impossible, or that the subject reacted to many allergens, since the activity was found throughout the chromatogram. The lack of correlation between protein concentration and allergenic activity was to be expected, since it has been estimated that greater than 90% of the protein in RWE is nonallergenic. Some tubes with a zero optical density gave a four plus scratch test. However, negative readings at 280 mμ would not exclude the possibility that small amounts of protein are present.

As seen in Figs. 5 and 6, allergenic activity did not completely adsorb to the column at pH 7.5. Fraction I was allergenic and contained all the antigenic components for rabbits of the original extract when tested on Ouchterlony plates with rabbit anti-ragweed serum. This fraction was used in further experiments as the ragweed preparation which did not adsorb to DEAE-cellulose.

2.2.5 Column Chromatography of Serum Globulins from Untreated Allergic Individuals on DEAE-Cellulose

Twelve experiments (G1-G12) were carried out using reaginic globulins precipitated with 50% or 55% ammonium sulfate. The globulins were redissolved in the starting buffer, and the ammonium sulfate was removed either by dialysis or by passage through a Sephadex G-25 column. The reaginic globulins were then applied to a DEAE-cellulose column at pH 7.5, 0.01M phosphate and fractionated by gradients of decreasing pH and increasing ionic strength identical with those used for fractioning RWE. As shown in Fig. 7, three major protein peaks were obtained. The first peak contained proteins not adsorbed to the column at 0.01M, pH 7.5 and probably consisted of gamma globulins. A very small peak was obtained during the first gradient

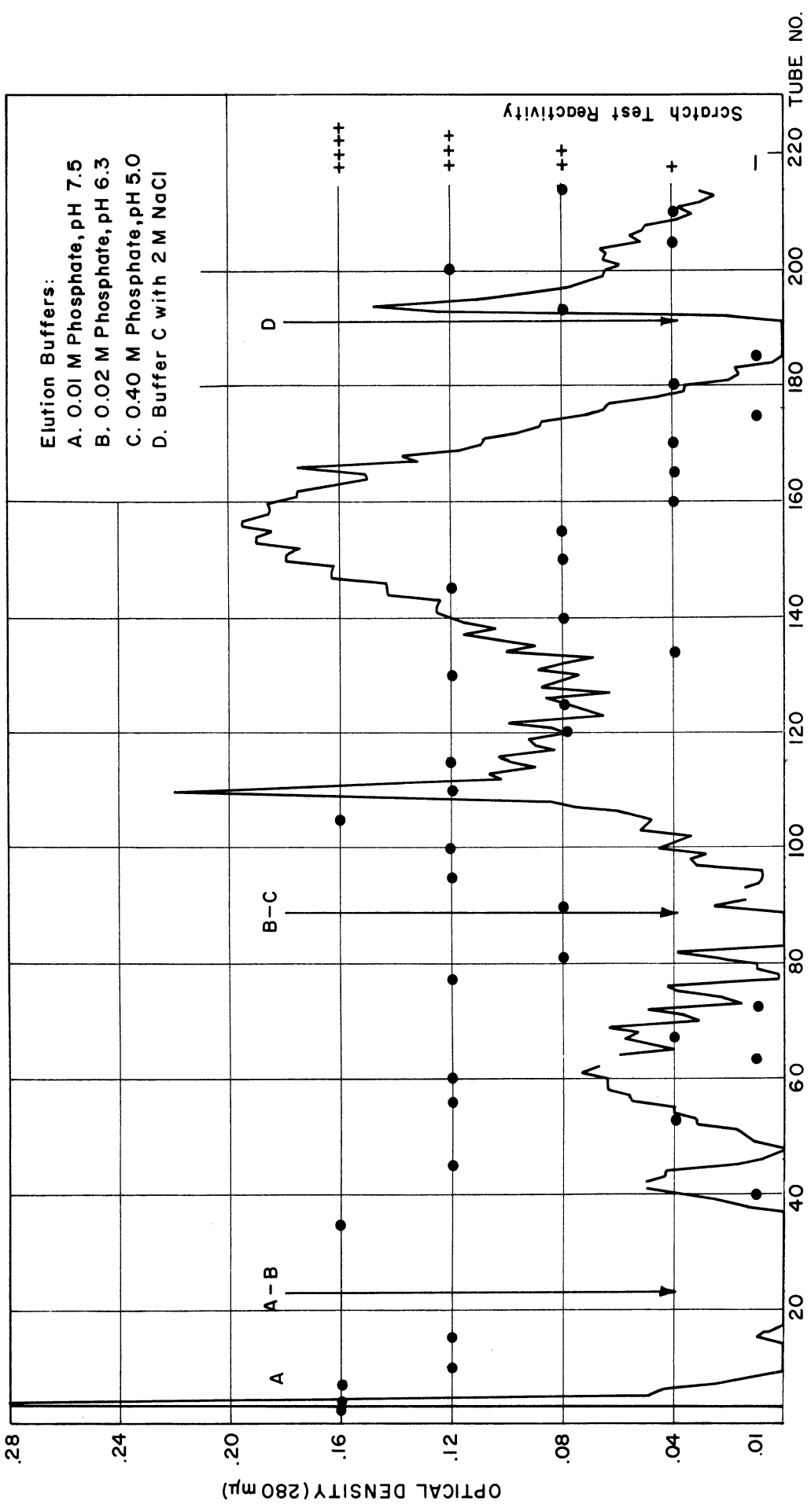

Fig. 6. Column chromatography of 1.4 ml of 15% ragweed pollen extract on DEAE-cellulose (Exp. E-4).

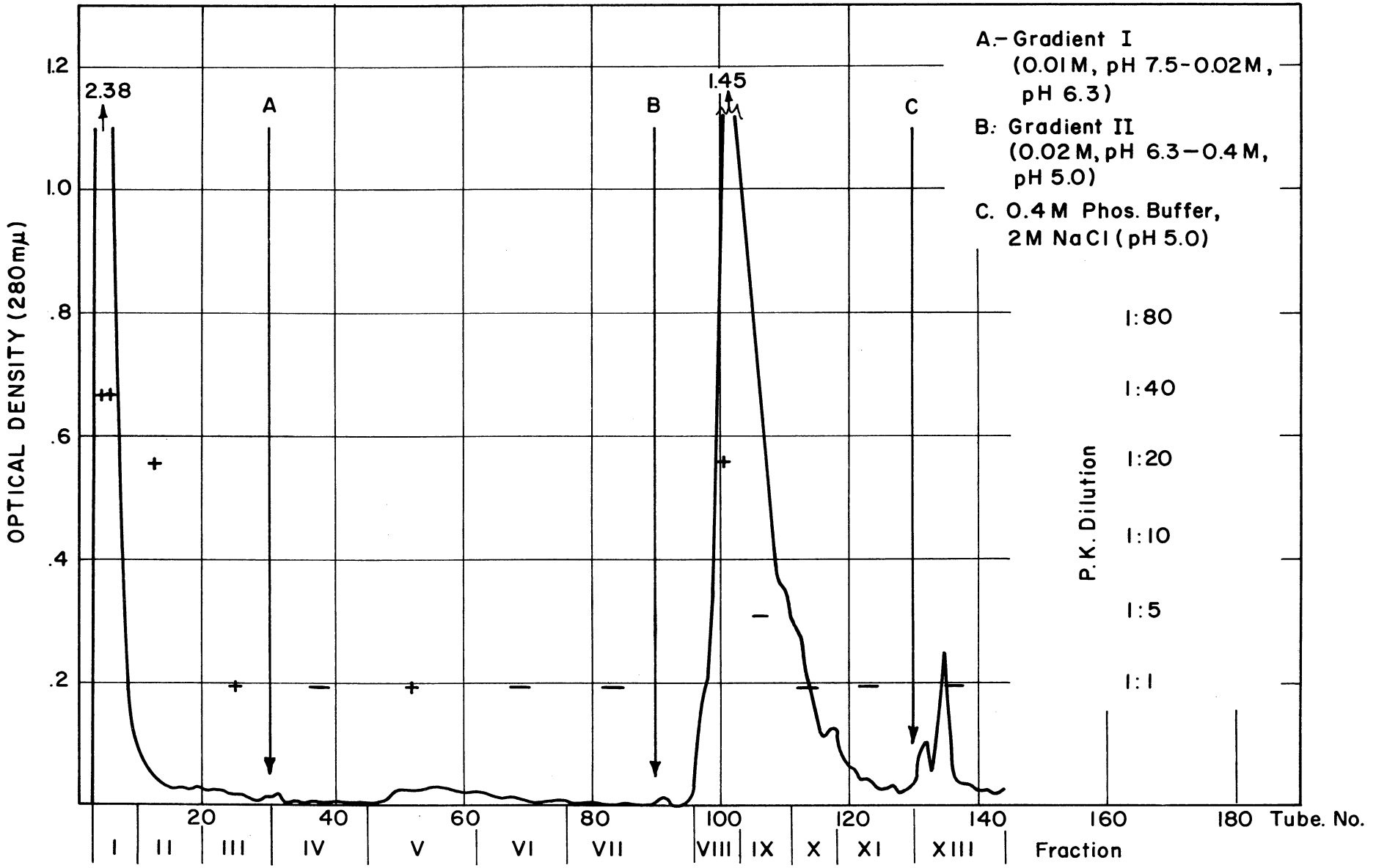

Fig. 7. Column chromatography of globulins from 5 ml of reaginic serum on DEAE-cellulose (Exp. G-12).

elution. A large peak with a sub-peak was obtained during the second gradient elution. Finally, a small peak was obtained with the 2 M NaCl buffer. The tubes were pooled into fractions, lyophilized and reconstituted with a minimal amount of water, and dialyzed against 0.85% saline. In two runs an aliquot was taken from each fraction for Kjeldahl nitrogen determination. Recovery of nitrogen from these columns was 76% and 77%. In three other columns, recoveries of protein were calculated from a nomograph for optical density readings at 280 mμ and 260 mμ and were found to be 60%, 61%, and 72%. Each fraction was sterilized (HA Millipore filter) and tested for reaginic activity by the Prausnitz-Küstner reaction (P-K test or P-K titer). Various dilutions of the active fractions were also tested.

Column G-12 (Fig. 7) shows a typical run in which 5 ml of reaginic globulins were fractionated on 3 g of DEAE-cellulose. The P-K reaction of the highest positive dilution of each fraction is indicated. Most of the reaginic activity was present in the initial unadsorbed fraction I. The wash fractions (II and III) contained less activity. The first gradient was devoid of any reaginic activity except for the small protein peak (V) which gave a one plus reaction with no dilution. This reaction was observed in three out of five columns. The large protein peak obtained during the second gradient elution usually had considerable P-K activity with titers from 1:20 to 1:50. The final fraction eluted with the 2M NaCl buffer had variable reaginic activity of low titer. Augustin and Hayward[4] also reported quite variable amounts of reagin eluted with the high-ionic-strength buffer (0.4M phosphate, 2 M NaCl). In one column their principal reaginic fraction was eluted by this buffer.

The presence of reagins in the unadsorbed fraction was unexpected, since Humphrey and Porter[5] and Augustin and Hayward[4] reported complete adsorption of grass and mold reagins to DEAE-cellulose under similar conditions. We tested overloading by increasing the ratio of cellulose to globulins, but reaginic activity in fraction I was not decreased. Our final ratio of 1 g of adsorbent to 1 ml of serum is well within the capacity limit used by other workers. Denaturation of the globulins during their preparation was apparently not involved since reagins were also obtained from fraction I of whole serum. Several globulin preparations from two allergic individuals were used and reagins were always present in fraction I. This phenomenon was not peculiar to ragweed reagins, since P-K sites sensitized with fraction I gave a positive reaction when challenged with a mixed grass extract. (The donor of the serum was sensitive to grass pollen as well as ragweed pollen.) At present, we cannot explain our findings except to suggest that perhaps the unexpected presence of reagins can be ascribed to the source and treatment of the DEAE-cellulose itself.

2.2.6 Absorption of Ragweed Allergens by Serum Globulins on DEAE-Cellulose

A. Absorption by Reaginic Globulins. After the adsorption of reaginic serum globulins to DEAE-cellulose at pH 7.5, a ragweed preparation that had not adsorbed to the cellulose was passed through the column in the hope of obtaining allergens specific for human reagins. If the allergens were eluted during the first gradient (pH 7.5 to pH 6.3; 0.01M to 0.02M phosphate), they would be relatively free of reagin. If they were eluted during the second gradient (pH 6.3 to pH 5.0; 0.02M to 0.4M phosphate), they would probably be complexed with reagin. A complex would render isolation of the allergenic components difficult.

The ragweed preparation used in the globulin and extract combination columns (GE) was prepared by first passing a 15% extract through a Sephadex column to exclude smaller molecules of ragweed allergens. The large molecules could be tested for homogeneity with rabbit anti-serum. Accordingly, 2 ml of a 15% RWE was passed through a Sephadex G-25 column at 0.01M, pH 7.5. Eluates showing precipitation with anti-ragweed serum were then pooled and passed through a DEAE-cellulose column equilibrated with the same buffer. The unadsorbed, proteinaceous material was lyophilized. This material (57 ml) was reconstituted to one-tenth the original volume and stored at -20°C; a portion was diluted for use. The concentrated preparation gave a four plus scratch test and produced 3 to 4 lines on Ouchterlony plates against rabbit anti-ragweed serum. It contained 180 μg of N/ml and 127 μg of phosphotungstic acid-precipitable N/ml.

In experiment (GE-3 (Fig. 8) 5 ml (157 mg of protein) of a globulin preparation derived from the serum of an untreated ragweed-sensitive individual, was applied to 3 g of DEAE-cellulose at pH 7.5, 0.01M. The column was washed with the starting buffer until the optical density of the eluate became negligible. Then 1.5 ml of the concentrated ragweed preparation previously described was diluted to 15 ml (0.01M final concentration) and applied to the column. The column was again washed with the starting buffer and the wash tubes were scratch tested. When a scratch-negative tube was obtained, the first gradient elution was begun. The first eluates from the gradient gave a one plus reaction and were pooled into fraction IV. Fraction V consisted of tubes which were scratch-negative. When the fractions were concentrated 15-fold by lyophilization, the scratch reactions from fraction IV became three plus and those from fraction V became two plus. However, these fractions contained no precipitins against rabbit anti-ragweed sera when tested with Ouchterlony plates and capillary precipitin tubes. Fractions VI through X were scratch-negative. All fractions gave negative P-K reactions except for fraction I and VIII. The reduction of P-K activity in this column was probably due to neutralization of reagin by the allergens present.

Experiment GE-3 looked promising because allergenic activity had been demonstrated in the fractions from the first gradient elution. This exper-

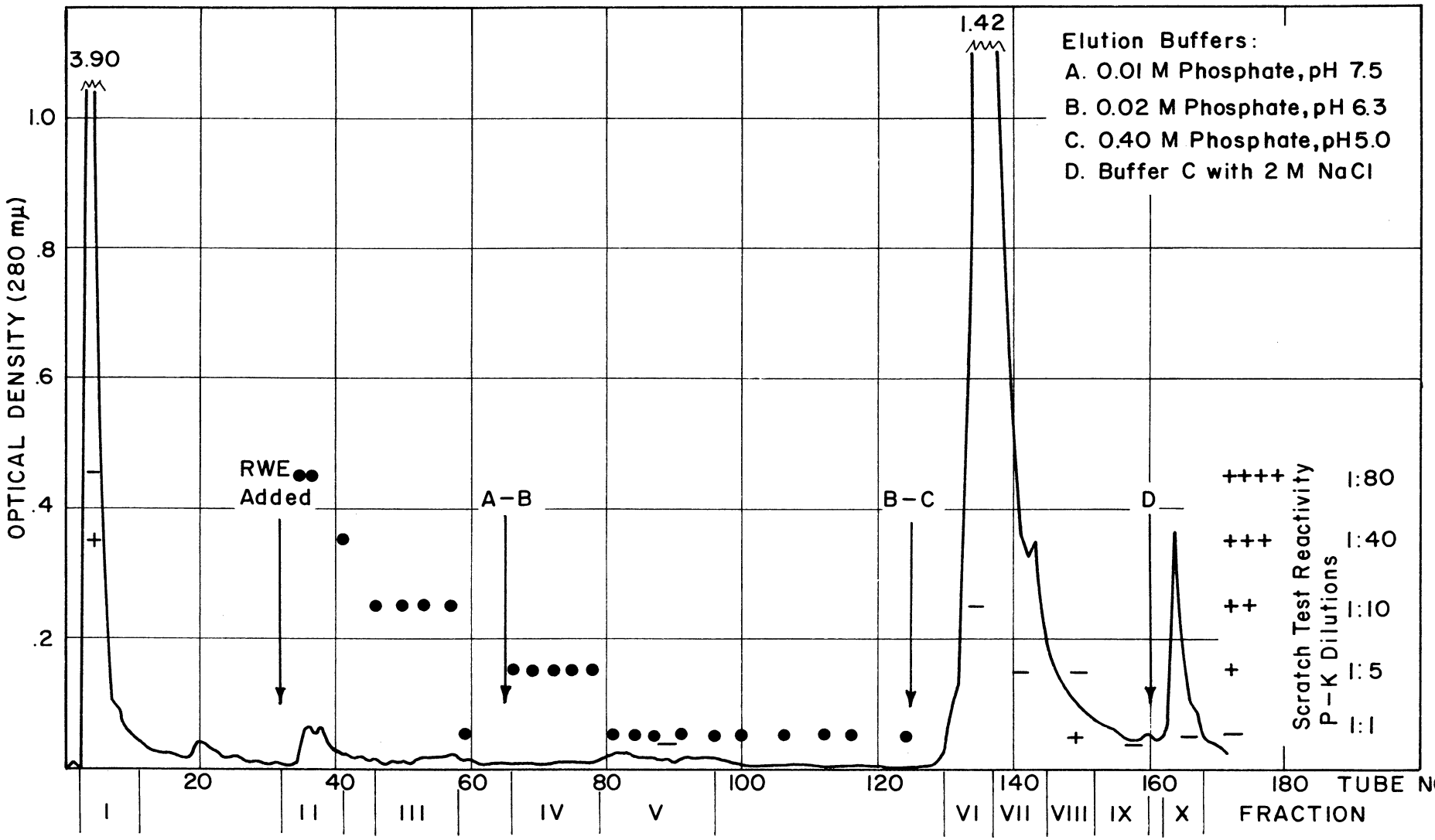

Fig. 8. Absorption of ragweed allergens by reaginic globulins adsorbed on a DEAE-cellulose column (Exp. GE-3).

iment was repeated in GE-4 (Fig. 9). In order to be certain that the presence of allergen in the first gradient elution was not the result of incomplete washing after the RWE was applied, 15 scratch-negative tubes were collected before the first gradient was begun. This time the eluates from the first gradient were found to be scratch-negative (except for two random tubes which were one plus) even after concentration. Subsequent fractions were also scratch-negative except for a one plus reaction with fraction XI. All the concentrated fractions from GE-4 elicited four plus reactions by intradermal testing on a sensitive subject. It is possible that the ragweed-preparative columns were overloaded so that not all components which adsorb nonspecifically to the DEAE-cellulose were removed. Therefore we decided to reduce the amount of RWE used for the GE columns and to use the more sensitive intradermal test. The following method of preparation of RWE was finally adopted as satisfactory: 15% RWE was passed through a DEAE-Sephadex (A-25) column and was washed through with 0.01M phosphate buffer, pH 7.5. The eluate tubes giving one or more lines in Ouchterlony plates with rabbit anti-ragweed sera were pooled, dispensed in several portions, and stored in the dry state. An amount equivalent to that derived from one-eighth ml of the original 15% extract and containing 99 μg total N and 64.5 μg phosphotungstic acid-precipitable N was passed through a 2.5-g DEAE-cellulose column and then washed with the buffer. The scratch-positive tubes were pooled and served as the source of allergen for control RWE columns and GE columns.

Each control RWE column was run through the chromatogram exactly like the GE columns but the first step of adsorbing globulins to the cellulose was eliminated. The concentrated fractions from this control column were tested intradermally concomitantly with fractions from the combination column. The fractions from the gradient elutions of the control columns were usually negative, or, at most, one plus when tested intradermally on a sensitive subject. In order to demonstrate significant elution of allergen from the GE columns, therefore, a two plus or greater reaction was necessary.

In experiment GE-6 (Fig. 10) the tubes were pooled into fractions, and a portion was tested intradermally. The remainder of each fraction was concentrated 15-fold by lyophilization. The concentrated fractions were invariably scratch-negative (except the early wash fractions after the RWE is applied) and did not form precipitates with rabbit anti-serum. The fractions were dialyzed and tested for reaginic activity by the P-K test. Activity was found in fractions I, XI, and XII. As in GE-3, the P-K titers of fractions from the second gradient elution were low, probably as a result of neutralization by allergens.

The results of intradermal testing on the donor of the reaginic globulins can be compared with those from control RWE column (E-9). There was no significant increase of reactivity of the GE-6 column over the control E-9 column in any of the fractions from the first gradient elution or in the final fraction. However, the reactivity of the second gradient elution fractions was significantly greater in the GE column than the control column.

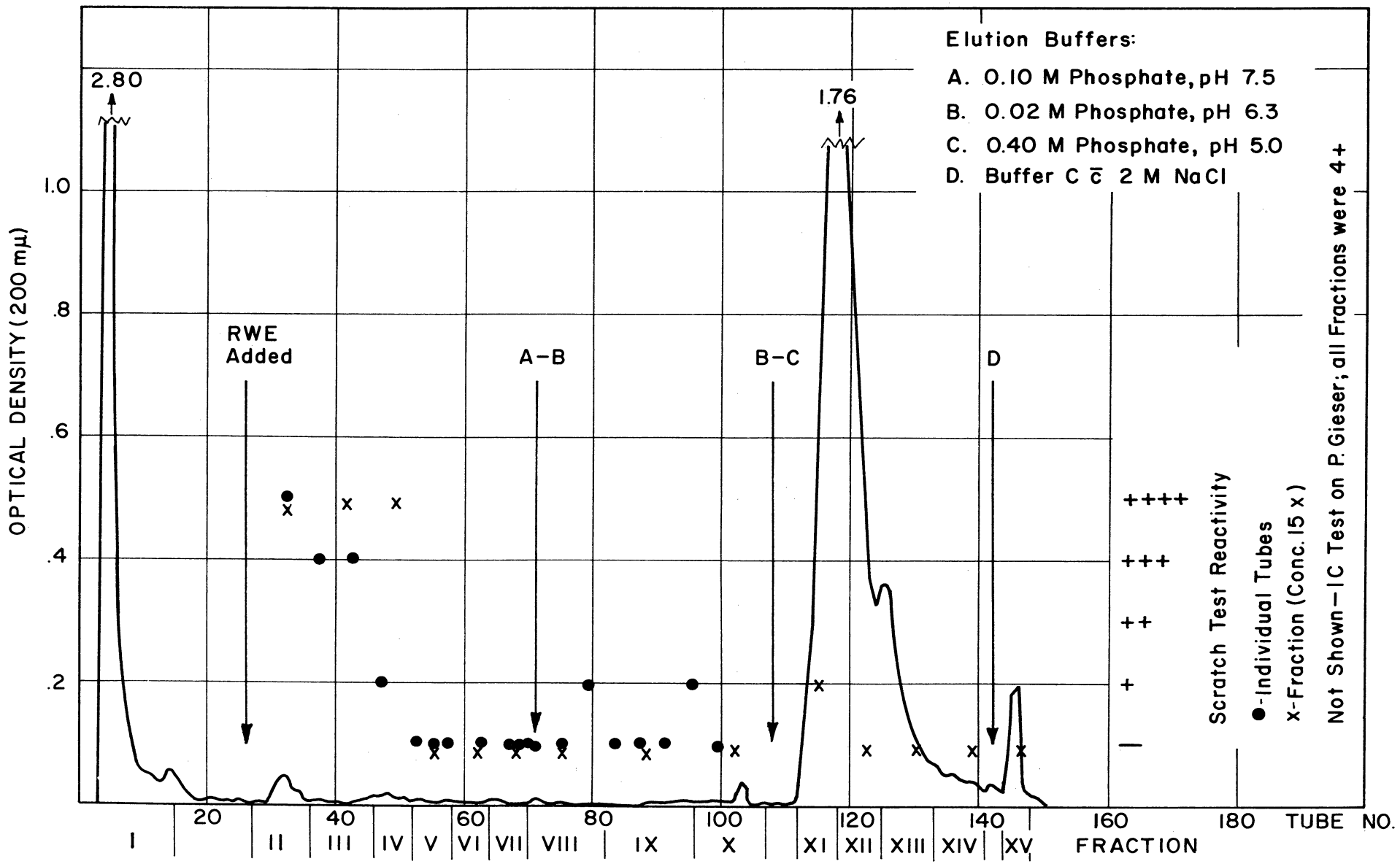

Fig. 9. Absorption of ragweed allergens by reaginic globulins adsorbed on a DEAE-cellulose column (Exp. GE-4).

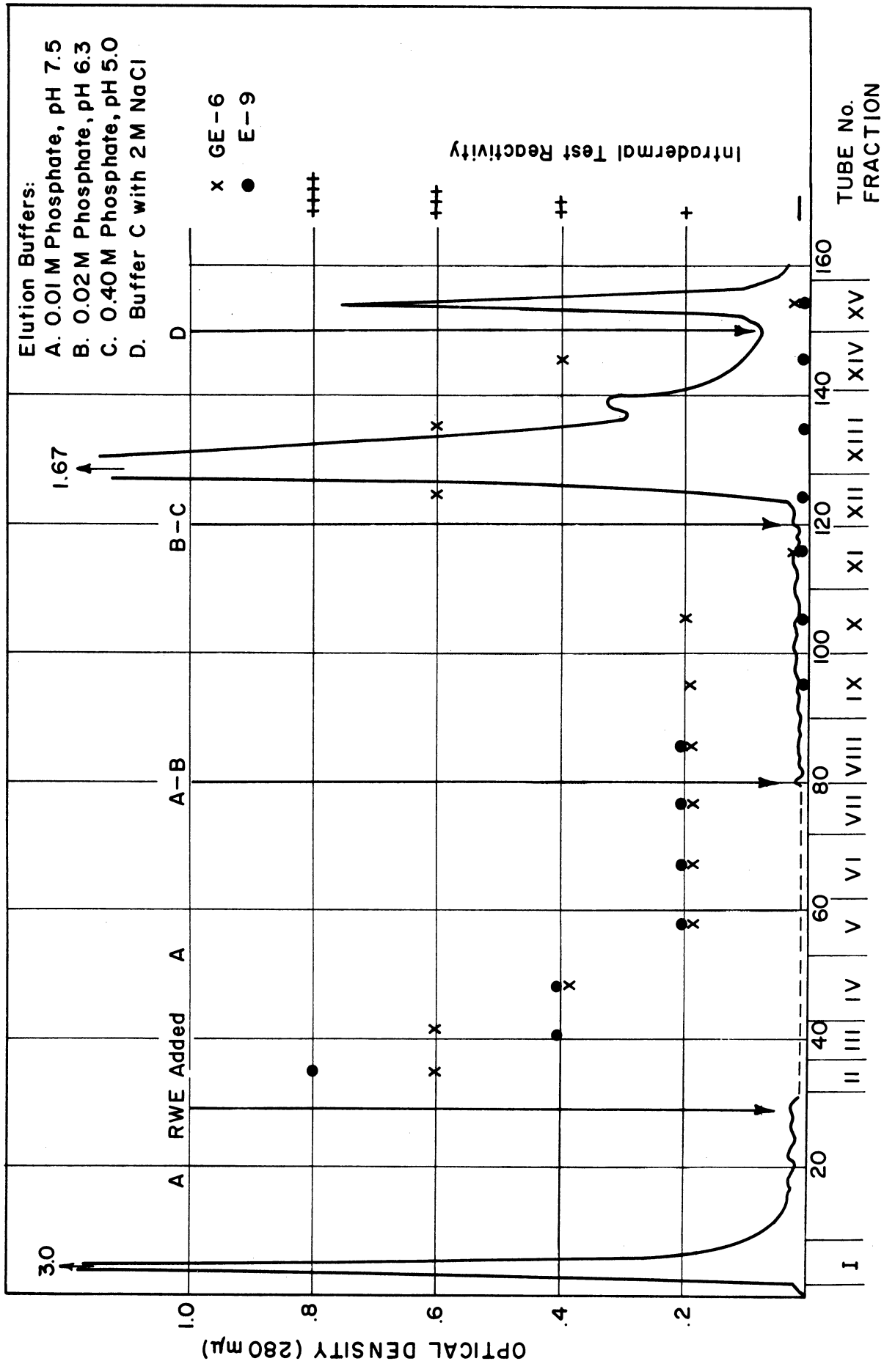

Fig. 10. Absorption of ragweed allergens by reaginic globulins adsorbed on a DEAE-cellulose column (Exp. GE-6; control column E-9).

Fractions XII and XIII were three plus and fraction XIV was two plus, whereas the control column fractions were negative. This difference was noted again in skin tests on another allergic subject. Similar findings were obtained in another experiment. Thus, reaginic globulins on DEAE-cellulose appeared to absorb small amounts of ragweed allergens. These allergens were eluted with the bulk of the globulins (containing reagins). The amount of allergen eluted with the globulins was insufficient to elicit a positive scratch test or precipitate rabbit antisera in Ouchterlony plates.

B. Absorption by Normal Globulins. To ascertain whether the absorption of ragweed allergens by ragweed reagins was specific (GE-6), the experiment was repeated with normal (nonreaginic) globulins. In experiment GE-9 (Fig. 11) there was no difference in the chromatograms between normal globulins and reaginic globulins, and elution of allergenic activity was again obtained with the second gradient. The skin tests were checked in a second subject with similar results. Normal globulins from a nonallergic individual also absorbed ragweed allergens nonspecifically.

The allergens eluted during the second gradient were not studied further for several reasons:

(1) They were present in extremely minute quantities.

(2) They were contaminated with serum proteins, including reagin.

(3) They were probably contaminated with nonallergenic constituents since they appear to be nonspecifically absorbed by normal serum globulins.

There are many possible reasons why we were unable to demonstrate a higher degree of absorption of ragweed allergens by reagins. Some of the more obvious ones are:

(1) Conditions—e.g., low temperature (4°C), short exposure time, unsuitable adsorbent (DEAE-cellulose), low concentration of reagin—were not conducive to combination. Difficulty in demonstrating the combination of allergen with reagin in vitro has been encountered by many workers. It is possible that, due to peculiar properties of reaginic antibodies themselves, the antigen-antibody complex is unstable, particularly when reagin is not attached to cells.

(2) Combination was limited to such an extent that (a) the allergen could not be detected in the presence of reagin, or (b) the allergen that had combined was diluted out or denatured.

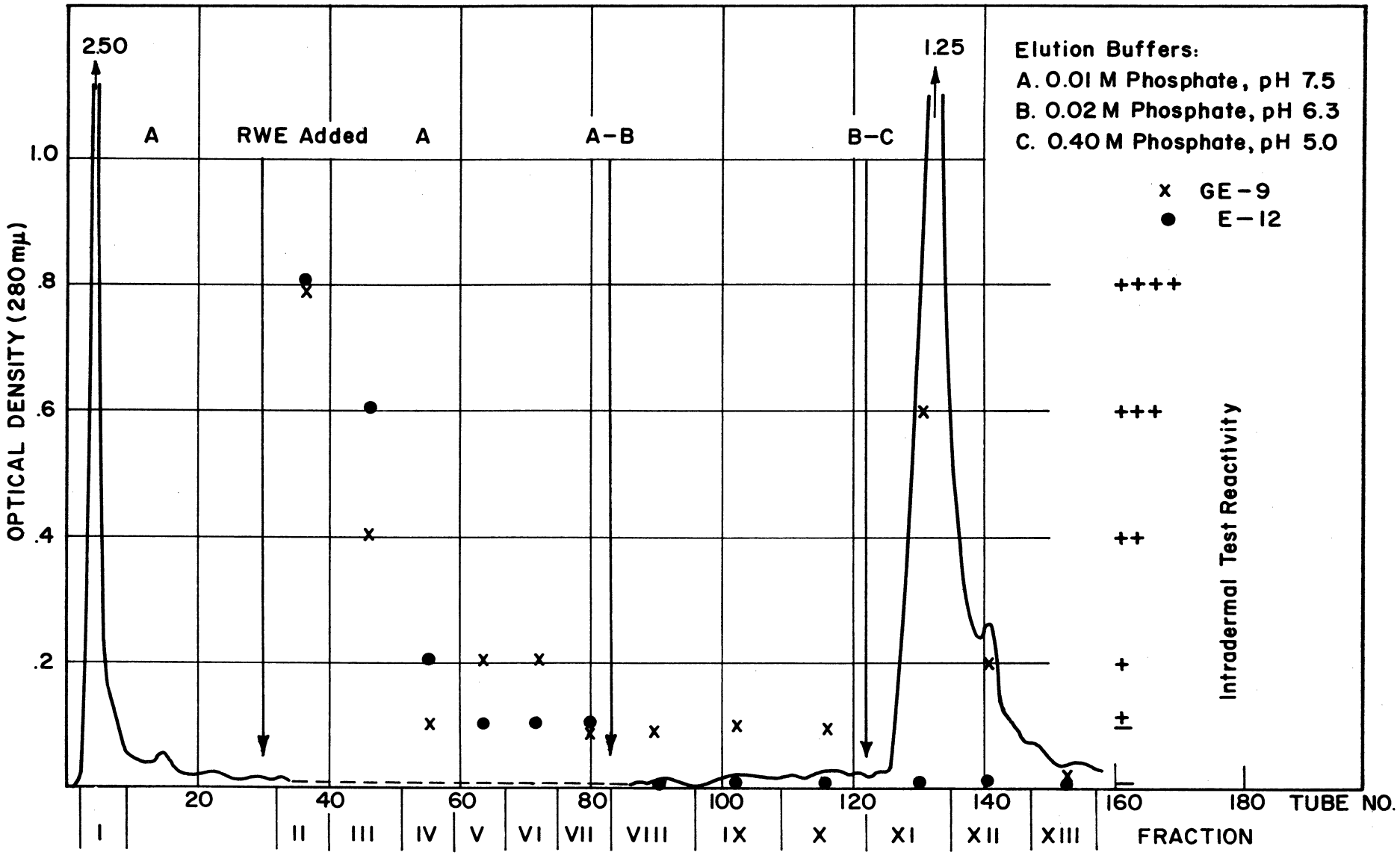

Fig. 11. Absorption of ragweed allergens by normal globulins adsorbed on a DEAE-cellulose column (Exp. GE-9; control column E-12).

2.2.7 Present and Future Studies

The use of blocking antibody from post-treatment serum instead of reagin to absorb the ragweed allergens was considered. Preliminary studies on the fractionation of post-treatment serum were carried out by using carboxymethyl cellulose (CM-cellulose), an ion exchanger. Blocking antibody to ragweed was detectable in the serum by using the passive transfer neutralization technique and the inhibition of histamine release from leukocytes of atopic individuals. The gamma globulin fraction (which contains blocking antibody) was prepared by passing dialyzed serum through a column of DEAE-cellulose at pH 6.3, 0.0175M phosphate according to the methody of Levy and Sober.[6] The gamma globulin fraction was then adsorbed to CM-cellulose at pH 6.3. Stepwise elution was undertaken by using four buffers of increasing concentration and decreasing pH. The fractions were dialyzed and concentrated by pervaporation. No blocking activity was detected in any of the fractions by the P-K neutralization techniques or by the blocking of histamine release. The difficulty and insensitivity of the assay for blocking antibody and the emergence of the gamma globulins from the column at relatively low ionic strength buffers (0.1M phosphate) made this approach for isolating allergens seem unpromising. Other possible adsorbents for antibody are being investigated.

2.3 ABSORPTION OF SKIN-SENSITIZING ANTIBODY FROM ALLERGIC SERUM BY INTACT WHOLE RAGWEED POLLEN GRAINS (A. I. Terr)

Preliminary experiments were carried out in an attempt to use ragweed pollen as an immunologically specific absorbent of skin-sensitizing antibody from the serum of subjects allergic to ragweed.* A 1% (w/v) suspension of undefatted Ambrosia eliator pollen in 0.9% saline was shaken on a mechanical shaker for 2 hours to thoroughly suspend the pollen grains. The suspension was then dialyzed against frequent changes of 0.9% saline for 48 hours. The suspension was then washed three times with saline, resuspended to the original volume, and the concentration of pollen counted in a standard hemocytometer. Varying amounts of the suspension were placed in separate conical tubes, centrifuged, and the supernatant fluid decanted and discarded. The packed pollen in each tube was suspended in 1.0 ml fresh serum from a ragweed-sensitive subject, and incubated for 30 minutes in a 37°C water bath, with frequent shaking by hand to keep the pollen well dispersed in the serum. After incubation, the tubes were centrifuged and the serum decanted and filter-sterilized through millipore membranes by using a Swinny filter. Prausnitz-Küstner passive transfer of serial dilutions of these serum samples, as well as of dilutions of untreated serum of the same allergic subject, was performed on a nonallergic individual, with a standard 1:500 dilution of ragweed extract in buffered saline used for challenge. The untreated control serum gave a positive P-K test to a titer of 1:320. Serum "absorbed" with washed ragweed pollen in a concentration of 250,000 pollen grains per ml

*After this work was initiated, Campbell and Sussdorf[7] reported experiments along similar lines.

serum, or greater, produced negative P-K reactions, undiluted. "Absorption" by 100,000 pollens per ml serum gave a slightly positive reaction, undiluted; lower pollen concentrations gave progressively greater reactions.

It appears from these results that "absorption" of skin-sensitizing antibody from allergic serum might be accomplished by using the intact ragweed pollen grain as a specific immunosorbent. Nonspecific destruction or removal of antibody was ruled out by demonstrating that such treatment with ragweed pollen did not affect the titer of skin-sensitizing antibody to timothy grass pollen (Phleum pratense), to which this subject is also sensitive. It remains to be shown, however, that the results are due to absorption of the antibody onto the pollen grain and not to neutralization of the serum by the release of soluble ragweed allergen into the serum during the 30-minute incubation period with the pollen. Three types of experiments were done in an effort to answer this question:

A. The pollen grains, after incubation with serum, were washed with saline and then treated with fluorescein-labelled rabbit anti-human globulin antiserum, to detect the presence of human globulin—presumably skin-sensitizing antibody—on the surface of the pollen. These experiments were thwarted, however, by the fact that untreated ragweed pollen itself emits an "apple-green" fluorescence indistinguishable from the specific fluorescence of fluorescein isothiocyanate, using the standard conditions of fluorescent-antibody microscopy.

B. The serum, after incubation with ragweed pollen, was tested for ragweed allergen activity by skin-testing on the donor subject, since skin-sensitizing antibody does not neutralize its allergen when mixed in vitro. In several experiments, serum which was completely "absorbed" of its ragweed skin-sensitizing activity by the minimal amount of pollen gave negative skin reactions by the prick-test method but three plus reactions by the more sensitive intracutaneous test. It appears therefore that some soluble ragweed allergen is released into the serum, although it has yet to be shown whether this amount is sufficient to result in complete neutralization of the skin-sensitizing activity.

C. Repeated serial absorptions of the same aliquot of pollen with the use of fresh samples of serum showed that the pollen was apparently "saturated" with skin-sensitizing antibody after the first 30-minute absorption, since the treated pollen was thereafter unable to affect the skin-sensitizing activity of the second or third sample of serum incubated in the same way.

These experiments are only preliminary, and further work on this problem is planned for the coming year. Experiments to be done include: (1) More refined semi-quantitative skin-testing procedures to show that absorption, and not simply neutralization, has occurred; (2) verification of these results with the use of other ragweed-sensitive sera; (3) demonstration of

skin-sensitizing antibody on the pollen, with the use of fluorochromes other than fluorescein; and (4) immunologic characterization of the serum component(s) absorbed onto the pollen, with the use of immunoelectrophoretic analysis. It is hoped that the use of ragweed pollen as an immunosorbent for skin-sensitizing antibody may permit eventual elution and characterization of the antibody.

2.4 STUDIES OF HUMAN LEUKOCYTES WITH FLUORESCENT ANTIBODIES (W. L. Kopp)

2.4.1 Introduction

The release of histamine from leukocytes from individuals with atopic disease after a specific allergen is added to suspensions of their leukocytes indicates that reagin may be associated with these cells. If reagin and antigen form a coherent bond in this reaction, an antibody prepared in rabbits against this antigen and conjugated with fluorescein might demonstrate the interaction. Nonspecific fluorescence, however, has limited investigations of leukocytes with fluorescent antibodies. Most investigators have studied dried films of leukocytes with this technique. A recent study[8] of blood group antigens with suspensions of erythrocytes and fluorescent antibodies suggests that suspensions of white blood cells exposed to fluorescent conjugates might not fluoresce nonspecifically.

Rappaport[9] has found that anti-human globulin conjugated with flurescein is fixed specifically to sections of skin of egg-sensitive donors, but not of normal donors. This suggests that a comparable procedure might identify globulin in association with leukocytes of individuals with atopic disease.

2.4.2 Materials and Methods

A. Antigens and Antisera. Short ragweed antigen and its rabbit antisera had previously been prepared for other studies of the project, as described in Section 2.2. For the present studies, human globulin was prepared from normal blood by 55% saturation with ammonium sulfate. Hyperimmune serum was prepared by immunization of rabbits with an emulsion of the globulins in Freund's adjuvant.

B. Fluorescent Conjugation. The antisera to human globulin and to ragweed were conjugated with 0.015 mg of fluorescein isothiocyanate per mg of serum protein and fractionated by passage through diethylaminoethyl cellulose.[10] After dialysis to pH 7.4, the conjugated globulins were concentrated by dialysis against 10% polyethylene glycol to final concentration of approximately 30 mg of protein per ml. Double diffusion in agar indicated immunologic specificity of the conjugated globulins. Normal rabbit serum was similarly conjugated with fluorescein, fractionated, and concentrated.

C. White Blood Cells. Leukocytes were separated from the heparinized blood of donors with ragweed hay fever who had not been hyposensitized. The blood of these donors had been shown to release histamine after addition of ragweed antigen. After the erythrocytes had settled at 37°C with or without the addition of 3.5% polyvinylpyrrolidinone to accelerate sedimentation, the supernatant plasma was centrifuged at 600 rpm at 4°C for 10 minutes. The plasma was discarded and the leukocytes were resuspended and washed three to six times in buffered saline or in Tyrode's solution, pH 7.4. Siliconed glassware was used.

D. Observation of Fluorescence. Suspensions of washed cells were observed after incubation with fluorescent globulins; a Zeiss microscope with an Osram HBO 200 lamp, BG 12 exciter filter, OG 5 barrier filter, and dark-field condensers were used.

2.4.3 Reactions of Leukocytes and Fluorescent Conjugates and Results

A. Normal rabbit globulin conjugated with fluorescein was incubated with suspensions of washed leukocytes for 30 minutes at room temperature. The cells were washed again three times. Because fluorescence did not occur many instance, further investigation with this technique was feasible. Absorption of fluorescent conjugates with tissue powders was not necessary.

B. Ragweed antigen in amounts from 80 to 1000 protein-nitrogen units was added to 10 ml of heparinized blood. The mixtures were inverted before and after incubation at 37°C for 15-30 minutes. Leukocytes were separated and washed three times. To equal aliquots of the final suspension of leukocytes, fluorescent anti-ragweed globulin was added in varying amounts. After incubation and repeated, mild shaking for 30 minutes at room temperature, the cells were washed two or three times. The cells of four ragweed-sensitive donors did not exhibit fluorescence.

C. Washed leukocytes were incubated for 30 minutes at room temperature with fluorescent rabbit anti-human globulin. The cells were washed again as many as five times. The leukocytes of all atopic and of all normal individuals similarly showed irregular small globules of fluorescence, which seemed to adhere to the outer surface of the cell membrane. Small clumps of fluorescent leukocytes occurred, but cells outside the clumps fluoresced. The addition of an equal amount of human serum to the fluorescent anti-human globulin precipitated the specific antibody. Incubation of washed leukocytes with the supernatant yielded no fluorescence. Other controls are being carried out.

2.4.4 Discussion

The failure of this technique to detect reagin to ragweed in association with leukocytes of individuals with ragweed hay fever might have been the result of lysis of reagin-containing cells by antigen-antibody interaction. Technical inadequacy, such as excess washing of cells or impotent antiserum, might also have been a cause. Still another possiblity is that ragweed antigen and reagin do not combine avidly, and thus one would not anticipate specific fluorescence under these conditions.

The observation that washed leukocytes of normal donors fluoresce after incubation with fluorescent rabbit anti-human globulin is compatible with Dausset's[11] observation that one absorption with washed leukocytes decreases slightly the titer of Coomb's serum.

2.4.5 Conclusions

A. Suspensions of human leukocytes which have been incubated with rabbit globulin conjugated with fluorescein isothiocyanate do not fluoresce non-specifically.

B. This technique did not demonstrate reagin in association with leukocytes of persons with ragweed hay fever.

C. Globulin has been identified in association with washed leukocytes of individuals with and without atopic disease.

2.5 THE EFFECT OF ARTIFICIAL UNIPOLAR AIR IONIZATION ON CERTAIN PHYSIOLOGIC PROCESSES IN LABORATORY ANIMALS (P. P. Barlow and J. A. McLean)

2.5.1 Introduction

A number of persons have suggested that atmospheric ions apparently can affect man's emotional state, efficiency, and health. Many of the reports written on this subject imply that an increase of negative ions over the level normally present in outdoor air exerts some beneficial effect on the respiratory tract of humans and of animals; in addition, some suggest that negative ions may affect microscopic airborne contaminants such as pollen. Generally, it is reported that positive ions exert a detrimental influence on humans and animals.

The object of this work was to determine whether exposure to high concentrations of either positive or negative ions would produce gross effects in laboratory animals. Three possible results of exposure to artificial unipolar air ionization might be anticipated: harmful effects, beneficial ef-

fects, and no effect. The observation of beneficial effects would suggest that human pollinosis might respond favorably to such treatment.

2.5.2 Materials and Methods

In order to confine significant unipolar ion densities within a small space, small animal chambers were constructed and air ions of either positive or negative polarity were passed through ports from Wesix ionizers which use a tritium source. Exposed guinea pigs were observed for gross changes in health and activity. Exposed mice were observed for changes in the normal weight-gain pattern of young mice.

A. Ionizers. "Ionaires" were obtained from the Wesix Electric Heater Co. in San Francisco. The Ionaire consists of a base through which current is passed and an ionizer head consisting of tritium foil and a charged plate mounted in a small plastic protective holder. The tritium emits beta particles of low energy which, in turn, create both positive and negative air ions on collision with air molecules. The charge plate serves as a polarizing electrode and is connected to a small rectifier. If the plate charge is negative, positive ions are attracted to it, allowing negative ions to be conveyed out of the ionizer head by following lines of electrostatic force. Sixteen ionizer heads were obtained, and the relative output of negative ions was measured for each of the heads. A Wesix Mark IV ion collector was operated at a collection potential of approximately 218 volts, and a Hewlett-Packard Model 425A current meter was used to measure the collected ion current. The ten ionizer heads having the highest output of unipolar ions were used for this entire study.

B. Guniea Pigs. Male albino guinea pigs weighing approximately 600 g were used for study of gross effects.

C. Mice. Young, male albino mice of the Spartan strain weighing 9-1/2 to 10-1/2 g were used in the study of weight-gain patterns. Most of these animals were two weeks old and were selected from similar litters.

D. Chambers. Cylindrical wooden containers measuring 17 inches in diameter and 6-1/4 inches in height were used as chambers for exposure of experimental animals. The tops of the containers were removable and had screened air holes; the sides of the containers had equi-distant two-inch openings covered by copper screening in which 1/2-inch openings were made in order to permit insertion of the ionizers. Ten ionizers were placed at equi-distant intervals around the circumference of the test chamber with the ionizer heads all facing the center and adjusted to be 14 inches from the radially opposite ionizer head. An identical chamber without the ionizers was used for housing control animals.

E. Scale. The "Shadowgraph," manufactured by the Exact Weight Scale Co. of Columbus, Ohio, and having an accuracy of 100 mg was used in all weight measurements.

2.5.3 Results

A. Physiologic Effects. Normal adult guinea pigs were individually placed in the test chamber and exposed to maximum unipolar air ionization for one-week intervals. The ionizer heads were so arranged that an animal had to be within at least three inches of one of the ionizer heads. Individual animals were exposed to positive and negative ions. After exposure, each animal was observed for six weeks for possible changes in activity, weight, alertness, regularity of breathing, and smoothness of fur.

Exposure to negative ions caused no observable changes, nor did exposure to positive ions.

B. Weight-Gain Patterns. Young, male albino mice were obtained and observed for two days in order to be certain that they were healthy and gaining weight normally. Thereafter the animals were weighed, marked, and randomly assigned to either the control or the treatment group. There were twelve mice in each group. The animals were handled identically except that the chamber in which the treatment group was housed had ten ionizer heads aligned radially. The animals were weighed daily, the percentage of weight gain over the weight at the start of the experiment was calculated individually for each mouse, and the results averaged for each group.

Four separate experiments were performed, with treatment groups being twice exposed to negative ions and twice to positive ions. Generally, it was found that the weight-gain pattern of the treatment group was slightly below that of the control group, possibly because the animals were more closely confined by the protruding ionizer heads. Nevertheless, weight gain proceeded at a normal slope and there was no significant difference between the weight-gain patterns of animals exposed to positive ions and of those exposed to negative ions. Specifically, negative ions did not exert a beneficial effect and positive ions did not exert a detrimental effect on the weight-gain pattern of young mice.

2.5.4 Discussion

The extensive literature on this subject has been reviewed, and a number of authors postulate that unipolar air ions exert certain effects on both humans and animals. Some suggest that large densities of positive ions exert very detrimental and grossly observable effects upon the organism. Alternatively, it is postulated by some that high negative ion concentrations cause grossly observable beneficial effects.

In this study, we were concerned whether unipolar air ionization would exert any grossly observable effect on experimental animals. To enhance the possibility of observing such effects, animals were exposed to maximally available air ion concentrations (by using the ten ionizer heads with high-

est output) and at close distances to the source of ionization (guinea pigs being a maximum of 3 inches and mice a maximum of 7 inches from the ionizer). In addition, the exposure to unipolar ions was relatively long, usually being continuous for 7 days. As is evident from this work, unipolar air ionization fails to exert a gross effect upon experimental animals.

2.5.5 Summary

Artificial unipolar air ionization of positive or negative polarity failed to exert gross physiologic effects on guinea pigs and on the weight-gain pattern of young mice in spite of long exposure at minimum distances to the ionizer heads.

2.6 ANIMAL TOXICITY STUDIES OF REPOSITORY ANTIGEN PREPARATIONS (P. O. Barlow, J. A. McLean, C. L. Larose, B. P. Ling, and H. Rodriguez)

2.6.1 Introduction

In order to investigate the local irritative reactions observed in some humans treated with repository allergenic emulsions, animal toxicity tests were applied to the evaluation of irritative properties of allergenic emulsions.

2.6.2 Materials and Methods

Two types of animal tests were used: guinea pig intradermal tests and mouse intraperitoneal tests.

A. The guinea pig intradermal test was performed by injecting 0.1-ml volumes of test materials and a control (saline) intradermally into normal, adult, male, albino guinea pigs. Six guinea pigs were used in each test and the skin on the back was prepared by close clipping of the hair. The area was divided into nine sites and the sites were injected in a Latin square pattern. The following observations were made daily over a 14-day period: (1) the diameter of erythema was measured to the nearest millimeter; (2) the intensity of erythema was graded; (3) induration of the injection site was measured by determining skin-fold thickness with the use of calipers; and (4) gross changes, such as blanching or necrosis, were noted.

B. The mouse intraperitoneal test consists of determination of weight-gain patterns of groups of mice. Young, male, albino mice weighing 9-10 g were randomly assigned to groups of 12 mice each, individually marked for identification, and weighed to an accuracy of 0.1 g. After two days of observation to assure that all mice were in a growth phase of active weight gain, each group was randomly assigned to a specific treatment, and individual

mice within groups were each injected intraperitoneally with 0.25-ml volumes of assigned material. Since the quality of mice could have varied from one shipment to another, a saline control group and a saline emulsion control group were included in each test. The mice were individually weighed two, three, and seven days after injection, and then sacrificed on the tenth day and examined for evidence of peritonitis or peritoneal irritation.

2.6.3 Results

A. Guinea Pig Intradermal Tests. Nonirritative materials usually caused erythema and induration during the first 3-5 days of the test, but subsequently the skin returned to normal. Toxic materials showed increasing erythema and induration over most of the 14-day period of the test. Aqueous pollen extracts containing more than 100,000 pollen units per ml usually evoked a mild irritative response in these tests. It was also found that Arlacel A which has been heated or exposed to light and air for several months may show an irritative reaction. All emulsions tested in this way, including those emulsions made with isotonic saline as the sole aqueous component, exhibited dermal irritation.

B. Mouse Intraperitoneal Tests. Toxic preparations injected intraperitoneally inhibited the normal weight-gain pattern of young mice and showed evidence of chemical peritonitis. The weight-gain pattern was considered to be inhibited if the average daily weight increment of a test group was not within 10% of the average daily weight increment of a saline control group. A number of tests in which saline or saline emulsions were used as the only injectables confirmed this 10% variation. The volume of material injected affected the weight-gain pattern, and tests showed that up to 0.4 cc of emulsion or 0.7 cc of aqueous material may be injected intraperitoneally without affecting the weight-gain pattern if the materials are not toxic.

This test was retrospectively applied to the treatment emulsions used in 1960. Of 64 patients who had been treated with ragweed emulsions, four developed subsequent swelling and pain. (One progressed to abscess formation.) These four patients had received ragweed emulsion containing 10,000 pollen units of weeds per 0.3 ml of emulsion. Six other patients had also received this emulsion but showed no reaction. Samples of the emulsion were subjected to the mouse intraperitoneal tests and found to be toxic in that they inhibited the normal weight-gain pattern of young mice. When the allergen in emulsion was subsequently diluted to contain 5,000 pollen units per 1 cc of emulsion, it was found that this preparation was no longer toxic to mice.

Toxicity tests were performed on all treatment emulsions and rejections made on the basis of the results. Maximum dosage of various allergens was determined by means of these tests. Generally, emulsions do not show toxicity if the following concentrations of aqueous allergenic extract are not

exceeded: (1) grass and ragweed extracts—10,000 pollen units per 0.1 ml; (2) Alternaria—500 PNU per 0.1 ml; (3) Endo house dust—0.0125 mg house-dust solids per 0.1 ml.

2.6.4 Discussion

The guinea pig intradermal test is of value in studying the irritability of components of emulsions. However, all emulsions when tested in this manner exhibit dermal irritation, which suggests that great care must be taken to avoid deposition of allergenic emulsions into the skin of patients.

The mouse intraperitoneal toxicity test seems to be the most adaptable to evaluation of allergenic emulsions. Inference suggests that the finding of an inhibited weight-gain pattern in mice may be associated with the occasional occurrence of a delayed irritative local reaction in some individuals treated with repository emulsions. It is of interest that no irritative reactions have been noted in the 350 patients treated after the initiation of this test for screening all emulsions to be used in the treatment of humans. Although allergenic emulsions which are toxic in mice are not necessarily toxic in humans, at our present state of knowledge, one should be extremely cautious in administering them to humans.

Animal toxicity studies are currently in progress to investigate some of the factors which may be important in emulsion toxicity. Preliminary results suggests that total dose of allergen, in addition to the concentration of aqueous allergenic extract, may be important.

2.6.5 Summary

Guinea pig intradermal tests were found useful in evaluating the irritating properties of components of emulsions; but all emulsions exhibit dermal irritative properties, which suggests that care should be taken to avoid injection of emulsions into the skin of patients. Mouse intraperitoneal toxicity tests appear helpful in the evaluation of irritative properties of allergenic emulsions. From results obtained in testing emulsions which cause an irritative reaction in patients, it is inferred that emulsions toxic in mice may prove to be toxic in humans. It is believed that animal toxicity studies performed on allergenic emulsions prior to clinical use may be of considerable value in avoiding subsequent undesirable irritative reactions in humans.

2.7 CLINICAL STUDIES ON REPOSITORY ANTIGEN PREPARATIONS (P. P. Barlow, J. A. McLean, J. M. Sheldon, F. Miller, C. L. Larose, and A. Bortz)

2.7.1 Introduction

For over 50 years, the injection of specific aqueous allergenic extracts

has been used in the treatment of certain allergic conditions, particularly hay fever and asthma. In recent years, emulsification of aqueous allergenic extracts into mineral oil has evolved into the so-called "repository treatment," in which there is a delayed, slow and gradual release of antigen from the site of injection to the immune mechanisms of the body; an adjuvant effect has been demonstrated in certain instances. The purpose of this work is to study the clinical effects of repository antigen administration in humans. This work was started in 1960 and continues currently.

As background to this work, a number of experiments were carried out in the Allergy Laboratory; the experience gained applied to the clinical study. It was found that good emulsions were prepared equally well by hand and by machine. Isotopic studies showed that the aqueous phase of a good emulsion is released rather rapidly during the first 48 hours after injection; e.g., 5% of the total antigen is released within 24 hours and 20% within 48 hours. Laboratory toxicity tests showed that increasing antigen concentration in the aqueous phase of emulsion is associated with increasing animal toxicity; that an irritating aqueous antigen is associated with animal toxicity; and that the emulsifying agent, Arlacel A, may be associated with animal toxicity, whereas mineral oil is not. Autopsy studies in mice revealed that apparently good emulsions may be prepared with an aqueous phase containing phenol or other preservative, but that these emulsions break down very rapidly upon injection into experimental animals.

The work of others in the field has also been applied to this program. Salk has shown that untoward local reactions to incomplete adjuvant emulsions may be avoided by using (1) low viscosity mineral oil, (2) purified Arlacel A, (3) intramuscular route of injection, and (4) nonirritating antigen. The U. S. Commission on Influenza reports good clinical results when adjuvant adenovirus-influenza immunization is used. Untoward local reactions to the virus emulsions occurred most frequently in Negroes and in children. The Commission's laboratory work revealed that the immunologic response equivalent to that of aqueous antigen is obtained by significantly smaller doses of antigen when it is incorporated with adjuvant materials. It was noted that a single dose of adjuvant virus vaccine produces significant antibody levels after 5-6 weeks, and a "booster" effect is noted when a second injection of adjuvant virus vaccine is given 12 weeks after an initial injection; high antibody levels persist for a long time thereafter.

Several workers in the field of clinical allergy have reported induction of immediate and delayed skin hypersensitivity in both atopic and normal individuals. This phemonenom occurs in persons who are neither clinically- nor skin-sensitive to the repository antigen administered. The adjuvant effect noted with virus vaccines has not been noted with allergenic emulsions as studied by laboratory determinations of skin-sensitizing antibody, hemagglutimating antibody, and blocking antibody. However, other workers report that results of treatment with allergenic emulsions are similar to those obtained with the use of conventional multi-injection aqueous treatment.

2.7.2 Materials and Methods

A. Patients. Patients for repository treatment were selected by physicians, and the following were excluded absolutely: children under 3 years of age, Negroes, patients with negative skin tests to allergens being considered for treatment, patients with serious disease other than clinical allergy, and nonatopic individuals. The following generally excluded: patients who had sufficient treatment and were asymptomatic, patients with history of previous reaction to repository treatment, and patients with significant skin test reactions but dubious clinical allergic symptomatology. Therefore patients selected were those who had respiratory allergy primarily due to one or more of the following, as shown by positive skin tests: grass, weeds, Alternaria, and house dust. In order to be selected, the patients had to agree to cooperate in evaluation of this form of treatment.

B. Oil Phase of Allergenic Emulsions. Drakeol 6-VR was obtained from the Pennsylvania Refining Co. and mixed with specially purified Arlacel A obtained from the Atlas Powder Co. in the ratio of 9:1. This mixture was sterilized by Seitz filtration and portions were subjected to animal toxicity studies prior to clinical use.

C. Aqueous Phase of Allergenic Emulsions. Aqueous grass and weed pollen extracts were prepared in the Allergy Laboratory. Pollen was exhaustively defatted in the Soxhlet apparatus and fractionally extracted (60% of the total extracting fluid used in the initial extraction) in the cold, with the use of phosphate buffered saline containing no preservative. Ten-percent extracts were prepared in this manner and then lyophilized and kept frozen. They were reconstituted and diluted just prior to preparation of emulsions. The aqueous extract mixtures were as follows: (1) grass mixture: 50% timothy grass, 50% orchard grass; (2) weed mixture: 80% dwarf ragweed, 20% giant ragweed; (3) grass and mold mixture: 50% grass mixture, 50% Alternaria extract; (4) grass and weed mixture: 50% grass mixture, 50% weed mixture; (5) grass, mold, weed mixture: 33% grass mixture, 33% weed mixture, 33% Alternaria extract; (6) weed and mold mixture: 50% weed mixture, 50% Alternaria; (7) weed and house dust mixture: 50% weed mixture, 50% Endo house dust extract; (8) mold and house dust mixture: 50% Alternaria extract, 50% Endo house dust extract. A lyophilized aqueous extract of Alternaria containing no preservatives was obtained from the Center Laboratories for use in preparation of mold emulsions. A 2.5% extract of house dust solids in distilled water with merthiolate added to a final concentration of 1:10,000 was obtained from the Endo Laboratories; it was subsequently diluted with buffered saline to prepare house dust emulsions. Prior to use in preparing emulsions, the pH of aqueous extracts was adjusted to 6.8 to 7.1. All extracts were sterilized by Seitz filtration.

D. Preparation of Repository-Treatment Emulsions. Emulsification was accomplished by combining two volumes of sterile Drakeol-Arlacel mixture with one volume of sterile aqueous allergen solution via a 3-way BD stop-

cock. This procedure involves attachment of a 10 cc Luer-Lok syringe on each side of a stopcock, closing the orifice to the passageway common to both sides so that considerable resistance is felt, "seeding" the aqueous phase into the oil phase, and completely emulsifying the ingredients by repeatedly pushing the contents from one syringe into the other. Physical tests of emulsification were performed on all treatment emulsions; these included tests for gross appearance and microscopic appearance, the water drop test, centrifugation, and bioassay. In addition, sterility tests on the emulsions were performed with the use of thioglycollate medium and brain-heart infusion broth. Concurrently, intraperitoneal mouse toxicity studies were initiated on treatment emulsions.

E. Mode of Repository Treatment. In general, repository treatment for a specific allergen involves two injections: a primary treatment followed by a "booster" treatment. In the case of multiple allergen sensitivities, the various allergens were all combined in the primary treatment and subsequent booster injections given approximately 6 weeks prior to the date of anticipated exposure. Most patients, therefore, received 2-4 repository injection treatments per year. Each treatment consisted of 0.3 ml of allergenic emulsion administered intramuscularly at one site. The emulsion consisted of two parts of oil phase (consisting of Drakeol 6-VR and Arlacel A in the ratio of 9:1) to one part of aqueous phase. Primary repository treatment was administered approximately 12 weeks prior to a booster treatment, which preceded the onset of an allergic season by approximately 5-6 weeks. Thus, in the case of weed emulsions, the primary treatment was administered early in April and the booster treatment late in June. Treatments were administered into the tricops muscles by means of 21-gauge, 1-1/2 inch needles attached to disposable syringes containing the emulsion; the overlying skin was cleaned with an iodine preparation.

F. Dose of Repository-Treatment Emulsions. The allergen dose of the repository injections was determined for each individual on the basis of tolerated aqueous allergen dose, which had been determined by actual injection prior to treatment. Based on the radioactive "release" studies reported by Sheldon,[12] it was found that the average allergic individual may tolerate a repository dose 20-25 times that which he can tolerate on administration of aqueous material. In the event that a patient's aqueous antigen tolerance was low and higher doses of repository allergen were desired, a pre-treatment aqueous allergen injection program was instituted. In 1960, repository allergen treatment doses tended to be high, generally containing 5,000-10,000 pollen units of allergen per treatment injection. In 1961, repository allergen treatment doses tended to be low, generally containing 500-2500 pollen units of allergen per treatment injection. Dose response is currently being evaluated by administering either 500 or 5,000 pollen units per treatment injection by means of the double blind technique.

G. Animal Toxicity Controls. Prior to the preparation of repository-treatment emulsions, the emulsion ingredients and prepared nontreatment emul-

sions were evaluated in animals for toxicity. These tests included mouse intraperitoneal tests and guinea pig intradermal tests, which had not been performed on the emulsions used in 1960.

H. Other Studies. Other studies performed with statistically significant numbers of patients included quantitation of urinary steroids before and after treatment; quantitative counts of circulating eosinophils of the peripheral blood before and after treatment; and titration of skin reactivity before and after treatment and at the end of the season.

2.7.3 Results

In the three pollen seasons from 1960 through 1962, approximately 410 patients were administered approximately 1,000 repository emulsion treatments. Of these patients, approximately 378 received an emulsion containing weed extract, either alone or in combination with other allergenic extracts.

A. The therapeutic results of repository treatment were evaluated by in-season and post-season evaluation visits of the patients and by daily or weekly symptom cards mailed by the patient. Although objective meausres of therapeutic efficacy are not available, the available data indicate that respository treatment is therapeutically at least equivalent to the conventional form of aqueous injection treatment. A majority of patients treated had previously been treated with aqueous injections in one form or another; on comparing the therapeutic efficacy of the two types of treatment, approximately 60% of the patients thought that repository treatment was better than aqueous injection treatment, 20% thought that it was equivalent and 20% thought that it was not as effective. In subsequent years and for a variety of reasons, including convenience and efficacy, more than 90% of the repository-treated patients have usually preferred repository treatment. The therapeutic results of the 1961 treatment program were approximately equivalent to those of 1960 but not as generally dramatic—perhaps because lower dosages of antigen were administered in 1961. No relations have been noted between therapeutic results of repository treatment and the patients' age, sex, duration of illness, nature of illness (hay fever or asthma), previous treatment, timing of repository treatment, number of repository treatments, pre-treatment skin reactivity, or dose of allergen. The following apparent correlations have been noted: (1) therapeutic results are specific for the allergen sensitivity treated; and (2) decreased skin reactivity after treatment is associated with good clinical results.

B. Evaluation of urinary steroids and of circulating eosinophils before and after repository treatment shows that there is no statistically significant change.

C. Three types of adverse reaction to repository treatment have been noted. Approximately 60% of the patients note mild pain and tenderness at

the site of injection for several hours after treatment, a reaction which is believed to be like that observed with any other intramuscular form of treatment. A mild, systemic, allergic reaction consisting primarily of urticaria has been observed in four patients; two of these reactions were caused by administration of an incompletely emulsified emulsion to very sensitive individuals, one was caused by error in dosage, and one (in an extremely sensitive individual) had been predicted. These allergic reactions occur about two hours after administration of the treatment. Four individuals have experienced a delayed local reaction generally consisting of swelling and tenderness at the site of injection. These reactions usually occurred one or two months after treatment, were associated with high-dosage treatment, and were noted in individuals with increased local muscular activity or trauma to the site of injection. In two of these individuals, the swelling spontaneously subsided after one week and has not recurred. In one individual the swellings persisted intermittently over a period of nine months and spontaneously disappeared; before they disappeared an abscess had developed in each arm requiring surgical drainage and curettage. The abscesses were sterile, however, and the pathological report suggested a foreign-body type of irritative reaction.

2.7.4 Discussion

The results of treatment with repository allergenic emulsions indicate that this newer and more patient-acceptable form of treating pollinosis is therapeutically comparable to the conventional form of multi-injection aqueous treatment. Although opinions differ widely, a majority of investigators share our opinion of the therapeutic status of this form of treatment. Laboratory studies have shown that emulsions do retard the release of antigen from the site of injection. In addition, clinical results suggest that mineral oil emulsions exert some adjuvant effect on allergenic extracts since the total dose of allergen administered in repository form is generally less than or equal to the allergenic content of one aqueous injection. The specificity of the therapeutic response again suggests an immunologic basis for the specific treatment of pollinosis.

Although objective measurements of therapeutic response are not available, a majority of patients (e.g., 67 out of 72 in one follow-up study) state that repository form of treatment is significantly better than no treatment at all. As noted, approximately 60% of the patients prefer repository treatment to aqueous treatment on the basis of therapeutic efficacy.

Since patients were carefully selected for this form of treatment, we have not had the opportunity to observe induction of sensitivity. It is planned to continue this type of selection in order to obviate any possible future harmful effects.

Results are not yet available to determine whether there is a dose response. However, animal toxicity studies indicate that moderate rather than high doses of allergenic emulsions are preferable. Since the routine testing of emulsions in laboratory animals was instituted, no significant irritative reactions such as abscesses or swellings have been noted in patients treated with emulsions.

Experience gained with the repository form of treatment suggests that it is particularly efficacious in the seasonal form of respiratory allergy and may even be indicated for two types of patients: (1) the very sensitive allergic individual who is not able to tolerate adequate doses of aqueous extract, and (2) the relatively insensitive allergic individuals who have not been significantly helped by the conventional form of aqueous treatment and can tolerate maximum doses of aqueous extract without significant local reaction.

2.7.5 Summary

Approximately 1,000 allergenic emulsions have been administered to over 400 allergic individuals since 1960 with significant clinical improvement and a low incidence of untoward side reactions.

2.8 THE POLLINOSIS TEST CHAMBER (J. A. McLean, C. E. Cookingham, W. R. Solomon, and R. von Morse)

The pollinosis test chamber was conceived as a means of better testing the variable effects of changes in pollen concentration, temperature, humidity, and other factors on the atopic individual sensitive to ragweed pollen. Earlier experience at the Jackson Prison during two ragweed seasons, the extraseasonal ragweed exposure at Willow Run Airport, and the wind tunnel extraseasonal ragweed exposures all yielded results but with multiple variables which could not be eliminated or individually evaluated. Thus a setting was proposed which would provide a control over temperature, humidity, pollen concentration, duration of exposure, and patient activities.

Although the original chamber was conceived as a unit which could be disassembled and transported from place to place, such mobility will probably not be necessary and, with the modifications to be discussed, not even possible. The chamber proper has an inside floor area of 9 x 8 feet and an inside height of 8 feet over-all; it is situated in the Allergy Laboratory in the Kresge Medical Research Building. Honeywell controls for humidity and temperature are situated inside the chamber and controlled from the outside. Perforated air ducts run beneath the floor grid for admission of air, and outlet vents and ducts are located in the ceiling (see Fig. 12). There are six air and pollen inlet tubes, each tube having a 6-inch diameter and multiple 3/4-inch-diameter perforations. The six air vent exits in the ceiling each measure 12 inches by 12 inches. A small fan recirulates the

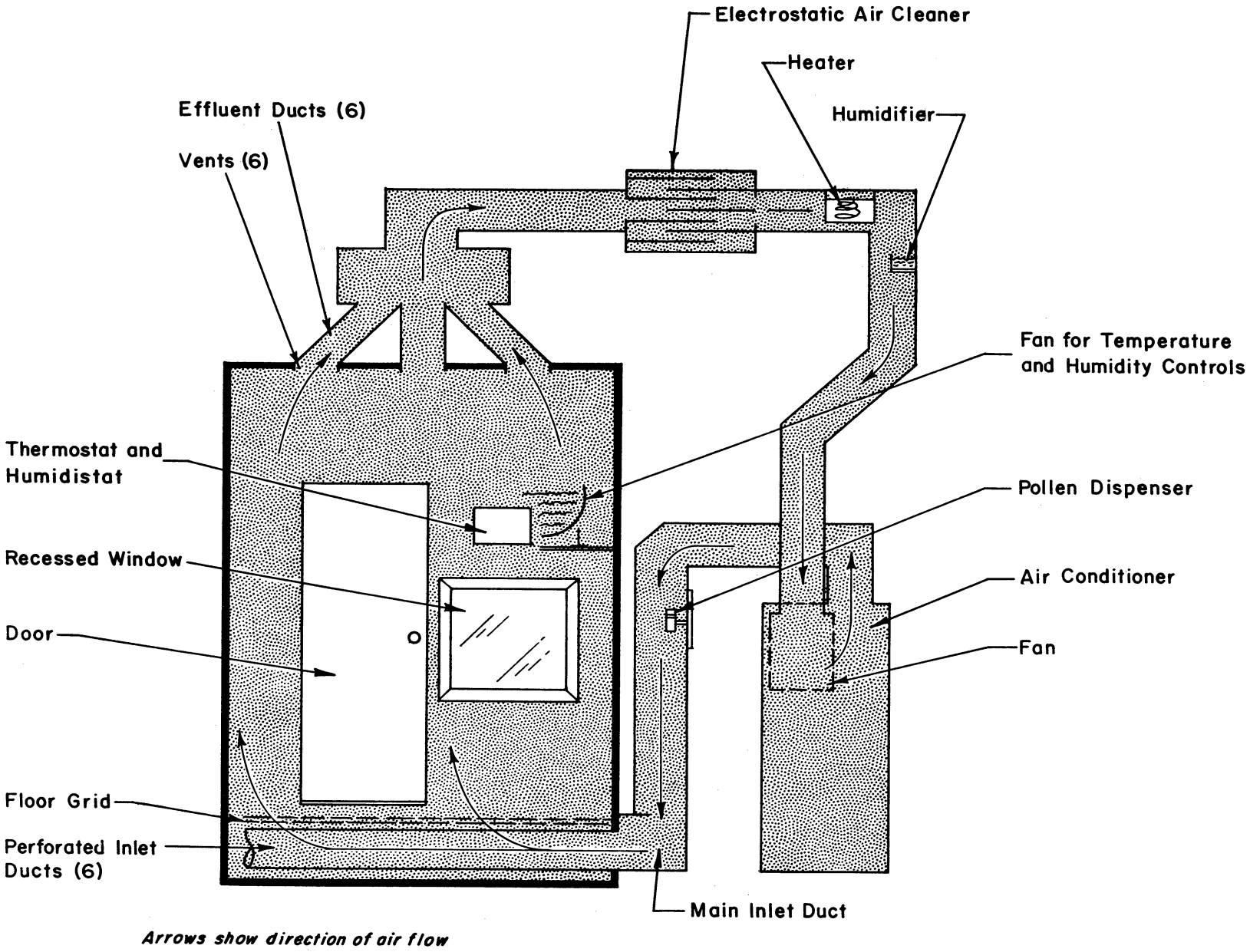

Fig. 12. The pollinosis test chamber.

air in the area of the humidity and temperature controls to insure a steady monitoring effect.

Outside the chamber are the pollen dispersion unit (the Wright Dust Feed Mechanism--see Fig. 13), the Trion Electrostatic Air Cleaner Unit, the two Humidimatic (Model 400) electric humidifiers, the Frigidaire refrigeration unit (reciprocating hermatically-sealed compressor, water-cooled condenser, and direct-expansion evaporator) and the heating unit (5000 watt, 230 volt with two Indeeco elements). Air from the chamber enters the duct system through the roof vents, is cleansed, and then either heated or cooled, depending on the desired temperature and humidity. It then receives the measured amount of pollen and circulates through the chamber, entering via the six perforated air ducts along the flooring. The speed of upward air flow is considerably in excess of the fall rate of the pollen.

Since the chamber was constructed, problems of standardization and maintenance have assumed major proportions. Rust has formed in the electrostatic precipitating unit and on some of the metal effluent grates in the chamber. The electrostatic air-cleaner precipitating plates were replaced and the metal grates painted with rustoleum. The Styrofoam sheeting used to insulate the air ducts outside the chamber has been reapplied to the sheet metal ducts with the use of a special adhesive. The air conditioning unit has been completely resealed to prevent outside air from entering the chamber beyond the electrostatic precipitating unit. Additional admission of fresh air to the chamber at apoint proximal to the electrostatic precipitating unit may be necessary in the future, especially if a completely air-tight duct system is achieved; the installation of a small exhaust fan distal to the air cleanser would than be required. Replacement of the perforated floor ducts, which are not rust-proof, by aluminum ducting has been considered.

Preliminary testing by the meteorological and engineering groups had shown that the chamber equipment should predictably maintain a pollen concentration in the range normally encountered by the allergic individual. However, this range is wide and our studies at the Jackson Prison had suggested the initiation and modification of allergic symptoms by changes in pollen concentration of less than 1000 grains/M^3. It was felt, therefore, that a finer control of pollen concentration in the chamber would be necessary before meaningful data could be derived from work with human subjects.

The pollen dispenser unit (see Fig. 14) was initially conceived as a cylindrical unit with a fritted glass filter disc designed to disperse pollen by the upward flow of air through a fluidized pollen bed. This was found to be unsatisfactory and was replaced by a Wright Dust Feed Mechanism supplied by L. Adams, Ltd., London, England (see Fig. 13).

The Wright Dust Feed Mechanism[13] is designed primarily for particles of approximately 10 microns. Considering the uniformity of size and regular spherical shape of ragweed pollen grains, however, an extended trial of its

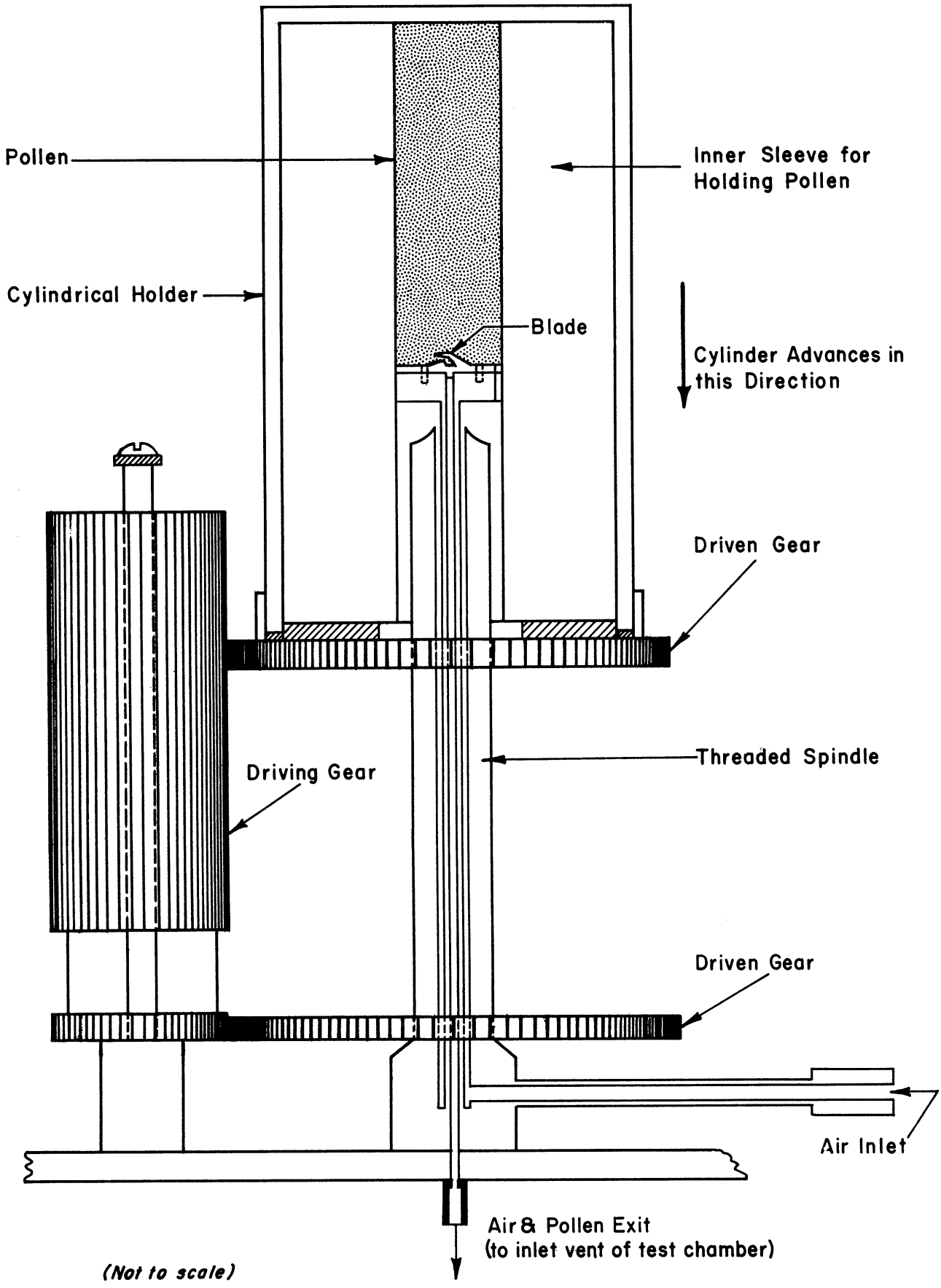

Fig. 13. The Wright Dust Feed Mechanism.

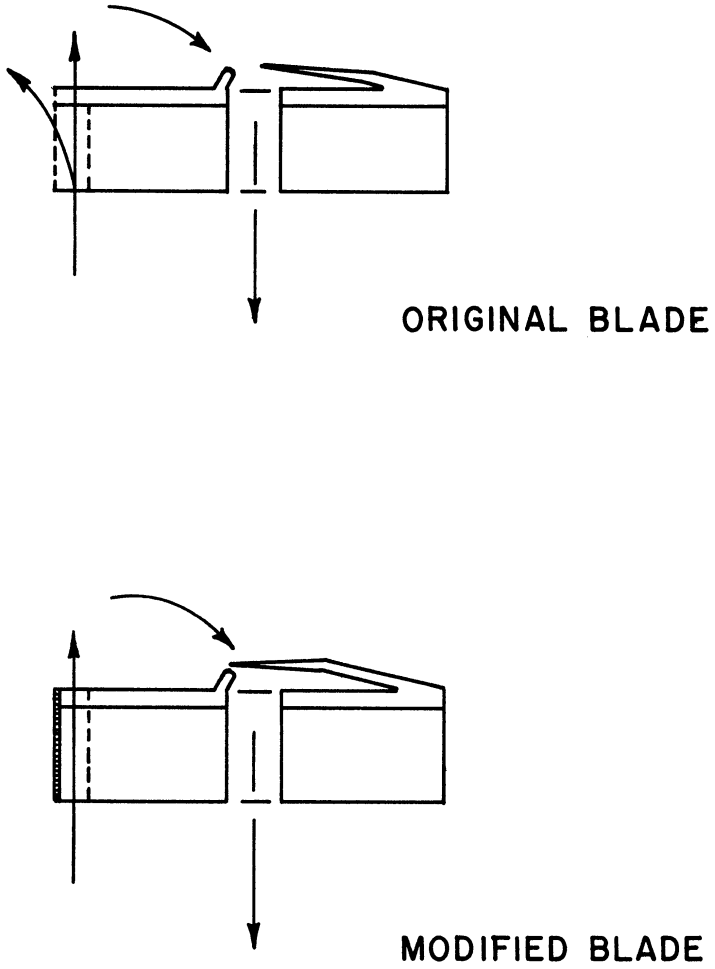

Fig. 14. The pollen dispenser blade before and after modification.

use with the test chamber seemed justified. The lightly compacted grains are contained in a cylindrical holder of 1/2-inch internal diameter which is rotated about a threaded spindle by the action of a differential gear. An electric clock motor drives the mechanism through a sequence of change wheels such that a range of relatively slow rotational speeds may be obtained. The threaded spindle carries, at its end, a knife blade corresponding in length to the internal radius of the holder. Grains freed from the compacted pollen by the knife blade, in the course of its rotation, are dispensed into an air stream which passes medialward along the blade then through the hollow core of the spindle to the main inlet duct of the chamber. When the mode of packing of the pollen and the temperature, humidity, and air flow employed remain constant, the amount of pollen presented to the chamber, per unit time, should vary predictably with the speed of rotation of the cylinder.

In the initial testing of the Wright Dust Feed Mechanism, the engineering group used defatted short ragweed pollen for dispersion and suggested several modifications because of the nonuniform counts recorded during succeeding sampling periods. Initially, the unit was inverted to offset the adverse effects of gravity; a short jet nozzle was added to insure pollen dispersion perpendicular to the exit duct by a Venturi effect. The problem of achieving optimal packing of the ragweed pollen in the Wright Dust Feed Mechanism was also noted by the engineering group. Flow rates of 1160 cc/min through the dispenser and 29 L/min through the jet nozzle were suggested as satisfactory. However, a significant variability in the pollen counts in the chamber was still observed, even when sampling was carried out over successive 10 and 20 min periods. Consultation with members of the Mechanical Engineering Department and a lengthy process of trial and error resulted in several further modifications of the mechanism. The chamber was grounded to prevent erratic release of charged pollen grains from the metal duct system to which they might adhere because of static charge. The blade of the dispenser unit was changed in two aspects. The slot allowing direct linear communication of the pollen bed with the inner dispensing cavity was blocked by extending the blade length, and the side slot allowing ingress of air to the pollen bed was laterally bounded (see Fig. 14) to prevent air erosion of the pollen bed. By using an air flow of 1160 cc/min through the dispenser and reverting the unit to its original position as pictured in Fig. 13 so that gravity could tend to foster pollen dispersion, the jet nozzle was rendered unnecessary. The flow used is a portion of the air stream distal to a flow divider and pressure regulator set at 150 inches of water pressure, with the remaining portion of the divided flow being channeled through a 20 L/min limiting orifice connected in series with the flow meter to minimize transmitted variations in the line pressure.

With these modifications, recent runs have shown more consistent pollen counts per unit of time. For example, succeeding 20-min counts at a temperature of 72°F and relative humidity of 50% vary within 1-3000 g/cu M/hr. The initial high concentrations of over 200,000 pollen g/cu M/hr, found within 30 min after the dispersion was started, have now been abolished. Table 6 gives an example of such data, obtained with the use of defatted ragweed pollen.

TABLE 6

Variations in Pollen Concentration in The Test Chamber
During Six Hours of Dispersion and Sampling

Rotobar No.	Count Begun	Duration of Count (Minutes)	Pollen Concentration (Grains/M^3 Calculated for 1 hr)	Comments
1	10:05	20	123	
2	10:26	20	162	Dispenser and its
3	10:46	20	102	air flow OFF; back-
4	11:06	20	93	ground count
5	11:27	20	69	
6a	14:30	20	31,508	Dispenser ON; with
b			36,271	air flow 1.480L/min;
7a	14:50	20	3,357	Temp. 72°F; Rel.
b			3,540	Hum. 50%. The cham-
8a	15:10	20	5,429	ber fan and all equip-
b			5,010	ment except for the
9a	15:31	20	4,267	dispenser were turn-
b			5,429	ed ON at 0800 hr.
10a	15:51	20	5,877	The pollen dispenser
b			5,401	and its air flow were
11a	16:11	20	4,972	set in motion just
b			4,846	prior to the first
12a	16:33	20	5,353	sampling period. After
b			5,143	the initial serial
13a	16:53	20	5,143	background counts,
b			5,419	sampling was done
14a	17:13	20	5,877	with paired rotobars
b			6,029	(a+b) at position
15a	17:33	20	7,823	"2" (the approx. cen-
b			7,975	ter of the chamber,
16a	20:28	20	5,810	at a level 3' 6" from
b			6,134	the floor grating).
17	21:21	20	219	Dispenser and its air flow OFF at 20:50; background count.

The representative data were derived by using the Wright Dust Feed Mechanism with the modified blade and no jet nozzle. Rotobars were sprayed and counted on the day of the test. The solution used consisted of one part of rubber cement and ten parts of thinner; it had been prepared several weeks before and stored in the dark in an air-tight container.

Several features of the data are noteworthy. Correspondence of the pairs of counts during any one sampling period is satisfactory. The initial high concentration obtained is probably related to the need, before using the dispenser, of advancing the blade manually and without direct visualization until some resistence (denoting contact with the compacted pollen), is felt. Undoubtedly, considerable pollen is mobilized in this maneuver, dispersed by the first flow of air past the blade and measured in the initial sampling period. A fair uniformity of concentration in successive test periods is seen. Although it still falls short of the uniformity desired, it is vastly improved over earlier studies in which the unmodified blade, jet nozzle, and the dispenser were used in an inverted position.

Table 7 shows successive counts obtained with all chamber units in operation and air flowing through the dispenser (1,480 cc/min) as before. For these counts, however, although the blade was initially advanced to engage the pollen, the dispenser motor was left OFF and the cylinder did not rotate during sampling. The high concentration at the outset is once again seen. Thereafter, the counts approximate normal background counts, suggesting that erosion of compacted pollen by the air stream does not play as significant a role in pollen dispersion as it did before. Objective evidence of such erosion had been present when the unmodified blade and jet were used, and counts had been considerably higher and more variable than the normal background. At the lowest possible gear combination, pollen counts of several thousand grains/cu M are still obtained (Table 6), and the clinical significance of this pollen concentration has yet to be determined. If symptoms result, a lower gear total ouflow from the pollen dispenser into the chamber.

Besides the problem of maintaining uniform, consistent, predictable pollen dispersion, there is the problem of keeping background pollen counts of the chamber constant or of changing them as preplanned. The control of counts requires an efficient air cleanser distal to the outflow ducts from the chamber. When significant background concentrations are obtained under the conditions previously mentioned, i.e., with the air flow fan engaged but the pollen dispenser immobile, there are several possible explanations. Either pollen is leaking passively from the pollen dispenser (a condition which occurs but is thought to be minimal, as evidenced by examination and lack of erosion of the pollen in the dispenser holder), the electrostatic precipitating unit is deficient, or pollen grains which accumulate on surfaces in the air duct system within the chamber itself or in the duct system outside the chamber are erratically released. Although grounding the chamber and duct system has been helpful, some pollen accumulation still occurs. Table 8

TABLE 7

Pollen Concentration in the Test Chamber Due to Air Flow Alone*

Rotobar No.	Duration of Sampling Period (Minutes)	Count Begun	Concentration (Grains/M^3 Calculated for 1 hr)
8a	20	09:45	1068
b			1227
9a	20	10:06	104
b			75
10a	20	10:27	84
b			93
11a	20	10:47	96
b			108
12a	20	11:08	60
b			93
13a	20	11:29	63
b			66

*Temperature setting: 72°F; relative humidity setting: 50%; chamber fan and dispenser air flow set in motion at 09:45 hours; pollen dispenser motor OFF.

shows the effect of agitation in the chamber tending to dislodge adherent pollen. Background counts were determined at 5- and 10-min intervals for a total of 50 min after an initial high concentration had been established. At this time, i.e., 50 min after the pollen dispenser had been disengaged, the operator stamped on the floor gratings in the chamber and beat on the chamber walls for approximately 5 min. Thereafter, two more pollen counts were taken with a resultant four-fold increase in the pollen count.

Another factor of paramount importance is the air flow within the chamber itself. Pollen counts in the chamber, and more importantly, the patient's pollen exposure, are directly related to the pattern of air flow; therefore it seemed pertinent to undertake air flow studies. Vertical air flow was measured with the use of a nondirection-sensitive velometer (Anemograph Corporation of America) which was sensitive to velocities of air flow greater than 5-10 feet per minute. Each velocity map was made for a particular vertical level in the chamber (22 inches from the floor grating, 63 inches from the floor grating and 6 inches down from the ceiling vents); these points were chosen because they corresponded to the nearest and farthest points from the floor and to a height which was anticipated to be near the subject's nose. Figures 15(a), 15(b), and 15(c) show the velocity map for each of these particular levels; the flows are probably vertical in nature and are correct to a ± 5 feet per minute. No areas of total dead space were discovered. In the future, a chemical smoke will be used at several horizontal points on the flooring to establish true air-current directions.

TABLE 8

Effect on Pollen Concentration of the Electrostatic Air Cleaner and of Disturbance Within the Chamber*

Test Ending	Test Duration (Minutes)	Rotobar No.	Actual Count	Time Factor	Grains/M^3/hr Calculated to One Hour
19:57	5	2	1253	12	15,036
20:03	5	3	103	12	1,236
20:14	10	4	55	6	330
20:25	10	5	73	6	438
20:36	10	6	46	6	276
20:47	10	7	24	6	144
AGITATION					
20:53	5	8	198	12	2,376
20:57	3	9	27	20	540

*Temperature setting: 72°F; relative humidity setting: 50%; sampling made at a constant point with single rotobars; chamber fan and all units except pollen dispenser were turned on at 1750 hours; pollen dispenser (with gears 36 - 36 - 54 - 72 and flows of 1450 cc/min through dispenser and 29L/min through jet) was operated from 1952 hours to 1957 hours; unused rotobars sprayed and taken from the storage box have shown no ragweed pollen grains.

Much of the previously described work was carried out at a standard temperature (72°F) with a relative humidity of 50%. A small fan is used in the chamber to adequately ventilate the temperature and humidity control area, since a projection of the chamber wall to house certain recording equipment might shield the controls from the main air stream. A Bendix-Friez Hygrothermograph (Model 594) is used to record the temperature and humidity. Satisfactory correspondence was found upon calibrating this instrument and comparing readings from it with readings from a well ventilated, calibrated, wet- and dry-bulb thermometer.

As mentioned in an earlier progress report,[14] the full range of temperature and humidity variation anticipated has not been realized. Reassessment of the range of temperature and humidity attainable in the chamber was made with the surrounding laboratory temperature at 72°F and the relative humidity at 35%. Findings were as follows:

(1) The average maximum temperature at any humidity was 87° with a range of 84-99°.

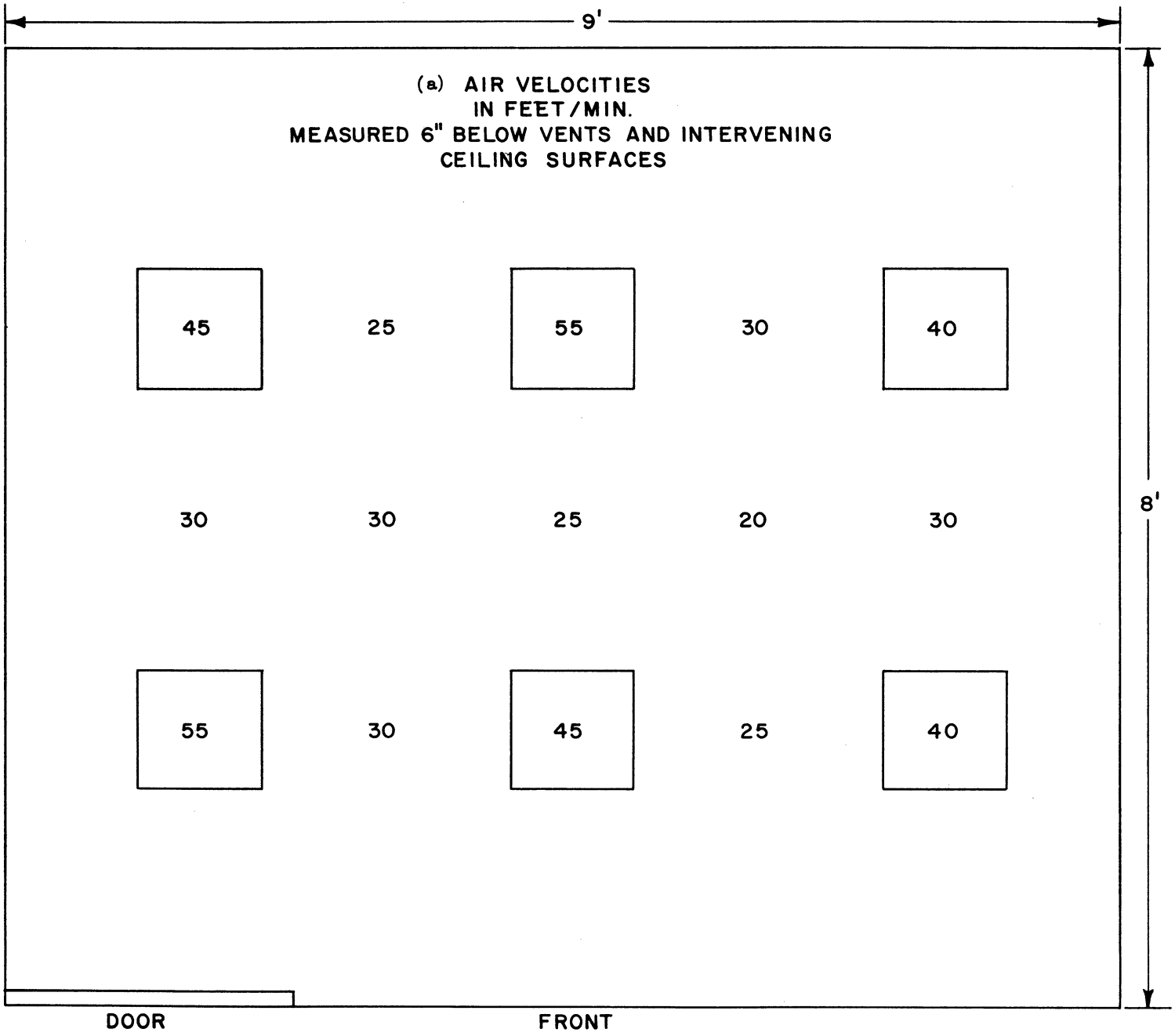

Fig. 15(a). Air velocities at horizontal levels in the Pollinosis Test Chamber.

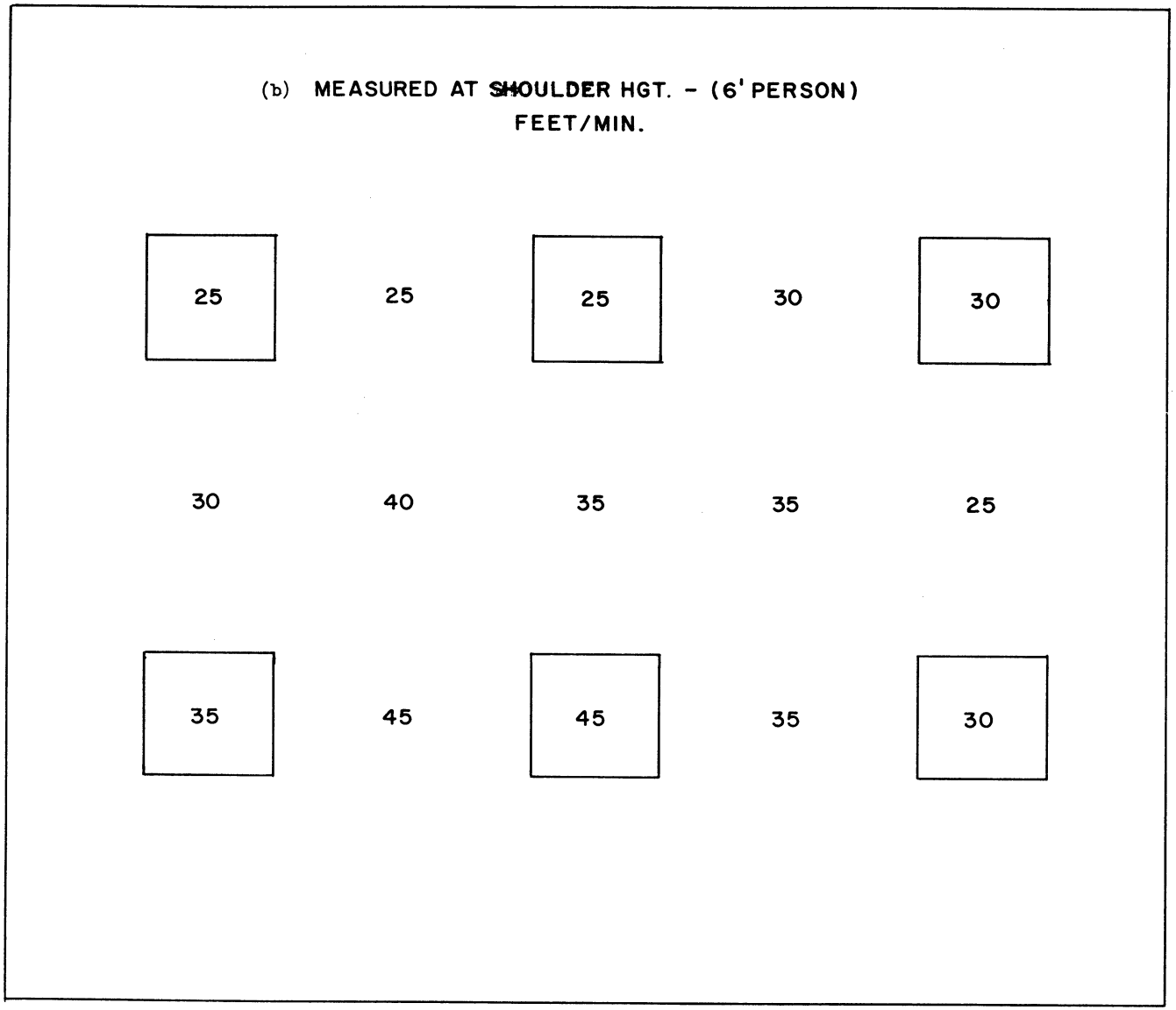

Fig. 15(b). Air velocities at horizontal levels in the Pollinosis Test Chamber.

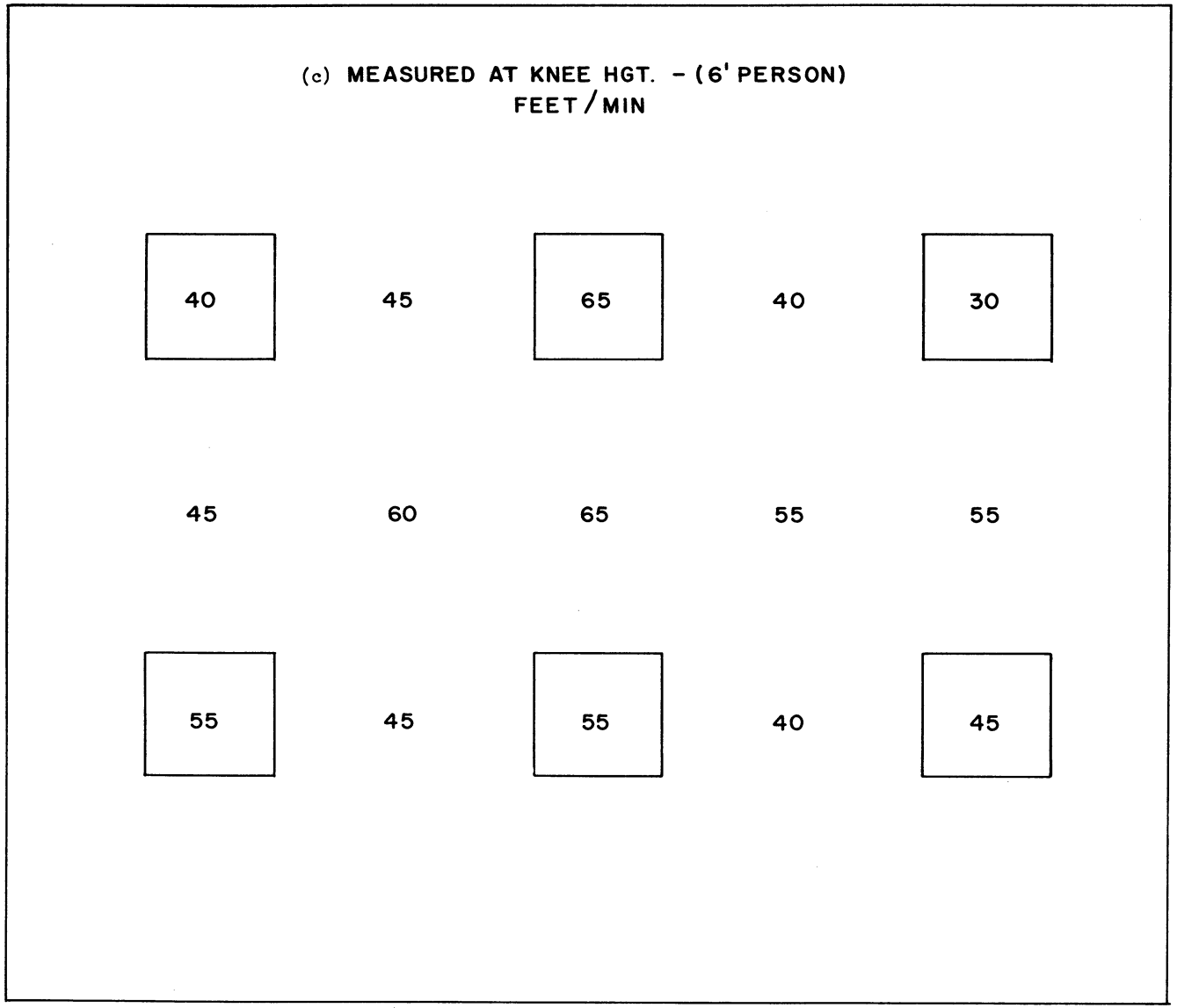

Fig. 15(c). Air velocities at horizontal levels in the Pollinosis Test Chamber.

(2) The average minimum temperature at any humidity was 50° with a range of 47-55°.

(3) At low humidities, less than maximal temperatures were reached; similarly, at high humidities, minimal expected temperatures could be produced.

(4) With the humidistat on "maximum" (over 80%), the highest relative humidity recorded (100%) was attained only with temperatures of less than 55°. With the same humidistat setting, and at 75°, humidities above 55% were not producible, whereas at 85° maximum humidity was 46%.

(5) The lowest relative humidity attained with the humidistat on "minimum" (less than 20%), was 22%; it was attained with temperatures above 80°. At progressively lower temperatues, the minimum humidity parameter gradually rose. At 52°, the humidity could not be lowered beyond 45%.

The method of pollen counting has been described in previous reports.[14] In brief, sampling is done with rotobars carrying No. 606 "Scotch" tape coated lightly (by means of a paint sprayer) with 1:10 Carter's rubber cement in thinner. The rotobars were coated within 12 hours of use and stored in a closed box. Four motors, rated 1800 rpm, are now available for monitoring concentrations at different points in the chamber simultaneously. Tapes are immediately mounted on 100 x 25 mm microscope slides and counted within 48, usually within 12 hours; a calibrated Whipple disc is used in the microscope eye piece to establish a 1 mm^2 field. With low concentrations, the entire sample is counted; with high concentrations, a minimum of 500 grains in one or two entire fields are counted. The result is multiplied by an appropriate factor to obtain a concentration in grains/M^3/hr. Where one position has been used for collection, the sampler has been at the center of the chamber approximately three feet above the floor grating.

The problem of quantitative evaluation of a patient's symptomatology has been given considerable thought and more definitive physiological recordings of the early changes of pulmonary malfunction were deemed necessary if quantitation of the effects of known pollen concentrations on the rapid shift from normal respiration to asthmatic breathing was to be recognized and measured. The simultaneous analysis of pressure, volume, and flow rates, dynamic compliance and airway resistance will be added to the previously mentioned pulmonary function parameters. The suggestion made to us that a similar set of recordings could be modified to measure nasal airway resistance and thus furnish objective data on the symptoms of hay fever led to development of the following procedure. Cyclic variations in pharyngeal pressure occur with specific phases of respiration. If the mouth is firmly closed, so that air flow is entirely through the nasal passages, determination of flow relative to the pressure difference between the pharynx and atmospheric pressure should allow a measure of airway resistance. Obviously, all connections must be as air-tight as possible. A standard, contoured

Collins face mask fitted with a clinical pneumotachygraph is applied to the subject's face so as to enclose the nose and mouth. A curved polyethylene pharyngeal catheter pierces the mask and is positioned such that its distal end is free in the oropharynx. The tubes' proximal end is attached to a differential pressure transducer which also receives a tube connection from the interior of the mask, allowing a measure of the resultant pressure difference and its inscription on a Sanborn 350 recording system. The simultaneous recording of flow with the pneumotachygraph allows determination of pressure at a specific flow, preferably in the laminar or turbulent range. Variations in pressure at a constant flow can then be related to variations in nasal airway resistance for a given subject.

With the use of this procedure, the type of data shown in Figs. 16 and 17 was obtained. The variation in pressure with flow is shown. Initial trials have suggested that with this procedure exposure to significant concentrations of pollen will produce, in a sensitive subject, airway obstruction of measurable degree. Just how early changes in resistance may become apparent with small exposures remains to be established. Because of the variability in nasal architecture and respiratory effort in the population, results obtained from any subject allow comparison only with his own baseline values. Unfortunately, not all subjects are able to tolerate the tip of the polyethylene catheter in their oropharynx; therefore these measurements cannot be carried out on them. Along with the value of objective measurements of nasal airway resistance during known pollen exposures in the chamber, the effects of variation in temperature, humidity, ion concentrations, and position can be ascertained. Emotional and physical stresses and the response to medication can also be evaluated.

2.8.1 Summary

A. After the construction of the chamber was completed, unexpected problems in maintaining equipment had to be met.

B. After the demonstrated failure of previously tried means of pollen dispersion, the Wright Dust Feed Mechanism was modified and used with moderate success.

C. Further extension of attainable temperature and humidity ranges and refinement of the control of these factors is indicated.

D. A method of measuring changes in nasal airway resistance was evaluated by means of simultaneous measurement of pressure drop and flow through the nasal passages. The method was found sensitive to rapid changes in the nasal airway induced by pharmacological agents and will allow objective evaluation of changes in subjects with hay fever.

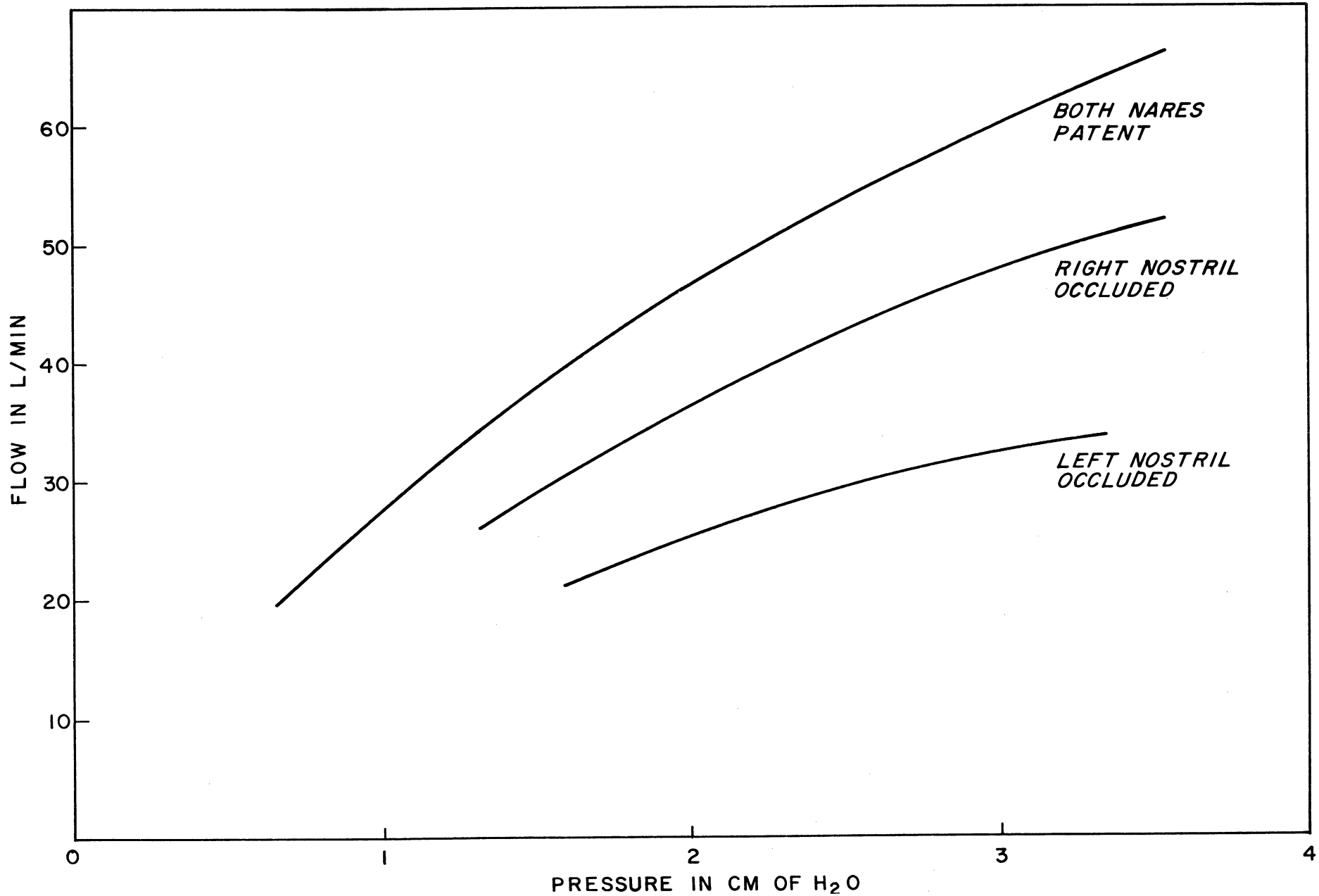

Fig. 16. Variations in flow and pressure with changes in nasal airway potency.

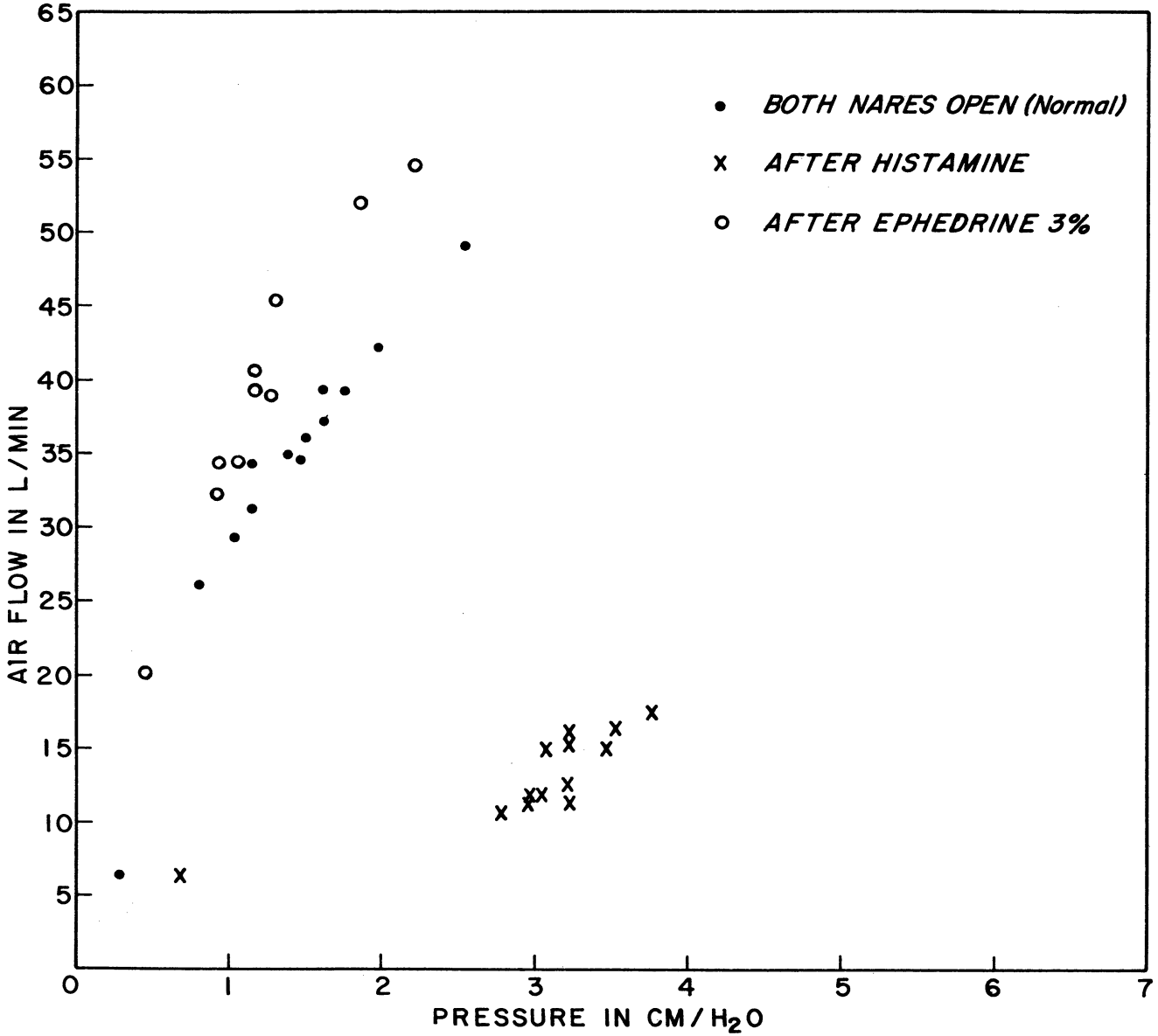

Fig. 17. Changes in the inspiratory pressure. Flow pattern with both nares patent produced by local application of histamine followed by topical ephedrine sulfate.

2.9 THE EPIDEMIOLOGY OF ASTHMA AND HAY FEVER IN A TOTAL COMMUNITY (Tecumseh, Michigan)* (I. Broder, P. P. Barlow, and R.J.M. Horton)**

2.9.1 Description of Study and General Findings

The Tecumseh Community Health Study is a continuing project which was started in 1957 by The University of Michigan School of Public Health. The general aim has been to study the epidemiology of health and disease in a total community.[15,16] This section presents a brief description of the study and the currently available data dealing with asthma and hay fever.

Tecumseh is in southeast Michigan and has a population of approximately 9800. A variety of socio economic and occupational group are represented by this community, and the ethnic composition is almost entirely Caucasian. The proportion of urban to rural households is estimated to be 4 to 1.

2.9.1.1 Method.--The study on which this report is based was begun in March, 1959, and completed in October, 1960. The purpose was to obtain on all residents a record of past and present illnesses, a family medical history, a complete physical examination, and certain physical and laboratory measurements. These data were collected by means of a detailed medical questionnaire and the facilities of a special clinic which was established in the community.

The questionnaires were administered in the homes by a group of ten trained interviewers. Persons aged 16 and over were questioned directly and data for those under this age were obtained from a parent or other close adult relation. Standard questions concerning asthma and hay fever (including questions regarding onset, treatment, and seasonal pattern of symptoms) were asked of all persons aged 8 years and older, of 70% of those aged 7, and of 34% of those aged 6. The remainder of the 6 and 7 year olds were not uniformly questioned about asthma and hay fever, and no persons under this age were so questioned. This entire group has, consequently, been excluded from consideration of this report.

After the questionnaire was completed, an appointment was made for each person to be seen at the special clinic which was established in the community hospital. These visits were arranged to last approximately 1-1/2 hours, during which each person was seen by a physician and certain physical measurements, a chest X-ray, an electrocardiogram, and blood and urine specimens were collected. The physicians were from either the Medical School or the School of Public Health of The University of Michigan and the majority were from the Departments of Internal Medicine and Pediatrics; most were at the level of instructor or higher and approximately 1/3 had worked at some time in the Allergy Section. The physician reviewed all positive sections

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*Presented in part before the American Academy of Allergy, in Denver, Colorado, Feb. 6, 1962

**We are grateful for the extensive assistance given by Mrs. Helen Metzner and Mr. John Napier in all aspects of this work. We also wish to acknowledge the helpful advice and discussion provided by Drs. F. H. Epstein, M. W. Payne, M. D. Kjelsberg, K. P. Mathews, B. C. Johnson, N. S. Hayner, and T. Francis, Jr.

of the questionnaire, asked appropriate additional questions, and carried out a complete physical examination. A list of diagnoses was then made which applied to all conditions present currently or at any time in the past. In order to minimize the possible loss of information, the physicians were encouraged to make diagnoses even where the indications were only suggestive. For each diagnosis recorded, the physician selected one of two possible categories of diagnostic certainty; if a given diagnosis was considered to be reasonably firm, the term "probable" was applied; if it entailed some degree of doubt, the term "possible" was applied. We have chosen to refer to cases in the "probable" category as the "more certain group" and those having the designation "possible" as the "less certain group."

The charts were finally reviewed by one of the staff physicians associated with the project and an independent list of diagnoses was made from the more complete information then available. On the whole, the reviewing physician tended to introduce some degree of uniformity into the diagnoses; he rarely excluded a diagnosis made by the examining physician and at times was able to include diagnoses which had been overlooked in the initial examination.

Although diagnostic criteria were not rigidly defined for this phase of the study, the term "asthma" was generally used in a broad context and applied to manifestations which were either brief or prolonged, occurring in either single or recurrent episodes and either alone or in association with other upper or lower respiratory symptoms. The term "hay fever" was applied to upper respiratory symptoms which were believed to be allergic in origin and occurred predominantly in the spring, summer, or fall.

The analyses to be presented were studied separately on the basis of diagnoses made by both the examining and reviewing physicians. Althought the number of cases found in the groups diagnosed by the reviewing physicians were consistently larger than in the comparable groups diagnosed by the examining physicians, all of the trends to be pointed out were similar regardless of which group of diagnoses was used. In order to simplifty presentation, only those data which are based on the diagnoses of the reviewing physicians will be shown in this report.

The data for the more certain and less certain diagnostic groups were also examined separately, and here again there were no critical differences in the trends shown. In order to preserve the greater reliability of the more certain group and yet retain the inclusiveness provided by the other, the various data are presented in two ways: (1) for the more certain group alone, and (2) for a "combined group" which includes all cases of a given diagnosis without regard to certainty.

As has been pointed out, the diagnoses were applied to conditions which were present at the time of the study or at any time in the past. In describing these data, the term "cumulative prevalence" rather than the used term "prevalence" is used to emphasize their cumulative nature.

2.9.1.2 Results.—In this community 9823 persons were known by census-interviews and examinations were completed for 88% of this total (Table 9). The age and sex distribution of the 6995 persons aged 6 and over on which this report is based are shown in Table 10.

TABLE 9

Total Population of the Tecumseh Area and Segment From Which Full Participation was Obtained

Persons	Male	Female	Total
Number in census	4868	4955	9823
Number interviewed and examined (Percent of number in census)	4238 (87.1%)	4403 (88.9%)	8641 (88.0%)
Number used in this report*	3403	3592	6995

*Applies to the persons aged six and over on whom the subsequent analyses are based.

TABLE 10

Current Age Distribution of the Interviewed and Examined Population

Current Age	Male	Female	Total
6-9	357	342	699
10-14	521	485	1006
15-19	286	316	602
20-24	181	257	438
25-29	274	328	602
30-34	333	365	698
35-39	346	340	686
40-44	243	262	505
45-49	225	212	437
50-54	170	169	339
55-59	162	158	320
60 & over	305	358	663
Total	3403	3592	6995

The total cumulative prevalence of persons with a more certain diagnosis of asthma and hay fever per hundred members of the population was found to be respectively 4.1 and 6.3, and that of the combined groups to be 10.2 and 9.7 (Table 11). Each disease occurred with equal frequency in males and females. As will be discussed later in this report, 128 persons in the more certain asthma and hay fever groups and 254 in the combined groups had both diseases diagnosed and therefore appeared both as persons having asthma and as persons having hay fever.

TABLE 11

Total Number of Persons With a History of Asthma or Hay Fever and the Cumulative Prevalence of Each Diagnosis

	More Certain Group					
	Male		Female		Total	
	Number	Cases per 100 pop.	Number	Cases per 100 pop.	Number	Cases per 100 pop.
Asthma	137	4.0	148	4.1	285	4.1
Hay Fever	213	6.3	226	6.3	439	6.3
			Combined Group			
Asthma	361	10.6	350	9.7	711	10.2
Hay Fever	322	9.5	359	10.0	681	9.7

The age-specific cumulative prevalence of the more certain asthma group in the general population was relatively constant, whereas that of the combined group showed an increase at both ends of the age scale (Table 12). In hay fever, both the more certain and the combined groups demonstrated identical trends; the cumulative prevalence was relatively low in the youngest groups, rose in the second and third decades, and then varied somewhat in the older groups (Table 13).

The age-of-onset data for both asthma and hay fever were more complete in the more certain than in the combined diagnostic groups (Table 11). Of those persons with a history of asthma for whom this information was available, the onset was reported to have occurred most frequently in the youngest age group; for subsequent age groups there was a steep decline in the frequency of onset (Table (14). The onset of asthma occurred before the age of ten in a higher percentage of males (58.7-59.6) than females (37.3-40.6).

TABLE 12

Age-Specific Cumulative Prevalence in the General Population of Persons With a History of Asthma

Current Age	More Certain Group					
	Male		Female		Total	
	Number	Cases per 100 pop.	Number	Cases per 100 pop.	Number	Cases per 100 pop.
6-9	17	4.8	16	4.7	33	4.7
10-14	21	4.0	16	3.3	37	3.7
15-19	10	3.5	10	3.2	20	3.3
20-24	6	3.3	7	2.7	13	3.0
25-29	8	2.9	19	5.8	27	4.5
30-34	16	4.8	14	3.8	30	4.3
35-39	11	3.2	15	4.4	26	3.8
40-44	11	4.5	12	4.6	23	4.6
45-49	8	3.6	5	2.4	13	3.0
50-54	7	4.1	8	4.7	15	4.4
55-59	7	4.3	6	3.8	13	4.1
60 & over	15	4.9	20	5.6	35	5.3
Total	137	4.0	148	4.1	285	4.1
			Combined Group			
6-9	54	15.1	47	13.7	101	14.4
10-14	75	14.4	47	9.7	122	12.1
15-19	25	8.7	22	7.0	47	7.8
20-24	14	7.7	21	8.2	35	8.0
25-29	19	6.9	34	10.4	53	8.8
30-34	31	9.3	26	7.1	57	8.2
35-39	29	8.4	33	9.7	62	9.0
40-44	27	11.1	25	9.5	52	10.3
45-49	21	9.3	24	11.3	45	10.3
50-54	13	7.6	11	6.5	24	7.1
55-59	15	9.3	18	11.4	33	10.3
60 & over	38	12.5	42	11.7	80	12.1
Total	361	10.6	350	9.7	711	10.2

TABLE 13

Age-Specific Cumulative Prevalence in the General Population of Persons With a History of Hay Fever

Current Age	More Certain Group					
	Male		Female		Total	
	Number	Cases per 100 pop.	Number	Cases per 100 pop.	Number	Cases per 100 pop.
6-9	15	4.2	10	2.9	25	3.6
10-14	26	5.0	25	5.2	51	5.1
15-19	28	9.8	17	5.4	45	7.5
20-24	12	6.6	24	9.3	36	8.2
25-29	18	6.6	32	9.8	50	8.3
30-34	22	6.6	24	6.6	46	6.6
35-39	16	4.6	26	7.6	42	6.1
40-44	16	6.6	20	7.6	36	7.1
45-49	17	7.6	9	4.2	26	5.9
50-54	14	8.2	16	9.5	30	8.8
55-59	5	3.1	3	1.9	8	2.5
60 & over	24	7.9	20	5.6	44	6.6
Total	213	6.3	226	6.3	439	6.3
			Combined Group			
6-9	21	5.9	26	7.6	47	6.7
10-14	44	8.4	39	8.0	83	8.3
15-19	36	12.6	26	8.2	62	10.3
20-24	16	8.8	34	13.2	50	11.4
25-29	35	12.8	42	12.8	77	12.8
30-34	32	9.6	44	12.1	76	10.9
35-39	25	7.2	40	11.8	65	9.5
40-44	28	11.5	28	10.7	56	11.1
45-49	24	10.7	16	7.5	40	9.2
50-54	16	9.4	19	11.2	35	10.3
55-59	11	6.8	11	7.0	22	6.9
60 & over	34	11.1	34	9.5	68	10.3
Total	322	9.5	359	10.0	681	9.7

TABLE 14

Age-of-Onset Distribution for Asthma

Age of Onset	More Certain Group					
	Male		Female		Total	
	Number	Percent*	Number	Percent*	Number	Percent*
0-4	38	36.5	31	26.3	69	31.1
5-9	24	23.1	13	11.0	37	16.7
10-14	11	10.6	11	9.3	22	9.9
15-19	7	6.7	17	14.4	24	10.8
20-24	4	3.8	9	7.6	13	5.9
25-29	4	3.8	10	8.5	14	6.3
30-34	3	2.9	7	5.9	10	4.5
35-39	8	7.7	7	5.9	15	6.8
40-44	2	1.9	6	5.1	8	3.6
45-49	3	2.9	2	1.7	5	2.3
50-54	--	--	3	2.5	3	1.4
55-59	--	--	1	0.8	1	0.5
60 & over	--	--	1	0.8	1	0.5
Total	104	99.9	118	99.8	222	100.3
			Combined Group			
0-4	98	41.7	71	31.7	169	36.8
5-9	40	17.0	20	8.9	60	13.1
10-14	21	8.9	17	7.6	38	8.3
15-19	9	3.8	21	9.4	30	6.5
20-24	9	3.8	16	7.1	25	5.4
25-29	11	4.7	16	7.1	27	5.9
30-34	10	4.3	12	5.4	22	4.8
35-39	10	4.3	11	4.9	21	4.6
40-44	8	3.4	16	7.1	24	5.2
45-49	5	2.1	7	3.1	12	2.6
50-54	7	3.0	10	4.5	17	3.7
55-59	2	0.9	3	1.3	5	1.1
60 & over	5	2.1	4	1.8	9	2.0
Total	235	100.0	224	99.0	459	100.0

*Of total group.

The onset of hay fever occurred more frequently at a later age, and here again a difference between the sexes was apparent: the peak for males occurred in the group aged 5 to 9, whereas that for females was 15 to 19 (Table 15).

TABLE 15

Age-of-Onset Distribution for Hay Fever

Age of Onset	More Certain Group					
	Male		Female		Total	
	Number	Percent*	Number	Percent*	Number	Percent*
0-4	19	11.5	14	7.7	33	9.5
5-9	32	19.4	23	12.7	55	15.9
10-14	30	18.2	31	17.1	61	17.6
15-19	19	11.5	38	21.0	57	16.5
20-24	15	9.1	22	12.2	37	10.7
25-29	9	5.5	21	11.6	30	8.7
30-34	12	7.3	11	6.1	23	6.6
35-39	9	5.5	7	3.9	16	4.6
40-44	10	6.1	7	3.9	17	4.9
45-49	1	0.6	1	0.6	2	0.6
50-54	3	1.8	3	1.7	6	1.7
55-59	1	0.6	--	--	1	0.3
60 & over	5	3.0	3	1.7	8	2.3
Total	165	100.1	181	100.2	346	99.9
			Combined Group			
0-4	24	10.6	21	8.3	45	9.4
5-9	43	18.9	26	10.3	69	14.4
10-14	36	15.9	41	16.3	77	16.1
15-19	27	11.9	56	22.2	83	17.3
20-24	20	8.8	26	10.3	46	9.6
25-29	11	4.8	29	11.5	40	8.4
30-34	20	8.8	15	6.0	35	7.3
35-39	13	5.7	13	5.2	26	5.4
40-44	13	5.7	10	4.0	23	4.8
45-49	6	2.6	3	1.2	9	1.9
50-54	4	1.8	6	2.4	10	2.1
55-59	3	1.3	1	0.4	4	0.8
60 & over	7	3.1	5	2.0	12	2.5
Total	227	99.9	252	100.1	479	100.0

*Of total group.

Information regarding the seasonal pattern of asthma and hay fever symptoms was available in over 80% of data for all but the combined asthma group. Symptoms were classified as perennial if they were present throughout the entire year and did not tend to flare in any season; otherwise, they were assigned to one, two, or three seasons (winter, December through March; spring, April and May; summer, June and July; fall, August through November) accordind to predominate tendencies of occurrence. In analyzing these data, spring and summer were combined in order to provide comparable seasonal groups of 4 months in each (Table 16). Perennial symptoms were reported in approximately 30% of persons with a history of asthma and 5% of those with hay fever. On dividing both disease groups into persons under the age of 30 and those 30 and over, it was found that the tendency for symptoms to follow this pattern did not increase significantly with age; these data are tabulated here. Of the three seasonal groupings, spring-summer and fall were commonly reported in both asthma and hay fever; a higher percent of persons with a history of hay fever than with a history of asthma was represented in the fall group. Winter symptoms were reported in 14 to 19% of persons with a history of asthma, but in only 2% of those with hay fever.

TABLE 16

Seasonal Distribution of Asthma and Hay Fever Symptoms*

	More Certain Group			
	Percent of Total			
	Perennial	Winter	Spring-Summer	Fall
Asthma	28.4	14.0	42.4	38.4
Hay Fever	3.8	2.0	48.0	72.4
		Combined Group		
Asthma	30.5	19.2	35.3	33.3
Hay Fever	5.1	2.4	50.3	65.6

*Persons with perennial symptoms are by definition excluded from seasonal groups. Any other persons might be represented in as many as 2 of the 3 seasonal group.

Information regarding treatment was reported in a high percentage of persons with a history of asthma and hay fever. Almost the entire asthma group but only 2/3 or less of the hay fever group had been seen by a physician (Table 17). Injection therapy was more frequently reported in the asthma group than in the hay fever group.

TABLE 17

Number of Persons Seen By a Physician and
Number Receiving Injection Therapy

	More Certain Group					
	Male		Female		Total	
	Saw a Physician	Injection Therapy	Saw a Physician	Injection Therapy	Saw a Physician	Injection Therapy
Asthma	99.2	40.1	98.6	46.0	98.9	43.2
Hay Fever	64.9	21.5	66.5	22.8	65.7	22.2
			Combined Group			
Asthma	99.7	23.2	97.7	33.5	98.6	28.1
Hay Fever	58.1	17.7	59.1	18.9	58.6	18.4

2.9.1.3 Discussion.—A good deal of information is available regarding the characteristics of asthma and hay fever in different populations. Much of this is summarized in 2 reviews[17,18] and no attempt will be made here to treat the existing data in detail. Previous studies have been based either on clinical experience, insurance examinations, student groups, or population studies combine the groups with asthma and hay fever rather than assessing the two as separate entities. At the level of the total community there is some lack of information based on diagnoses made by a participating physician.

A liberal diagnostic policy was emphasized in this study. "Asthma" is believed to have been applied in its broadest context, so that the term includes conditions which might be classified as acute bronchiolitis in the younger groups or bronchitis or emphysema with sheezing in the older ones. Less latitude for this type of diagnostic variation is likely in the hay fever group. The less certain groups with either disease contain a number of persons in whom the diagnosis was comsidered somewhat doubtful. This "looser" type of diagnosis appears to have been made more commonly in the oldest and youngest groups.

As the data presented here are cumulative in nature and most of those from other sources are not, it is difficult to make valid comparisons. However, data somewhat comparable to those in Table 11 are available from several studies. In a small community surveyed by an interviewer, Vaughn found that asthma had been present at some time in 6.2% and nasal allergy in 26.4% of the population.[19] Three studies of university students provide a further

source of information: Van Arsdel and Motulsky[20] reported that of a group at the University of Washington, 4.7% had a history of asthma and 14.7% a history of hay fever; at The University of Michigan, Maternowski and Mathews[21] found cumulative prevalence rates of 5.7% for asthma and 16.6% for hay fever; at the University of Wales, Grant[22] found that 3.3% had asthma either currently or at some time in the past. Although there are some differences among these results and our own, the various data are not grossly discordant.

In this study, the total cumulative prevalence rates of both asthma and hay fever were similar in males and females. However, on comparing the age-of-onset distribution in the two sexes, it was found that both diseases demonstrated a trend towards an earlier onset in males (Tables 14 and 15). This phenomenon has been observed by others in both asthma and hay fever.

The seasonal pattern of hay fever symptoms helps to clarify retrospectively one important criterion used to characterize this group. Perennial symptoms were found to be quite infrequent, and symptoms occurred predominantly in the spring-summer and fall in over 90% of cases (Table 16). As would be anticipated in this geographic area, fall was the most common season reported. In the asthma group, 42-50% of persons had symptoms which were either perennial or occurred predominantly in the winter.

The treatment data (Table 17) demonstrate that medical attention was sought in the asthma group considerably more often than in hay fever, and are in keeping with the expectation that people generally find symptoms of bronchoconstriction much more alarming than those of upper respiratory allergy. The fact that the lower respiratory symptoms were sufficient to warrant medical attention in almost all cases helps to enhance the acceptability of this diagnostic group. The term "injection therapy" was applied to any form of injectable medication and did not necessarily indicate that treatment with an allergenic extract had been reported.

2.9.1.4 Summary.—An epidemiological study of a total community has been described in which a detailed medical history, family history, physical examination, and certain physical and laboratory measurements were obtained in 88% of all residents. Cumulative prevalence rates for asthma and hay fever are presented which are based on the population aged 6 years and older. A diagnosis of asthma was made in 711 persons and of hay fever in 681; these included 285 persons with a history of asthma and 439 with a history of hay fever in whom the diagnoses were considered to be more firm than in the remainder. The age-specific cumulative prevalence of the more certain asthma group was relatively constant, whereas that for hay fever rose to a plateau in the second and third decades. Of those persons with a history of asthma, the first episode most commonly occurred under the age of 5 years; hay fever most commonly began at a later age. Although there were no appreciable sex differences in the total cumulative prevalence of asthma and hay fever, both diseases demonstrated a trend towards an earlier onset in males than in females. Of persons having a history of asthma, 42 to 50% had symptoms which

were either perennial or occurred predominantly in the winter; amost everyone with hay fever had symptoms in the spring, summer, or fall. Persons in the asthma group were more commonly seen by a physician than those with hay fever.

2.9.2 The Relationship Between Asthma and Hay Fever

2.9.2.1 Introduction.—Many authors have stated that asthma occurs in a range of from 22 to 75% (mean 43%) of persons having hay fever. This information is based on the clinical experience of physicians who have a special interest in this type of disorder and is generally found in a context which implies that if persons with hay fever are not treated with allergenic extracts, they are quite likely to develop asthma at a later time. Althought it is undoubtedly true that a high proportion of the persons with hay fever seen by allergists do have asthma, it does not necessarily follow that generalizations made from such highly selected experience can be validly extended to all persons having hay fever. Allergists may tend to see the more serious hay fever cases, and this could lead to their having a distorted picture of the natural history. Therefore it appeared desirable to examine the relationship between asthma and hay fever as found in the population of the total community of Tecumseh, Michigan.

The data to be presented are somewhat in keeping with clinical experience in demonstrating that asthma and hay fever frequently occur in the same persons. However, the relationship between the onset of the two diseases as defined in this study is somewhat different from that generally accepted. The data also imply, as suggested above, that allergists are exposed to a segment of the hay fever population in which the prevalence of asthma is disproportionately high.

2.9.2.2 Results.—A history of both asthma and hay fever was found in 128 persons of the more certain group and 254 of the combined group (Table 18). These data demonstrate that asthma and hay fever occurred in the same persons considerably more often than either disorder was separately diagnosed in the general population.

If this frequent association necessarily implied that hay fever is followed at a later time by asthma, the available data might be expected to show several relationships which they do not. There was no appreciable rise in the frequency of the onset of asthma following the peak of that for hay fever (Fig. 18). Similarly, although there was a two-fold rise in the cumulative prevalence of hay fever in the general population between the current age groups 6 to 9 and 25 to 29, there was no corresponding rise in the cumulative prevelance of asthma (Fig. 19). Most significantly, there was no rise in the cumulative prevalence of asthma in the successive current age groups of the hay fever population (Table 19).

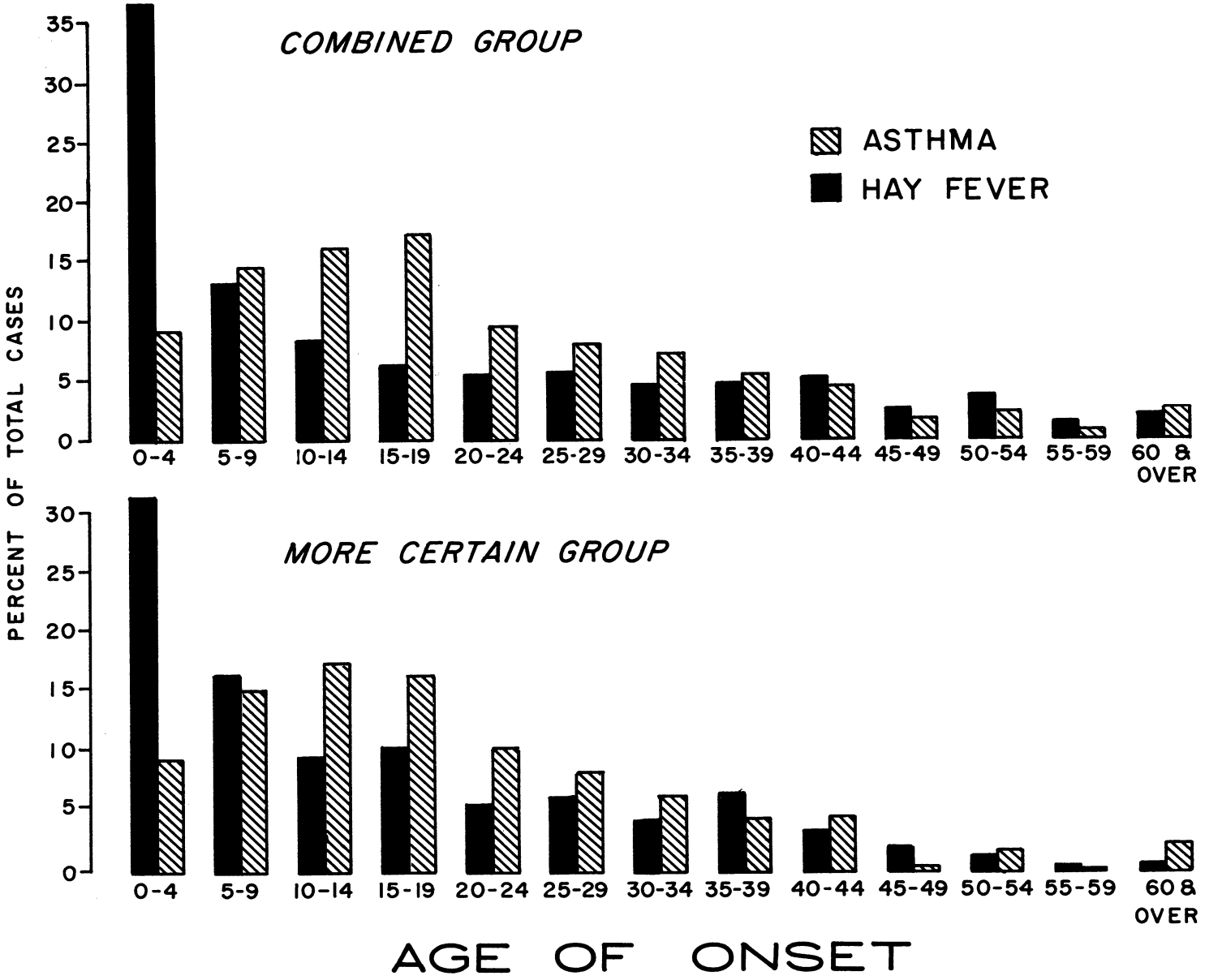

Fig. 18. The age-of-onset distribution for all persons with a history of asthma compared with that of all persons with a history of hay fever.

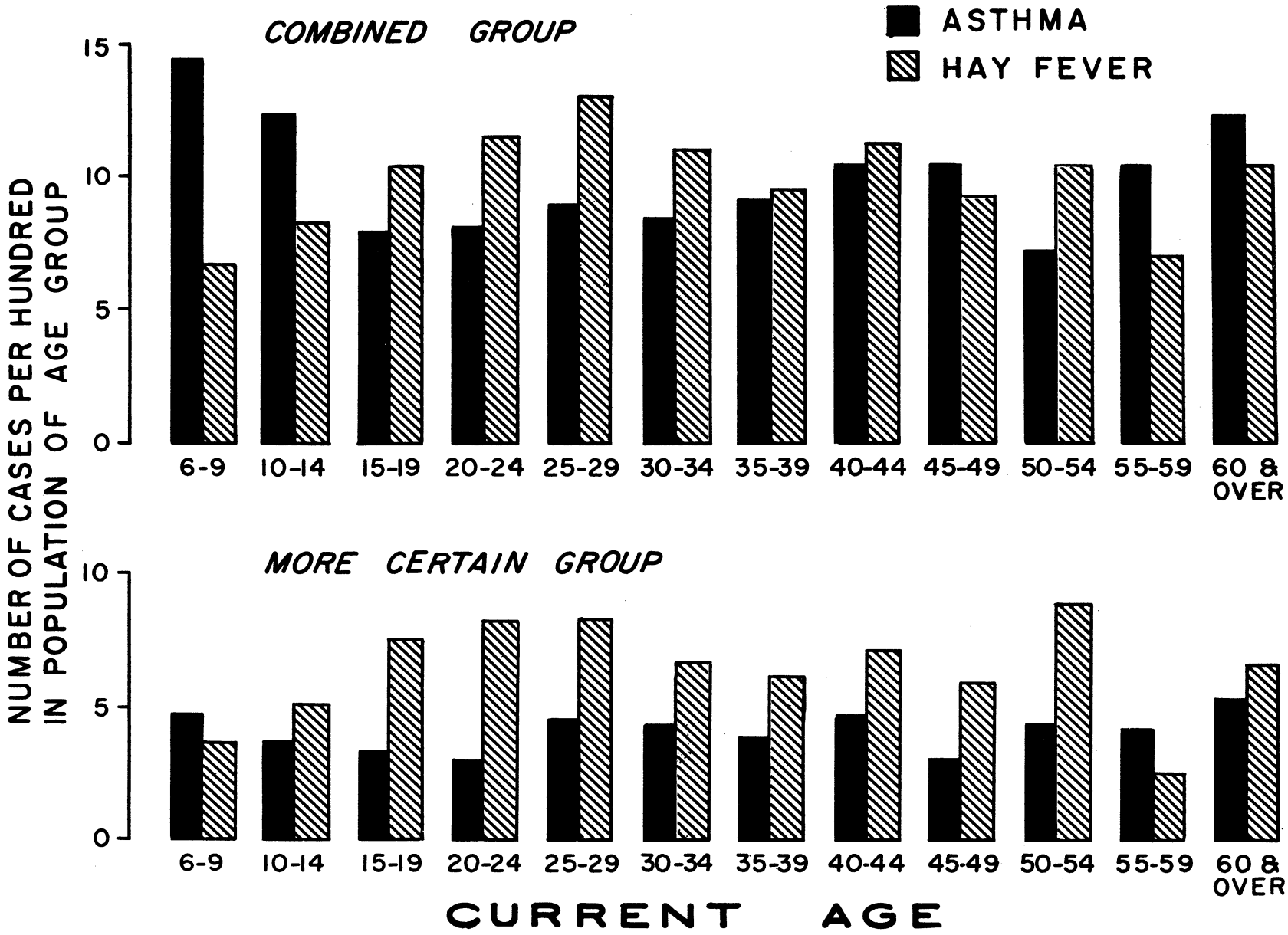

Fig. 19. Age-specific cumulative prevalence for all persons with a history of asthma compared with that of all persons with a history of hay fever.

TABLE 18

Number and Cumulative Prevalence in the General Population of Persons With a History of Both Asthma and Hay Fever*

	More Certain Group		
	Male	Female	Total
Number having a.+h.f.	56	72	128
Cum. prev. a.+h.f. in gen. pop.	1.6	2.0	1.8
Cum. prev. a. in h.f. pop.	26.3	31.9	29.2
Cum. prev. a. in gen. pop.	4.0	4.1	4.1
Cum. prev. h.f. in a. pop.	40.9	48.6	44.9
Cum. prev. h.f. in gen. pop.	6.3	6.3	6.3
	Combined Group		
Number having a.+h.f.	113	141	254
Cum. prev. a.+h.f. in gen. pop.	3.3	3.9	3.6
Cum. prev. a. in h.f. pop.	35.1	39.3	37.3
Cum. prev. a. in gen. pop.	10.6	9.7	10.2
Cum. prev. h.f. in a. pop.	31.3	40.3	35.7
Cum. prev. h.f. in gen. pop.	9.5	10.0	9.7

*The cumulative prevalence of asthma in the hay fever population is compared with that of asthma in the general population; similarly, the cumulative prevalence of hay fever in the asthma population is compared with that of hay fever in the general population. The prevalence rates are expressed as number of affected persons per hundred members of the population in the respective groups.

TABLE 19

Cumulative Prevalence of Asthma in Successive Age Groups of the Hay Fever Population

	More Certain Group					
	Male		Female		Total	
	No. With a.+h.f.	Cases per 100 h.f.	No. With a.+h.f.	Cases per 100 h.f.	No. With a.+h.f.	Cases per 100 h.f.
6-14	12	29.3	13	37.1	25	32.9
15-24	8	20.0	11	26.8	19	23.5
25-34	13	32.5	17	30.4	30	31.3
35-44	9	28.1	9	19.6	18	23.1
45 & over	14	23.3	22	45.8	36	33.3
Total	56	26.3	72	31.9	128	29.2
			Combined Group			
6-14	28	43.1	35	53.8	63	48.5
15-24	18	34.6	22	36.7	40	35.7
25-34	23	34.3	29	33.7	52	34.0
35-44	16	30.2	21	30.9	37	30.6
45 & over	28	32.9	34	42.5	62	37.6
Total	113	35.1	141	39.3	254	37.3

These negative findings are in keeping with those obtained from examining the onset sequence of asthma and hay fever in persons having a history of both diseases (Table 20). Inapproximately 75% of persons in whom the onset sequence was known, either asthma was present alone initially or the two diseases began within the same year of age. Table 21 shows that in persons with hay fever who were at risk of developing asthma (i.e., those in whom asthma was not present beforehand or in whom the two did not begin within the same year), asthma subsequently developed in only 5 to 10%. Although the rates appear to be somewhat higher in females than males, they are not significantly different (chi square values have p levels greater than 0.05).

The possibility was considered that treatment which might prevent complications of hay fever was given to a large enough segment of this hay fever population to have obscured the anticipated relationship with asthma. In the previous section it was shown that treatment information was available in 82 to 91% of persons having a history of hay fever, 18 to 23% of whom reported injection therapy. Table 22 shows further that of the group answering the question on treatment, 12 to 16% reported injection therapy alone, and 4 to 9% indicated injection therapy and skin tests. Although these data demonstrate that only a minority of the hay fever population received treatment

TABLE 20

Onset Sequence of Asthma and Hay Fever in Persons
With a History of Both Diseases

	More Certain Group					
	Male		Female		Total	
No. with a.+h.f.	56		72		128	
No. w/known sequence	38		49		87	
No. w/Known Sequence	Number	Percent	Number	Percent	Number	Percent
Hay fever first	7	18.4	13	26.5	20	23.0
Asthma first	14	36.8	9	18.4	23	26.4
Onset in same year	17	44.7	27	55.1	44	50.6
Total	38	99.9	49	100.0	87	100.0
	Combined Group					
	Male		Female		Total	
No. with a.+h.f.	113		141		254	
No. w/known sequence	56		68		124	
No. w/Known Sequence	Number	Percent	Number	Percent	Number	Percent
Hay fever first	8	14.3	20	29.4	28	22.6
Asthma first	21	37.5	17	25.0	38	30.6
Onset in same year	27	48.2	31	45.6	58	46.8
Total	56	100.0	68	100.0	124	100.0

TABLE 21

Cumulative Prevalence of Asthma Following Hay Fever
in the Hay Fever Population at Risk

	More Certain Group			Combined Group		
	Male	Female	Total	Male	Female	Total
No. with a. after h.f.	7	13	20	8	20	28
No. h.f. at risk (onset known)	134	145	279	179	204	383
Cum. prev. a./100h.f. at risk	5.2	9.0	7.2	4.5	9.8	7.3

TABLE 22

Persons With a History of Hay Fever Who Reported Injection Therapy

	More Certain Group*					
	Male		Female		Total	
	Number	Percent	Number	Percent	Number	Percent
Injection therapy alone	31	16.2	28	13.6	59	14.9
Injection therapy with skin tests	10	5.2	19	9.2	29	7.3
Total	41	21.5	47	22.8	88	22.2
	Combined Group*					
Injection therapy alone	36	13.6	34	11.5	70	12.5
Injection therapy with skin tests	11	4.2	22	7.4	33	5.9
Total	47	17.7	56	18.9	103	18.4

*Percentages based on the total persons for whom treatment information was available.

which might potentially prevent asthma, even this minority is enough to limit any conclusions regarding this point. However, of the total hay fever group reporting injection therapy alone, 51 to 53% had a history of asthma, and of the total reporting injection therapy and skin tests, 62 to 79% had a history of asthma (Table 23). As stated previously, the term "injection therapy" was not restricted to the administration of allergenic extracts. Since less than half the persons reporting this form of therapy were in a position in which asthma could potentially be prevented, there was little opportunity for injection therapy to appreciably alter the natural frequency of complicating asthma in this hay fever population.

An additional observation of interest may be made from the treatment data in Table 23. The cumulative prevalence of asthma in the group with hay fever reporting injection therapy and skin tests was more than twice as high as that in the group not reporting injection therapy. This difference in the frequency of asthma in the two groups is highly significant statistically (chi square p value less than 0.001). Making the assumption that those persons who reported injection therapy and skin tests represent the segment of the hay fever population seen by allergists, one can speculate that these allergists are exposed to a selected segment of the total disease group in which the prevalence of asthma is disproportionately high.

TABLE 23

Cumulative Prevalence of Persons With a History of Asthma in the Hay Fever Treatment Groups

	More Certain Group			Combined Group		
	Male	Female	Total	Male	Female	Total
Receiving Injection Therapy Alone						
Total number	31	28	59	36	34	70
Percent with asthma	38.7	64.3	50.8	36.1	70.6	52.9
Receiving Injection Therapy and Skin Tests						
Total number	10	19	29	11	22	33
Percent with asthma	50.0	68.4	62.1	90.9	72.7	78.8
Receiving Neither Injection Therapy Nor Skin Tests						
Total number	150	159	309	218	240	458
Percent with asthma	23.3	22.0	22.7	28.4	29.6	29.0

2.9.2.3 Discussion.—The question has been raised that the clinical experience of allergists may tend to be weighted with the more serious hay fever cases and may therefore lead to a distorted picture of the natural history. The foregoing data from an epidemiological study of a total community not only suggest that there is such distortion but also indicate that the cumulative prevalence of asthma following hay fever is considerably lower than estimates made from clinical experience. Support for these findings are available from a study of university students carried out by Maternowski and Mathews.[21]

Although the data from the present study appear to provide a more valid foundation for generalizations than existing information, their interpretation may be limited by several factors. First, incomplete data, even where they represent only a small percent of the total, may favor a particular group and therefore introduce bias. The completeness of the data has been indicated for the analyses which are presented in this and the preceding progress report. The analyses based on current age (Fig. 19 and Table 19) have complete data available for the entire population interviewed and examined, and are complementary to the other analyses based on less complete data. Thus, the incompleteness of the data with respect to these variables does not appear to have produced detectable bias.

A further limiting factor may be introduced by memory error, which is inherent in such retrospective data as those dealing with age of onset and

onset sequence. Age-of-onset data from other sources agree with those reported here, but onset-sequence data are not as readily available. We found that of a consecutive group of 41 new patients seen at the Allergy Clinic of The University of Michigan Medical Center who had both asthma and allergic rhinitis of known onset, in 21 either asthma had initially occurred alone or the two diseases had begun within the same year. Urbach reported that of a group of 88 patients having asthma and perennial allergic rhinitis, in 57 either asthma had initially occurred alone or the onset of the two conditions had been approximately simultaneous.[23] Although these studies are not exactly comparable to the present one, they confirm the finding that of persons having a history of the two diseases, a significant proportion either develop asthma first or have an approximately simultaneous onset of both.

It might be argued that the onset-sequence group in which both asthma and hay fever began within the same year of age is an artificial one, and that if it were possible to examine the sequence more carefully, the persons could be distributed between the individual groups in which either asthma or hay fever occurred first. This distribution might considerably enlarge the group in which hay fever is followed by asthma. In the present context, however, the "simultaneous occurrence" onset-sequence group is an important one to maintain. It is not usual for cases having hay fever alone to be presented as candidates for injection therapy during their first year of symptoms. More important, most cases of hay fever are confined to a relatively short period of a given year, particularly during their first season of symptoms. If asthma were to be associated with the hay fever and develop during the same year, the interval between the two would probably be short enough to render impracticable any thoughts of diagnosing the first and establishing effective therapy with the aim of preventing the second.

This is an initial report from a continuing project. In repeat study of this total community, now in progress, more detailed and prospective information is being collected which may make it possible to define more conclusively the relationship between asthma and hay fever. It is hoped that the questions raised here will stimulate other population surveys which, by contributing further data, will increase the number of valid generalizations.

2.9.2.4 Summary.—The clinical experience of allergists indicates that many persons with hay fever may develop asthma if not treated with allergenic extracts. The question has been raised that the experience of specialists may be weighted with the more serious hay fever cases and may therefore lead to a distorted picture of the natural history. Although initial epidemiological data from this study of a total community agree with clinical experience in indicating a frequent correlation between asthma and hay fever, they do not suggest that this relationship is dominated by hay fever leading to asthma; of persons having a history of both asthma and hay fever, in 75% either asthma initially occurred alone or the two diseases appeared within the same year; of persons with hay fever who were at risk to develop asthma,

the disease actually developed in only 5 to 10%. In this population it is unlikely that injection therapy has appreciably altered the tendency for hay fever to be complicated by asthma. The data also imply that allergists may be exposed to a segment of the hay fever population in which the prevalence of asthma is disproportionately high.

2.10 PUBLICATION OF RESULTS

(Most of the papers published since the last Progress Report [No. 4, 1960] refer to work reported in the previous Progress Report).

1. Mathews, K. P. and Spear, H.J.: A Comparative Study of the Hemagglutinating and Skin Sensitizing Activities of Ragweed Sensitive Human Sera, J. Immunol., 87, 274, 1961.

2. Delorme, P. and Mathews, K. P.: The Effect of Human Serum and Serum Globulins on Passive Transfer Tests for Skin Sensitizing Antibodies, The University of Michigan Medical Bulletin, 27, 28, 1961.

3. Field, R. C., Schulte, H. F., Jr., Mixat, D. R., Patterson, R. and Sheldon, J. M.: The Effect of Artificial Unipolar Air Ionization on Hypersensitivity Phenomena, The University of Michigan Medical Bulletin, 27, 269, 1961.

4. Maternowski, C. J. and Mathews, K. P.: The Prevalence of Ragweed Pollinosis in Foreign and Native Students at a Midwestern University and Its Implications Concerning Methods for Determining the Inheritance of Atopy, J. Allergy, 33, 130, 1962.

5. Broder, I., Barlow, P. P., and Horton, R.J.M.: The Epidemiology of Asthma and Hay Fever in a Total Community - Tecumseh, Michigan. I. Description of Study and General Findings. (Accepted for publication by the Journal of Allergies)

6. Broder, I., Barlow, P. P., and Horton, R.J.M.: The Epidemiology of Asthma and Hay Fever in a Total Community - Tecumseh, Michigan. II. The Relationship Between Asthma and Hay Fever. (Accepted for publication by the Journal of Allergies.)

7. McLean, J. A., Barlow, P. P , and Sheldon, J. M.: Reactions to Emulsion Therapy. (Being submitted for publication.)

8. Barlow, P. P., Miller, F. F., Larose, C L., McLean, J. A , Ling, B. P., and Rodriguez, H.: Animal Toxicity Studies on Allergenic Emulsions. (Being submitted for publication.)

3. METEOROLOGICAL PHASE

by

E. W. Hewson, D. F. Gatz, A. N. Dingle, and J. B. Harrington, Jr.

3.1 RURAL AND URBAN AIR POLLUTION BY RAGWEED POLLEN

3.1.1 Introduction

Ragweed pollinosis has been treated in several ways, one of the most effective being that of simply avoiding exposure to the pollen. This can be done by vacationing in a ragweed-free area for a period the time of which is determined by the geographical area and the length of which is determined by the sensitivity of the patient. Persons who cannot do so can still minimize their exposure if they are aware of the times and locales in which pollen concentrations are highest.

The object of the work reported here was to determine the spatial distribution of ragweed pollen concentration and also its variation with time along urban and rural roads. It was originally thought that most ragweed grows in weedy areas and along roadsides so that an automobile passenger travelling along a rural road or highway might be exposed to abnormally high pollen concentrations. If this hypothesis were true a simple spraying program along the roadsides would eliminate a large part of the ragweed menace.

3.1.2 Preliminary Study

Before the sampling program was initiated, a 43-mile route in and around Ann Arbor was selected which incorporated a variety of roads in urban, suburban, and country environments (Fig. 20). At precisely 1-mile intervals along this route, four 1-foot-wide strips extending across the roadside and 100 feet into the fields were marked out at predetermined distances before and behind the parked automobile. In these 400-square-foot areas and also in the roadside areas every ragweed plant was counted; where counts were exceedingly high, however, only a 100-square-foot area was counted.

This survey revealed that the ragweed density was usually determined by the vegetation. Virtually no low annual ragweed grew in deserted weedy fields, lush pastures, over-grown orchards, luxuriant clover or alfalfa fields, in swamps, or in woods. A modest number of plants could be found in and around corn fields, gardens, overgrazed pastures, poor feed crops, and housing developments. The vast majority of plants, completely overshadowing all other

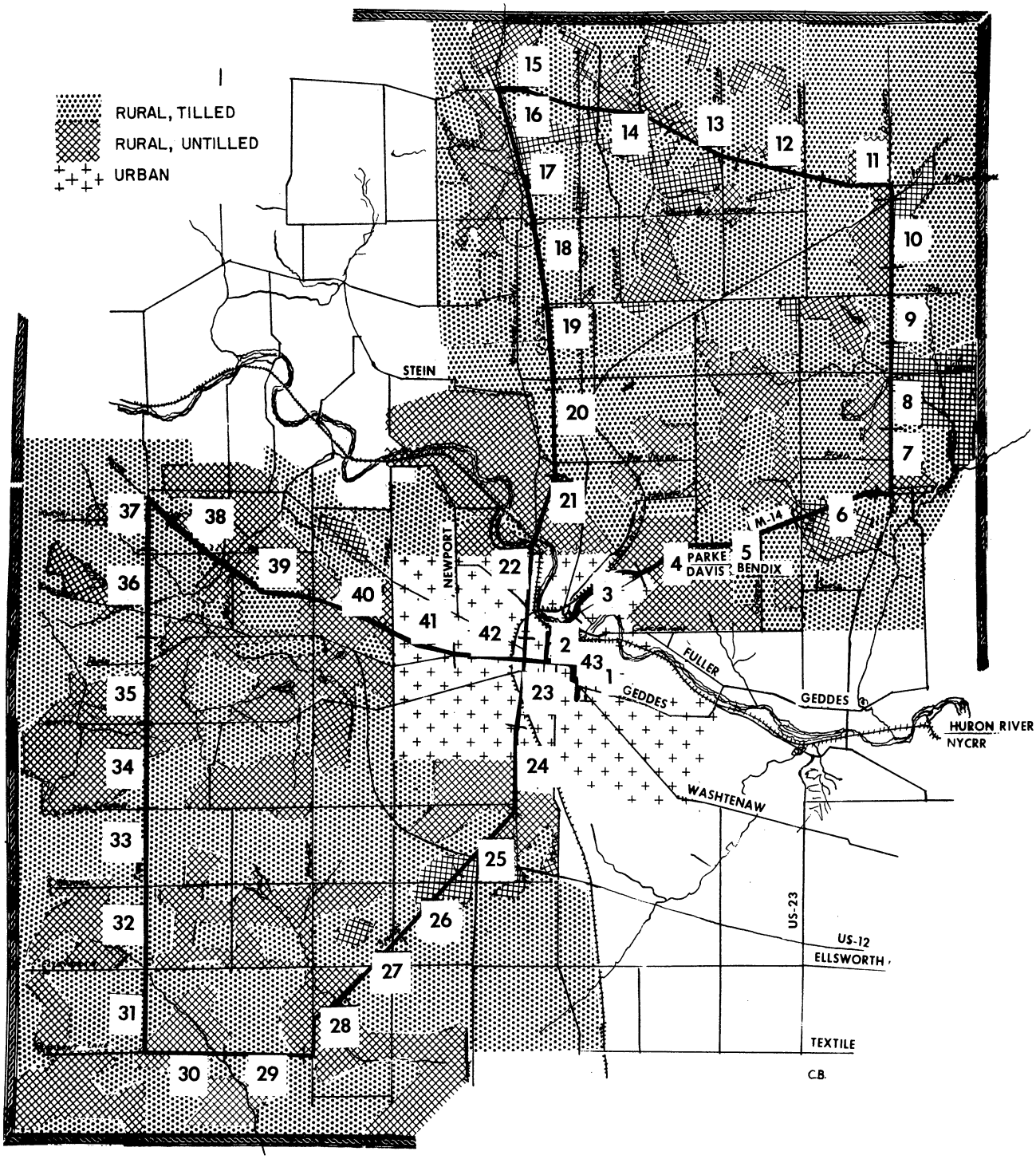

Fig. 20. Route of mobile sampling program.

sources, grew in cereal grain fields, the estimate running to well over 100,000 plants per acre in some fields.

Roadsides do not appear to be a major source of pollen. Some newly developed roads had dense ragweed populations along their margins, but in general ragweed grows only in a 6-inch-wide strip along the shoulder of the road where the gravel meets the ditch vegetation. Even within this strip, mowing often reduces ragweed plants to stunted miniatures of those growing in grain fields.

This preliminary survey has since been corroborated by a group of University of Michigan botanists who carried out an extensive survey of the ragweed population in the Tecumseh area of Michigan.[12]

Some of the objectives of the botanical study had already been fulfilled by the survey. It had definitely been determined that the roadside ragweed plants were inconsequential. Another conclusion of the survey—one of great significance—was that fairly simple methods of control could almost totally eradicate the problem of low annual ragweed. Were the farmer to spray his cereal crops for weeds in the spring, or to plow down the stubble directly after harvest, the ragweed hayfever problem would be virtually eliminated.

3.1.3 Auto Sampling Procedure

Rather than establish stationary sampling sites where the pollen concentrations would be extremely susceptible to local variations in the ragweed population, it was decided to sample from a moving automobile. The flag sampler[24] was used because it could be readily changed in a moving automobile, provided for easy counting, and had a known efficiency. The sampler was mounted near the end of an 18-inch aluminum rod fastened to the side of a car. The rod was located near the forward edge of the right front door, level with the lower portion of the window frame.

The automobile was usually driven at between 25 and 40 miles per hour to ensure high sampler efficiency without becoming a hazard on the highway. At wind speeds exceeding 20 mph the flag samples ragweed pollen with a fairly constant impaction efficiency, near 93%. However, this efficiency is reduced to approximately 70% by the lack of perfect adhesiveness of the sampling surface.

Ideally the sampler would have been changed every time the vegetation in the adjoining upwind field changed. This was not possible, however, because the fastest flag change took at least 3 seconds, which represents approximately 100 feet at the speeds being travelled. Furthermore, the number of grains collected in each sample had to be large enough to have statistical significance. Therefore it was decided to change the sampler every mile to minimize the errors caused by unavoidable variations in sampler-changing

time. This distance turned out to be quite satisfactory; the maximum count never exceeded 800 grains, and even during the evening when concentrations were low the count averaged about 25 grains.

Thirty-two runs were made over the same route between August 18 and September 12, 1958. Of this total, three 2-hour runs were made on each of 7 days, and from 2 to 5 runs were made on three additional days, as indicated in Table 24. Only data from the days on which three complete runs were made, have been used in the subsequent analysis.

TABLE 24

Date and Time of Auto-Sampling Runs (1958)

Date	Morning		Afternoon		Evening	
	Start	End	Start	End	Start	End
Aug. 19	0818	1006	1303	1455		
22	0756	0945	1325	1550	1902	2050
26	0835	1015	1355	1544		
27	0835	1010	1330	1517	1938	2116
28	0835	1022	1321	1541	1904	2055
29	0840	1043	1332	1555	1917	2105
30	0856	1036	1337	1549	1913	2122
31	0835	1045	1328	1526	1952	2200
Sept. 1	0918	1050	1324	1510	1930	2113
12*	0844	1100			1940	2128

*Also 0628-0825, 1123-1330, and 1632-1730.

3.1.4 Analysis

A series of minor corrections were applied to the raw pollen counts. First, those segments in which only a fraction of a mile was sampled were weighted accordingly. Second, all counts were divided by 0.7 to make a rough correction for the lack of adhesive efficiency of the sampling surface. Finally, the pollen counts were converted to concentrations.

The conversion from count to concentration requires a knowledge of the volume of air sampled in each 1-mile segment. When the winds are calm this volume is simply the product of the sampling area multiplied by mile. When there is a wind, however, the sampling area must be multiplied by the ratio of the mean air speed at the sampler to the automobile speed.

The air speed at the sampler is approximately the vector sum of the auto and wind velocities. The former was computed from the known segment orienta-

tion and auto speed. The latter was computed from the wind record of the anemometer at 160 feet on the meteorological tower in Ann Arbor, assuming a 1/7-power-low wind profile between that height and the ground.

In each segment the estimated concentration, χ, is related to the measured pollen count, C, by

$$\chi = \frac{U_c}{V}\left(\frac{C}{DAE}\right),$$

where U_c is the automobile speed, V the speed of air flow past the sampler, D the distance sampled, A the area of the sampler, and E the efficiency of the sampler.

The air speed, V, is computed from the vector components of the automobile speed and the wind speed on the meteorological tower, U_t, reduced to 4 feet, with the 1/7-power-low wind profile again used. Thus

$$V = \sqrt{(W_x + V_c \cos\theta)^2 + (W_y + V_c \sin\theta)^2} \quad ,$$

where

$$W_x = 0.59\ U_t \cos\phi$$
$$W_y = 0.59\ U_t \sin\phi$$

θ is the direction of travel on the road segment and ϕ the wind direction.

3.1.5 Results

The six days August 27 to September 1 were selected for analysis because they had uniformly high counts and spanned the peak of the ragweed season (Fig. 21). Figure 22 shows the average pollen concentration and the extremes along each one-mile segment of the 43-mile route for the six days selected.

First it will be noted that in the morning, pollen concentration varies markedly from segment to segment, and is considerably higher in the country (and in particular along paved roads) than it is in the city.

The very high concentrations along rural paved roads compared with rural gravel roads may be fortuitous, but on the other hand it may represent a more intensive cultivation of longer standing along the paved roads.

Suburbs in general do not have higher pollen concentrations than the nearby city except where the suburb lies on the windward side. In such a case the pollen concentration is more typical of that in the surrounding

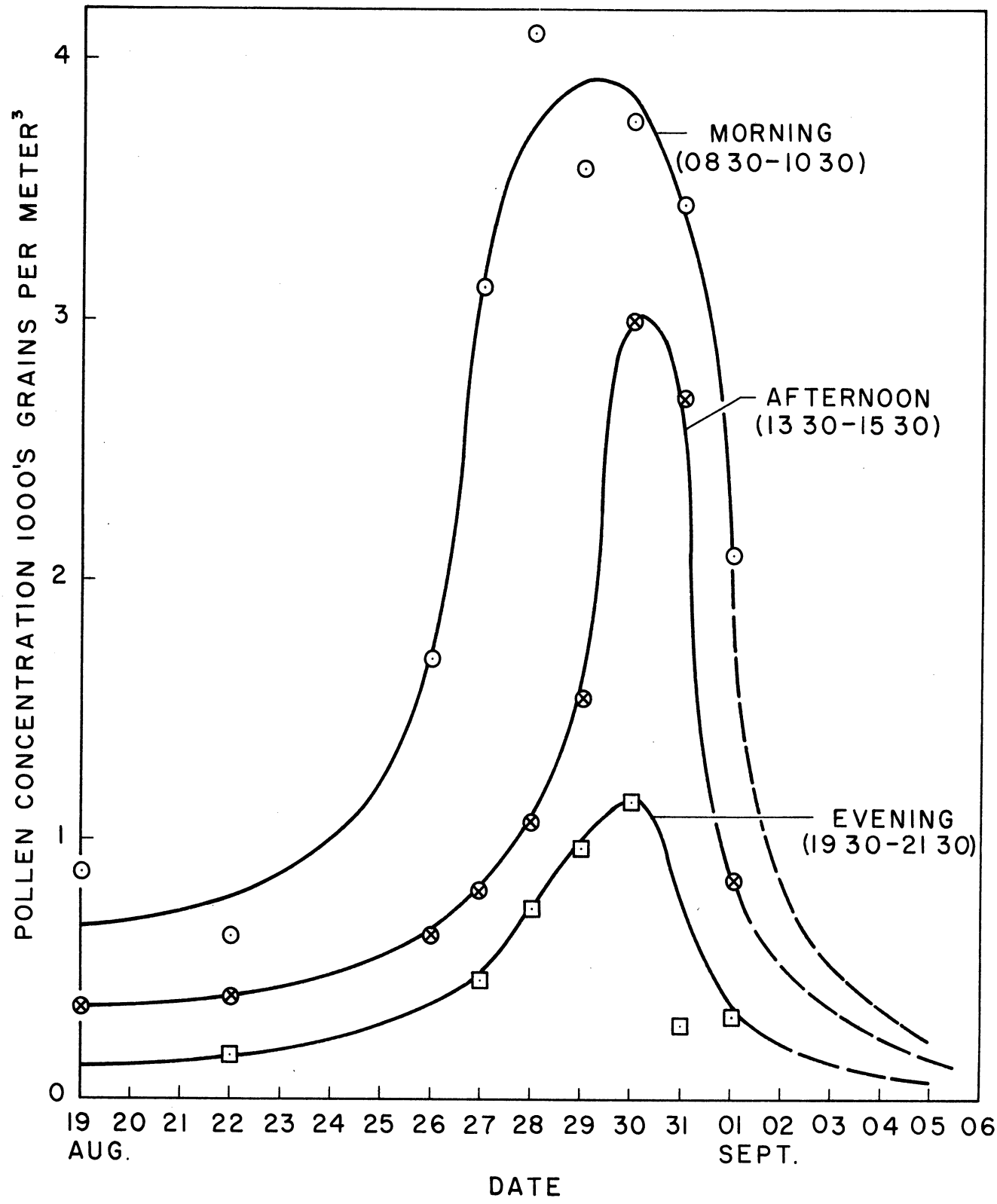

Fig. 21. Ragweed pollen concentrations during the 1958 ragweed season at and near Ann Arbor, Michigan.

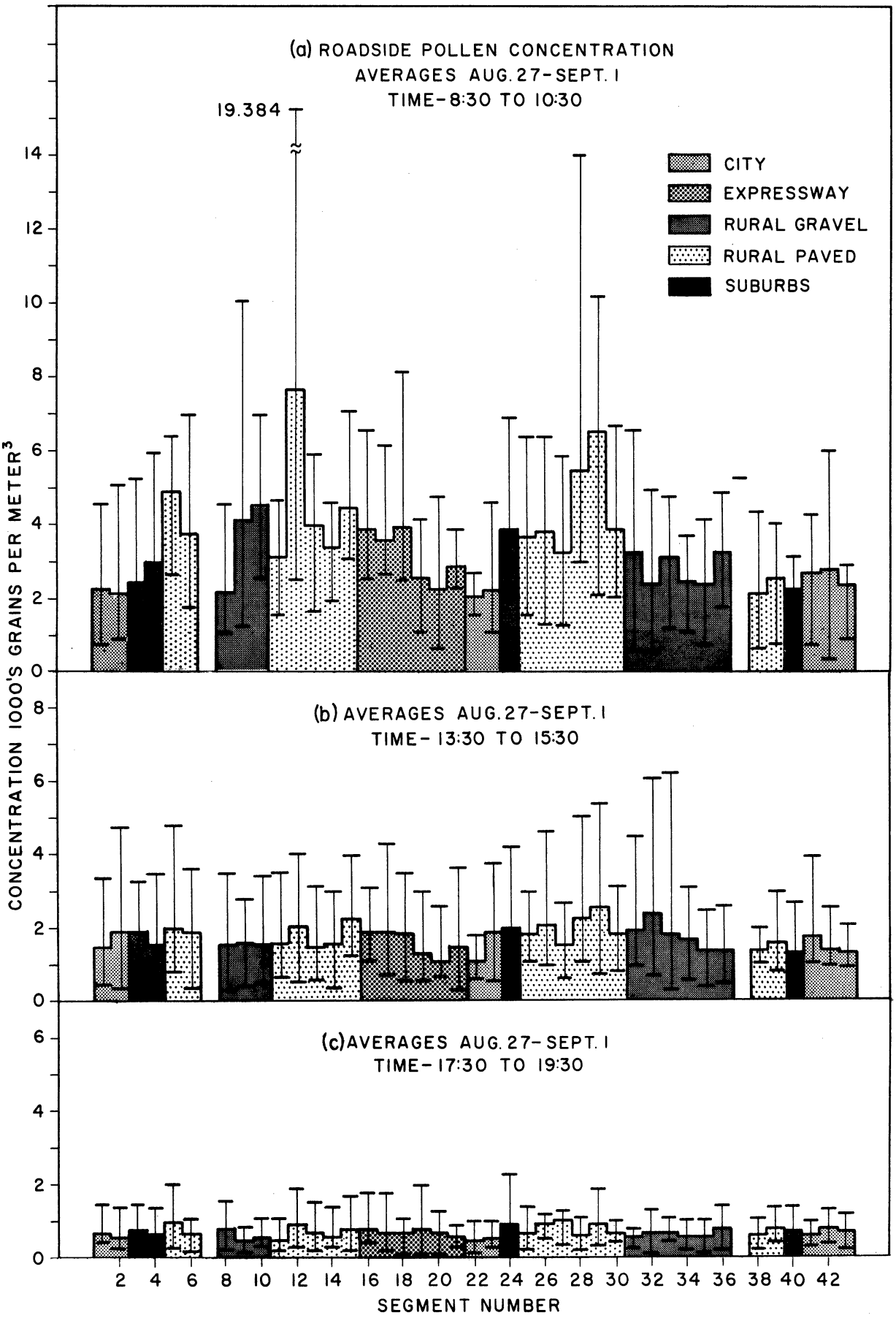

Fig. 22. Average pollen concentration and extremes along each 1-mile segment of the 43-mile route for the 6-day period 27 August—1 September 1958 in and near Ann Arbor, Michigan.

rural area. For example, during the period in question the most frequent wind direction was southwest; therefore one would expect segment 24 to have concentrations representative of rural conditions (Fig. 22(a)).

Segments 1, 23, and 43 were heavily trafficked sections of the city. The sampler was covered during stops; at other times the automobile speed was relatively slow. It is interesting to note that the counts in these sections of the city were not significantly different from those in other sections of the city where normal speeds could be maintained.

During the afternoon the variability of pollen concentration decreased and by evening there was no significant difference between rural and city concentrations (Figs. 22(b) and 22(c)). This could be expected since ragweed pollen is emitted early in the morning and by afternoon has become well mixed in a deep layer of the atmosphere.

The average concentration between 0830 and 1030 during the six days August 27 - September 1 was 3343 grains per cubic meter. The ratio of the 1330-1530 concentration to the morning value was 0.48, with a further decrease to 0.20 between 1900 and 2100. (The times are only approximate.) This rapid decay of pollen concentration with time is further illustrated in Table 25, where the morning, afternoon, and evening concentrations are classified according to road type.

TABLE 25

Average Ragweed Pollen Concentrations in the Vicinity of Ann Arbor
(August 27-September 1, 1958)

Time	City	Suburban	Expressway	Rural Gravel	Rural Paved
0830-1030	2341	2870	3182	2998	4143
1330-1530	1507	1624	1539	1656	1811
1900-2100	604	704	672	589	710

One would expect the highest pollen concentration to occur near the sources of pollen during the period of emission in the morning. If this expectation were valid one could further expect a rather high correlation between the fraction of cultivated land along a road and the pollen concentration. The mixing power of atmospheric turbulence is so great, however, that downwind of a source area even a narrow ragweed-free area materially reduces the ground-level concentrations. Broader roadside margins should be associated with lower concentrations during the morning, and the data of Fig. 22 and of Table 25 for Expressway certainly tends to confirm this thesis.

To obtain a more objective test each roadside segment was classified according to the fraction of cultivated land within 1000 feet of the road to both the left and right. The measured concentrations were then correlated with the fraction cultivated on the upwind side of the road. The results shown in Table 26 indicate that the correlation was not particularly strong, averaging 0.33. This value is just barely significant at the 5% level, suggesting that only 11 to 16% of the variance in the pollen count can be explained by the proximity of cultivated land.

TABLE 26

Correlation of the Fraction of Cultivated Land on the Windward Side of the Road With Concentration During the Morning

Date		Correlation
Aug.	19	0.36
	22	0.46
	26	0.54
	27	0.49
	28	0.40
	29	0.29
	30	0.01
	31	0.15
Sept.	1	0.28
Average		0.33

3.1.6 Sources of Error

The efficiency of the flag sampler has never been adequately tested over a wide range of wind speeds. However, wind tunnel tests have been performed in the range 3-13 mph which indicate about a 11% coefficient of variation in the flag-sampling efficiency.

Over a treeless, uniform surface a few tens of square miles in area, turbulent eddies will cause the wind speed a few feet above the ground to vary by a factor roughly proportional to the mean speed itself. The effect of averaging the speed over one-mile increments is to reduce this variability, since most of the wind energy near the ground lies in the short wave lengths. The exact amount of error caused by wind gustiness is not known and does not seem to have been treated in the literature, as simple as the problem appears to be. Other factors such as the presence of buildings and trees, the degree of instability, and undulation in the land will also affect the wind speed.

Although it is impossible to assess the effect of all errors on the estimate of wind speed, the maximum error in any one-mile segment would probably be no greater than the magnitude of the wind speed itself. Averaged over several segments this error would be reduced by a factor approximately equal to the square root of the number of segments.

During the 21 sampling runs for which the data were analyzed the computed wind at sampler height averaged 5.4 mph. A deviation of 5 mph from this value would produce a maximum error of 20% in the computed concentrations, which is relatively small compared with the 80% coefficient of variation of the segment concentrations for each trip.

3.1.7 Conclusion

Ragweed pollen concentrations could be effectively reduced by a few simple changes in farming practice. Since a great preponderance of low annual ragweed grows in cereal grain fields it could be controlled either by spraying in the spring or by cultivation soon after harvest.

Ragweed-sensitive people can minimize exposure to high pollen concentrations by avoiding rural areas or sources of pollen during the morning and early afternoon hours. During the late afternoon and evening the pollen is distributed uniformly.

Relatively narrow areas free of ragweed plants markedly reduce ground-level concentrations. The major evidence in support of this fact has been obtained from other unpublished experiments by J. B. Harrington; but it is also supported by the relatively low correlation between cultivated land along the roadside and pollen concentration.

3.2 WASHOUT OF RAGWEED POLLEN BY RAINFALL

3.2.1 Introduction

In this section, data gathered in two rains during the ragweed pollen season of August and September, 1961, are analyzed and some of the restrictions on the nature of washout of particulates from the atmosphere by rainfall are discussed. The data were obtained from samples of rain gathered at specified intervals during the two rains. These samples were then analyzed for pollen count.

3.2.2 Sampling Apparatus

The rain samples were collected in two large pans located on a flat roof approximately 12 feet above the ground at The University of Michigan's Willow Run facilities. The pans had a total surface area of 5.2 square meters. Three standard rain gauges positioned about the sampling area were used for determining total rainfall, and a tipping-bucket recording rain gauge was used to obtain data on total rainfall and rainfall intensity.

3.2.3 Sampling Procedure

Prior to each rain, the pans were scrubbed to remove dry fallout. This technique was found sufficient to remove nearly all the radioactive fallout from the pans, and can be assumed to have had the same effect for ragweed pollen grains. It is unlikely that any pollen not removed by scrubbing would be removed by the washing effects of rainfall. Our sampling procedure was to take alternate samples for radiochemical and ragweed pollen analysis, at least during the early and middle parts of each rain. One-gallon samples were taken for radiochemical analysis and 1-quart samples were taken for ragweed pollen analysis.

3.2.4 Pollen-Analysis Technique

The basic technique consisted of (1) the filtration of several aliquots, usually 100 ml each, taken from each sample, and (2) the preparation of a microscope slide from the filter paper of each aliquot. Each slide was then counted for pollen after being stained with Calberla's solution.* The mean pollen concentration in the sample was determined by averaging the results of the several aliquots.

Our first filtration technique was merely to shake each sample thoroughly before removing the aliquot. Initial results showed that ragweed pollen counts went up for each successive aliquot removed from the sample (see the footnote to Table 27). Therefore we suspected that pollens and other sediments were adhering to the walls and bottom of the polyethylene sample bottle, and that—since the violence of the shaking increased as the volume decreased—each shaking was producing an increased concentration of pollens dispersed in the rain water. What was needed, evidently, was a method for evenly dispersing the pollens in the rainwater.

At the suggestion of Dr. W. S. Benninghoff, of the University's Botany Department, we used potassium hydroxide to disperse the pollens and devised the following method:

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*One part glycerine, 2 parts alcohol, 3 parts water, and 1% basic fuchsin.

A. KOH was added to the sample to make a solution approximately 5 to 10% KOH by weight.

B. The inside surfaces of the sample bottles were well scrubbed with a rubber policeman, and the samples were well shaken before withdrawal of each aliquot.

C. Usually three 100-ml aliquots were withdrawn by volumetric pipet and each filtered through a 1-inch-diameter millipore, style AA filter.

D. In order to prevent the KOH from bleaching out the Calberla solution stain, it was necessary to dry each filter overnight in a desiccator before staining.

E. The filters were then mounted on glass slides and counted under the microscope.

3.2.5 The Storm of 1 September 1961

This storm gave us nearly 2 inches of rain and provided our most extensive set of data.

Examination of the synoptic situation shows that the rain which fell at Willow Run on this date was not associated with any frontal system whatever. The rain system resulted from an intensification of an easterly wave that progressed along the Gulf Coast and converged with an isolated cold mass of superior air moving northeastward from the Texas area. This peculiar combination resulted in the development of a fairly extensive isolated precipitation area across northern Alabama and Mississippi, western Tennessee, and eastern Arkansas and Missouri on the afternoon of 31 August. The surface trough associated with this rain system progressed northeastward and formed a weak low pressure center over Central Ohio by the morning of 1 September, at which time the precipitation area extended over most of the states of Ohio and Michigan and northward into Ontario. There is, therefore, good evidence that no air-mass change whatever took place on this occasion.

The rain began at 10:30 EST and continued until 13:12 EST. It was of showery character accompanied by occasional thunder and lightning and by highly variable winds. The three standard rain gauges at the station showed the total amount of rain as 1.99, 1.86, and 1.85 in., respectively. The tipping-bucket gauge recorded a total of 1.86 in. In all, we collected 29 gallon radiochemical samples and 15 quart pollen samples. Because some of the sample bottles had to be kept on hand for future use, only eight of the pollen samples were retained for analysis. The results of the pollen analyses are given in Table 27 and are plotted, together with the results of the radiochemical analyses, in Fig. 23.

TABLE 27

Results of Analyses for Ragweed Pollen in Rain Collected from Thunderstorm of 1 September 1961

Sample Code No.	Mid-Point of Sample Period (EST)	Accumulated Rainfall to Mid-Point (10^{-2} in.)	Total Sample Volume (ml)	Aliquot or Slide No.	Aliquot Volume (ml)	Raw Count (gr)	Pollen Concentration (gr/100 ml)	Mean Sample Concentration (gr/100 ml)
1 IX 1P	1044:32	5	913.2	1	100	19,716	19,716*	
				2	100	30,516	30,516*	
				3	100	53,422	53,422*	
				4	50	11,506	230,100$^{\Delta}$	
				5	50	8,981	179,620$^{\Delta}$	
				6	50	9,659	193,180$^{\Delta}$	146,300*
1 IX 3P	1046:55	16	1001.8	1	100	2,208	2,208*	
				2	100	2,277	2,277*	
				3	100	3,920	3,920*	
				4	50	2,144	4,288	
				5	50	2,034	4,068	3,746*
1 IX 5P	1048:36	24	906.8	1	100	856	856*	
				2	100	930	930*	
				3	100	1,224	1,224*	
				4	100	4,433	4,433	
				5	100	3,722	3,722	3,059*
1 IX 7P	1050:48	34	918.9	1	100	687	687	
				2	100	1,012	1,012	
				3	100	1,186	1,186	962
1 IX 11P	1055:16	47	1002.7	1	100	364	364	
				2	100	465	465	
				3	100	481	481	
				4	100	443	443	
				5	100	435	435	438
1 IX 19P	1103:51	78	917.0	1	200	193	96	
				2	200	230	115	106
1 IX 25P	1124:20	118	1005.1	1	200	1,472	736	
				2	200	1,104	552	644
1 IX 29P	1152:18	162	763.3	1	100	2	2	
				2	100	0	0	
				3	100	0	0	1

*The first three aliquots withdrawn from the samples coded 1 IX 1P, 1 IX 3P, and 1 IX 5P showed the necessity for better dispersion of the pollens in the sample, and led to the pollen-analysis technique described in the text. The original concentrations of pollen in these samples were calculated from the results of all withdrawn aliquots by the following method. X = initial number of pollen grains in the sample; G_1 = number of grains removed during initial filtration attempts, i.e., in aliquots 1-3; V = total sample volume in deciliters; and V_1 = volume removed during initial filtration attempts $V_1 = 3.00$ in each case. The observed average concentration, c, in the remaining aliquots, should then be related to X by the relationship $c = X-G_1/V-V_1$. This equation may be solved for X, which is then divided by V to obtain the initial concentration.

$^{\Delta}$Slides 4, 5, and 6 were prepared from an aliquot of the original solution diluted 10:1. Thus, the true concentration was calculated by multiplying the raw count of a 50 ml aliquot of the diluted sample by 20.

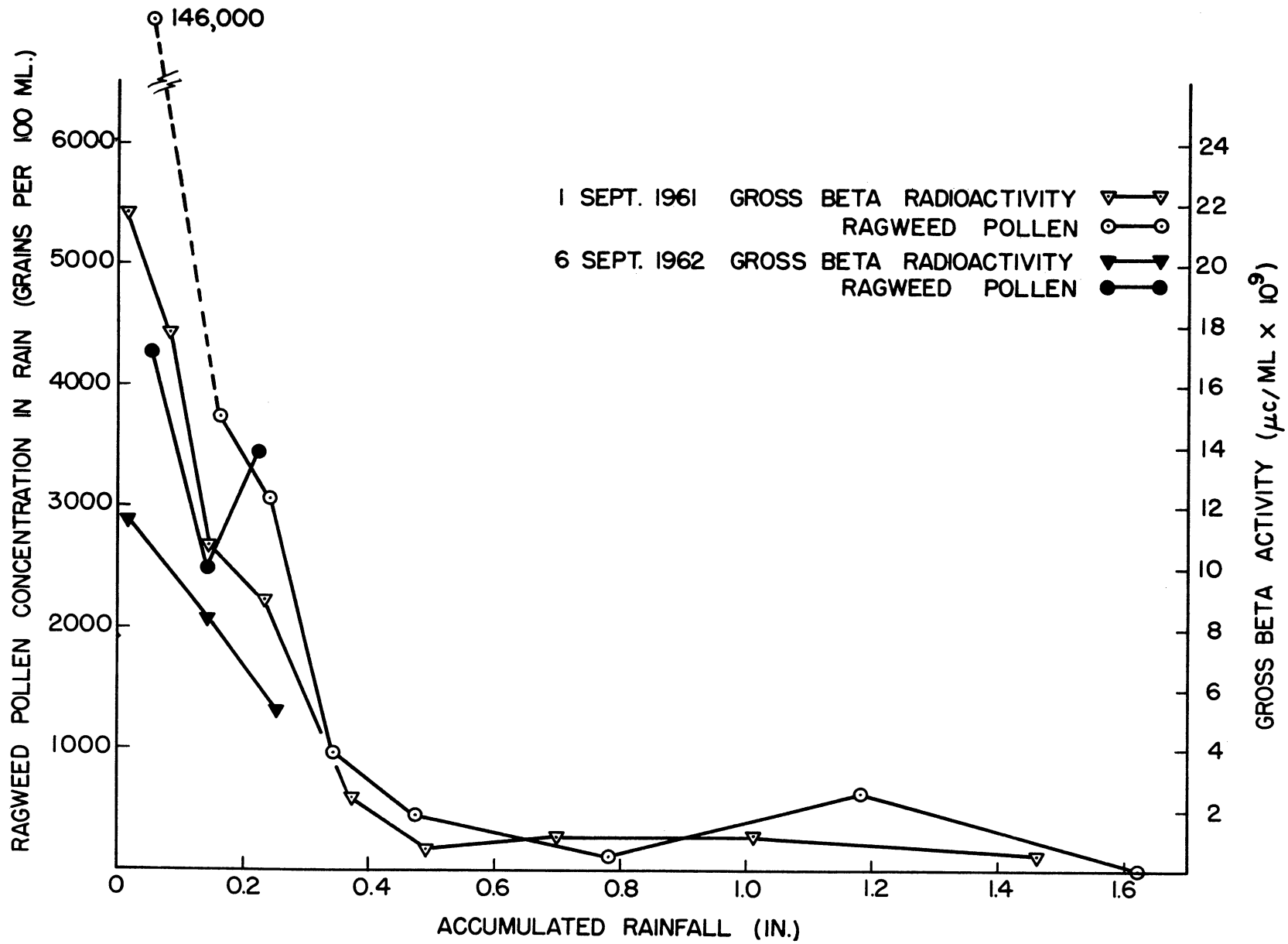

Fig. 23. Rain concentrations of contaminants vs. accumulated rainfall.

There is some possibility that the pollen grains found in the first sample were not accumulated exclusively by rainfall. Between 08:30, when the pans were scrubbed, and 10:30, when the heavy rain began, dry fallout of ragweed pollen may have contributed some of the pollen found in this sample. But on the other hand, calculations based on an air concentration of ragweed pollen of double the estimated value, and on a pollen fall velocity in still air of about 1 m/min, show that only about 624,000 grains could have been dry-deposited in the pans before the rain. Sample 1 IX 1P was collected after sample 1 IX 1 (a gallon sample consisting of the very first rain to fall during the shower) had been collected. If any of the dry-deposited pollens were going to be washed from the pan by the rain, one would expected that most of them would have come down in sample 1 IX 1. However, even the supposition that all of the dry-deposited pollens were removed from the pans into sample 1 IX 1P would account for no more than 43% (62,4000 grains) of the pollen concentration actually found in this sample (146,3000 grains). On the basis of these calculations we must conclude that a very substantial portion of the pollens found in sample 1 IX 1P was due to washout by rainfall.

Also significant is the increase in pollen concentration found in sample 1 IX 25P over that found in sample 1 IX 19P. Such an increase could result either from an influx of fresh pollen-laden air, or, assuming a static atmosphere, from an increase in removal efficiency. Note that in order for the latter to be the real cause, the magnitude of the increase must be large enough to compensate for a reduction in air concentration since the previous sample was taken. According to the method of calculation described in Section 3.2.7, for the period during which sample 1 IX 19P was collected, 74.0% of the pollen cloud remaining airborne at the beginning of the period should have been removed from the atmosphere. Another calculation, for sample 1 IX 25P, shows that 85.6% of the pollens in the air when the sampling period began should have been removed by the rain during this period. Although the percentage removed is seen to increase from the earlier to the later period, it is quite obvious (on the assumption that no fresh pollens were added to the atmosphere) that the increase is not large enough to compensate for the lower air concentration at the beginning of the later period. Thus, the observed increase in pollen concentration found in sample 1 IX 25P can only be accounted for by an influx of unwashed, pollen-laden air. Such occurrences cannot be accounted for by any present model for rain scavenging.

3.2.6 The Storm of 6 September 1961

The synoptic character of this rain is not clear from the U.S. Weather Bureau daily maps, but the description of the rain as recorded in the project log book suggests the character of a squall line. The rain began at 15:27 EST and ended at 16:18 EST. Despite a dark and turbulent appearance of the sky there was very little lightning and thunder, and the winds were relatively moderate. The total rain recorded by the standard rain gauges was

0.37, 0.32, and 0.33 in., respectively; the tipping-bucket gauge recorded a total of 0.34 in.

Concentrations of airborne ragweed pollen were determined before and after the rain by the rotobar sampler described by Harrington, Gill and Warr.[24] The air sample taken during the period from 15:16 to 15:36 EST shows an air concentration of 394 grains/m^3. Note that the rain began while the sample was being taken. Because air concentration is decreased by the scavenging effects of rain, the results should be taken as a lower limit of the true pre-rain air concentration. The air sample taken from 16:18 to 16:33 EST (immediately after the rain) shows an air concentration of 8 grains/m^3.

Three of the rain water samples taken during the storm were analyzed for their pollen content. The results of the analyses are given in Table 28, and shown in graphic form, together with the results of the radiochemical analyses, in Fig. 23. Because of the close agreement between the raw counts of the first two aliquots removed from samples 6 IX 1P and 6 IX 3P, it was decided that two aliquots were sufficient to determine the concentration. Because of the large difference between raw counts of the first two aliquots of sample 6 IX 4P, two more filtrations were made. Unfortunately, the additional filtrations did not help to resolve the anomaly—in fact, they appear to have increased it! No explanation for the variability of these results is available; in any case, the mean sample concentration calculated from these four filtrations should be treated with caution.

Because of the uncertainty associated with sample 6 IX 4P, no conclusive statements regarding the apparent increase in concentration from sample 6 IX 3P to sample 6 IX 4P can be made.

In comparing these results with those from the storm of 1 September, we immediately note the absence of the large pollen concentration in the first pollen sample taken. Here again, however, sample 6 IX 1P was taken after a gallon sample had been taken for radiochemical analysis, and it is quite possible that the gallon sample contained the high pollen concentration. Apparently a more detailed investigation of the initial stages of a rain is needed to clarify the actual process here. Such work will be undertaken during the coming ragweed pollen season.

3.2.7 Further Analysis of the Data

Our data for pollen grains washed from the air by rainfall have been compared with a model of below-cloud washout proposed by Greenfield.[25] With the aid of an IBM 709 computer, washout has been computed for a series of time intervals through the rain and compared with the actual washout for those time intervals for which we have data.

TABLE 28

Results of Analyses for Ragweed Pollen in Rain Collected from Storm of 6 September 1961

Sample Code No.	Mid-Point of Sample Period (EST)	Accumulated Rainfall to Mid-Point (10^{-2} in.)	Total Sample Volume (ml)	Aliquot or Slide No.	Aliquot Volume (ml)	Raw Count (gr)	Pollen Concentration (gr/100 ml)	Mean Sample Concentration (gr/100 ml)
6 IX 1P	1536:20	5	919.8	1	50	2,121	4,242	
				2	50	2,136	4,272	4,258
6 IX 3P	1539:40	14	898.9	1	50	1,375	2,750	
				2	50	1,114	2,228	2,489
6IX 4P	1547:27	22	906.3	1	50	1,078*	2,196	
				2	50	3,248*	6,496	
				3	50	656	1,312	
				4	50	1,893*	3,787	3,448

*Mean of two counts.

Greenfield has derived the following expression for the fraction $\bar{T}_d$ of particles of diameter d removed from a given cylinder of air in a given time by rainfall of known rate:

$$\bar{T}_d = 1 - [1 - \{\overline{E'(D)}\}_d]^n .$$

If distribution of the raindrop size is known or assumed, $\{\overline{E'(D)}\}_d$ can be determined by the relationship:

$$\{\overline{E'(D)}\}_d = \sum_D [P(D - \Delta D/2,\ D + \Delta D/2) E^*(D,d) (D/D_L)^2]$$

where $P(D-\Delta D/2,\ D+\Delta D/2)$ is the probability that a drop will have a diameter between $D-\Delta D/2$ and $D+\Delta D/2$, and $E^*(D,d)$ is the Langmuir collision efficiency, adjusted for particle density, for a drop of diameter D on a particle of diameter d. $E^*(D,d)$ has been computed[26] for drop sizes from 0.02 cm to 0.58 cm in increments of 0.02 cm, assuming a ragweed pollen diameter of 20μ and density of 1.3 g/cc.[27] We have followed the assumption of others[28] that each collision results in capture of the pollen grain by the raindrop, and have further assumed that electrostatic effects are negligible. D_L, the largest drop considered, is also the diameter of the cylinder of air from which washout is being computed. The number of raindrops to fall through the cylinder during the sample interval is n, and may be computed from

$$n = \frac{3}{2} \frac{D_L^2 \, Rt}{\sum_D [P(D - \Delta D/2,\ D + \Delta D/2) D^3]}$$

where R = rainfall rate in mm/hr, t is the duration of the sample interval in hours, and the other terms are as defined. Both $P(D-\Delta D/2,\ D+\Delta D/2)$ and D_L have been computed as a function of rainfall rate from the standard drop-size distribution given by Marshall and Palmer.[29] Drop-size spectra have been assumed constant with height. In such computations, the value of D_L has been arbitrarily taken as the mean diameter of the 0.02-cm-diameter interval (at the large-drop end of the spectrum) which accounts for just 1% of the total rainfall intensity in the time interval during which the particular sample was collected.

Average rainfall rates, R, have been computed for each sample period from data obtained from a recording tipping-bucket rain gauge. Duration of sample interval, t, is the actual length of time required to fill a sample bottle with rain water flowing from the collection pans.

At this point it is possible to calculate the total fraction of ragweed pollen grains removed from an imaginary cylinder of known diameter by rain-

fall of a known rate during a known period of time. Before an actual number of ragweed pollens removed in a given time can be calculated, however, we must specify the height of such a cylinder and then estimate the number of pollens in the cylinder. The number of pollens in the cylinder is most easily estimated by measuring the concentration at the ground and assuming a constant concentration with height. Such an assumption may approximate reality late on a fairly windy day, but the approximation becomes poorer as the wind decreases and sampling takes place earlier in the day. For our purposes we have assumed constant concentrations with height of 500 grains/m^3 for the air concentration of pollen before the rain of 1 September, and 400 grains/m^3 before the rain of 6 September. In the absence of a measured pre-rain concentration, the figure of 500 grains was arrived at by extrapolation of counts taken before and after the rain in Ann Arbor and after the rain at Willow Run; it is only a rough approximation at best. As mentioned earlier, the figure of 400 grains is derived from actual on-site measurement.

Obviously, the depth of atmosphere being cleansed by the washout process, i.e., the height of the cylinder, cannot be greater than the depth of penetration of the pollen. This probably means that it cannot be higher than the cloud-tops—the extent of convection; moreover, it should not be so high that our assumption of constant distribution of drop size is seriously in error. In view of the difficulty of estimating such heights in the absence of a nearby radiosonde observation, we have computed washout for heights of 1000, 5000, and 10,000 feet.

The analysis here described is clearly a preliminary attempt to see how closely our data agree with a proposed model which may be a gross misrepresentation of the actual state of the atmosphere. This possibility is evident a priori from the fact that the model cannot account for the increase in rain concentration of pollen observed from sample 1 IX 19P to 1 IX 25P. Of course the washout process is anything but static—the effects of continuity and replenishment of air contamination must be dealt with. Being as complex as it is, the process is extremely difficult to model adequately. We have been making some attempts to do so, based on the principle of continuity, but for our present purposes analysis in terms of a very simplified model is surely in order. It yields some rather interesting results.

The washout computations show that for both rains described above, more than 99.99% of the ragweed pollen should have been removed from the pollen cloud during the time in which the initial gallon samples were being collected. If this had actually occurred, then we should not have discovered any pollen at all in the samples we analyzed.

There are several possible explanations for the departure of the observed from the calculated washout:

A. The pollen may have been completely removed from the air during the period in which the initial gallon sample was collected. Because of the time required for a pollen grain deposited in a part of the pan remote from the drain to enter a sample bottle, the pollens which were deposited in the pans during the first sample interval might be found distributed over several sample intervals. This might well explain the large concentration found in sample 1 IX 1P, but hardly the pollens found in samples taken more than 10 min after the beginning of the storm. Furthermore, this would not explain the above-noted increase in concentration from one sample to the next.

B. Since the observed removal efficiency tended to be smaller than that predicted with use of the Greenfield model, it is probable that every pollen grain-raindrop collision does not result in the capture of the pollen grain (i.e., that collection efficiency is not 100%).

C. The observed concentrations of pollens in rain could result from the continual replenishment of the airborne pollen concentration by air which has not been in contact with the rain. This is believed to be the means by which pollens entered samples taken during the middle and later parts of the rains, and the cause for the increase observed in the rain concentrations of pollens.

It must be noted in conclusion that our observations are not in conflict with the Greenfield scavenging theory; however, they do indicate that the theory must be extended to account for replenishment of the contaminant with time.

4. STATISTICAL PHASE

by

R. D. Remington

This section describes work completed in the biostatistical phase of the Cooperative Study on Atmospheric Pollution by Aeroallergens during the 18-month period ending February, 1962. The general nature of the activities of the biostatistics group during this period was to provide statistical consultation to the investigators in the subject matter areas—botany, meteorology and allergy—concerning experimental and study design, data collection, data processing, statistical analysis, and the writing of research reports.

During this period there were no large sets of data analogous to those generated by the Jackson Prison studies and the pre-season test plot experiments, which in earlier periods required extensive and time-consuming processing by the statistical group. This is in part due to the fact that many of the data processing activities, at least in the meteorological phase of the study, are now being completed by the meteorologists themselves, several of whom have become skilled at writing computer programs. On the other hand, the ready availability of large-scale computing equipment has greatly simplified the data processing activities of the statistics group, and these activities now occupy a much smaller proportion of the total statistical effort than was expended during the studies mentioned above.

A few of the individual statistical consultations completed during this period will be described to indicate the diversity of activities. Because most of the projects involved are described in detail in other sections of this report, the basic emphasis here will be on the statistical problems and techniques.

The questionnaire study of foreign and native student groups at The University of Michigan carried out by Drs. Chester Maternowski and Kenneth Mathews in an attempt to elucidate possible hereditary factors and temporal influences on the development of ragweed sensitivity was completed. This study involved fairly extensive statistical consultation, as well as questionaire design, analysis of data, and completion of the research report.

A study by Willard Payne of the botany group investigated the comparison of three measurements of size of ragweed fruits in a group of contemporary fruits versus a group of ancient fruits found in Indian caches in caves in the southern United States. Mr. Jacob Keller, a graduate student in the Department of Biostatistics, completed the statistical analysis of the data. The analyses included an investigation of possible bimodality in the size distributions of the modern fruits apparently associated with geography, confidence-

interval estimates of the difference between the individual size parameters of modern versus ancient fruits, and a multivariate test of the difference between the corresponding vector means.

Alan Gebben, a botanist whose doctoral thesis project involves an investigation of ragweed growth and ecology, made extensive use of statistical and computer techniques with the assistance and consultation of the statistics group. Specific analytic techniques included chi-square partitioning and analysis of variance and covariance.

M. Anthony Schork, a doctoral student in biostatistics, has become associated with the group and has been involved in several studies with the meteorologists. This activity will expand with support by the continuing U. S. Public Health Service.

The group has acquired a large and varied set of statistical computer programs written by members of the Biostatistics Department of the University of California, Los Angeles—the BIMD program series. These were obtained on magnetic tape and will be available to the "Aeroallergens" group after a minimal amount of reprogramming to adjust for the requirements of The University of Michigan computer system. This program library should greatly facilitate the data processing and analytic activities of this project. An analysis of covariance program in the series has already been used in connection with Mr. Gebben's study.

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