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A SENIOR THESIS.

A REVIEW OF AIR STUDIES
IN THEIR RELATION TO HAYFEVER AND TO ASTHMA.

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PREFACE.

In this review of air studies in their relation to hay-fever and asthma I have been forced to limit my discussion to that part of the subject which is more or less directly related to the use of the counting chamber in air analysis.

This is a fascinating field where there is much room for further investigations.

I am deeply indebted to Dr. E.L. McQuiddy for inspiration and much appreciated aid in the preparation of this thesis.

Hugh H. Williams Jr.

HISTORICAL.

The hundred years from the first quarter of the 19th century to our own time encompass the historical development of our present knowledge of hay-fever. The recognition of the disease as a clinical entity, the presentation of experimental proof of its pollen etiology and the development of methods for its prevention and control have all been achieved during this period. Many of the physicians who contributed to this important knowledge were themselves sufferers of the malady.

On March 16, 1819 Dr. John Bostock, an English physiologist and clinician, read a paper before the Royal Medical and Chirurgical Society of London on a "Case of a Periodical Affection of the Eyes and Chest" (1.) in which he presented the history and clinical symptoms of a seasonal affection which had troubled him since childhood. Nine years later he gave a more detailed account (2.) of the disease applying to it the noncommittal name of "Catarrhus Aestivus" or summer catarrh, although the affection had, since his earlier publication obtained the popular name of "hay-fever". Even though Bostock's achievement is somewhat dimmed by his failure to discern that pollen was the cause of the disease, the credit for its first clinical recognition justly belongs to him.

The use of the popular name hay-fever shows that the conception of the complaint as produced by hay fields or attributable to hothouses and to the aroma of flowering grasses prevailed at large during the first quarter of the 19th century. That Bostock did not share in this opinion is clear from his statement: "I think myself fully warranted in asserting that, in my own case, the effluvium from hay has no connection with the disease". Bostock believed that his symptoms were brought on by the rays of the sun.

William Gordon, a contemporary of Bostock, took a different view. After

studying a patient suffering from hay-asthma he came to the conclusion that it was due to the "aroma emitted by flowers of the grasses" particularly from those of sweet vernal grass. In summing up his observations Gordon states: "I have said that sweet vernal grass seems to be the principle cause in exciting hay-asthma, and I am induced to come to this conclusion, first, because this plant is one of the most scented of the grasses; and second, because as soon as it begins to flower and not until then the asthma commences; as the flowers arrive at perfection the disease increases; and after they have died away I have remarked that the patient could pass through the most luxuriant meadow with total immunity. The disease then should rather be denominated a grass-asthma than hay-asthma since hay seems incapable of producing it". (3.)

John Elliotson (1786-1868) argues forcibly against Bostock's view. He suggests that hay-fever depends on the flower of grass and probably upon the pollen. (4.)

In 1859, a memorable one in the early history of hay-fever, Phoebus began the first monograph on the disease, Hyde Salter published his classical work describing cases of asthma and perennial hay-fever due to animal emanations, and Blackley commenced his ingenious experimental researches on the pollen etiology of hay-fever.

Phoebus (1804-1880) in his monograph which was published in 1862 (5.) was not inclined to accept any one agent as the sole cause of the disease. He accepted the view of Bostock that "the first heat of the summer is a stronger cause than all the grass emanations put together". He thought that the longer days produced a more continuous action of light and thus the reason for its presence during this time of the year.

Hyde Salter (1823-1871) in his classical work on asthma which first appeared in 1859 gives a classical description of hay-fever and asthma following exposure to animal emanations. (6.) His description of "cat-asthma" is probably the first in the literature.

In his monograph on hay-fever Phoebus described what is probably the first experiment carried out by a hay-fever patient upon himself. W.P.Kirkmann, a German physician, noticed a single plant of sweet vernal grass in his hothouse and it was loaded with pollen. He rubbed some of this pollen between his fingers and sniffed some up his nose. This brought on an attack of hay-fever which lasted about an hour.

It is, however, to the credit of Charles Harrison Blackley of Manchester England and Morrill Wyman of Cambridge Massachusetts, but more particularly to the former, to have given the first classical experimental proof that pollen is the cause of hay-fever. In analytic observation and ingenuity in devising experiments, Blackley's work first published in 1873 has no peer even in our time. (7) Year after year for some fifteen years he painstakingly and with discomfort to himself carried out the well conceived experiments upon himself. These observations were made and recorded during the busy days of an active practice. In 1859 Blackley noticed a bunch of one of the grasses in a vase in his home which had been placed there by a member of his family. In examining he disturbed the plant and a small cloud of pollen became detached and came in close proximity of his face and he began sneezing and had a short attack of his usual summer disorder. As this particular grass flowered early and as there was little or no grass in flower in the meadows at the time he was satisfied that his symptoms were due to the pollen that had escaped and from that time his investigations commenced. He tested himself on the pollen of nearly one-hundred different species of grasses and flowers in the fresh as well as the dried state and also, in some instances, in the form of the alcoholic extracts. He was probably the first to perform the skin test with pollen.

FIRST AIR STUDIES.

Having established the fact that seasonal hay-fever was caused by

pollen of plants, he turned to a series of experiments to find out the quantity of pollen that might be floating in the air, in low and high altitudes, and the relationship between this quantity and the severity of his symptoms. As for technic he adopted a simple plan which, he says, he afterwards found was recommended by Dr. Phoebus. This consisted of exposing slips of glass to the open air for a given length of time so as to allow any solid matter the air contained to deposit upon the glass. Each slip of glass had a cell formed upon it with black varnish so as to enclose a space one centimeter square. This square was coated with a layer of fluid which he prepared by mixing one part water, two parts of proof spirit, and one part of glycerine. The slips were exposed for twenty-four hours and then were placed under a microscope and the number of pollen granules counted.

The first series of experiments was made in meadow-land about four miles southwest of the city of Manchester. Pollen was found constantly upon the slides, and in large amounts during the whole of the hay-fever season, varying somewhat according to the temperature and rainfall. He observed that fully 95% of all the pollen collected on his glass slips belonged to the Gramineae. In the city he found there was, on the average, about 1/10 as much pollen as in the country. Inside his house, if the windows were closed, he found very little pollen.

Experimenting between the months of May and August 1866, he found that between May 30th and August 1st the quantity of pollen increased gradually up to the last week in June and then decreased. In his own case the hay-fever symptoms increased and diminished as the amount of pollen in the atmosphere rose and fell. The pollenometric charts of today differ in no essentials from those made by Blackley in 1866, 1867, and 1869.

Other observations were made by attaching the glass slide to

a kite, and thus examining the atmosphere at elevations from 500 to 1500 feet. One of the observations is thus described: "The altitude attained varied, according to the force of the wind, from 600 to 800 feet. The experiment occupied six hours. The wind blew all the time from the east, and consequently would pass over a large portion of the southern side of the city before it came in contact with the instruments. The nearest grass land would be from two and one-half to three miles distant. 584 pollen grains were deposited in the upper slide in six hours!" (Chapt.14, page 176)

Blackley wanted to find out whether other substances than pollen caused hay-fever, and if the property does belong to pollen, whether it belongs to the pollen of grasses only or to that of other plants and, if so, to which. He also asked himself whether it was due to fresh pollen alone or also to dry pollen, and, further, whether it's activity was due to a chemical or a physical agent.

As previously mentioned he experimented with air in town and compared it with that at the edge of town, and that of the hayfields a few miles out of town. He tried all sorts of grasses and flowers. He experimented with 35 different natural orders. He impregnated air in his home with both fresh and dry pollen. He also experimented with ~~the~~ air filtered through muslin impregnated with drugs, living in such a room in the middle of the country where air was filled with pollen. Also tried breathing air through a box in which there was a filter sieve. He fashioned some silver plugs which were shaped like shoes with gauze in the soles which were used in the nostrils.

Blackley also used glasses with adhesive surfaces so that the amount of pollen that would have fallen into the conjunctiva could be ascertained.

Blackley found that pollen alone would bring on attacks of hay-fever. Other agents would irritate susceptible people but would not

cause a typical attack. He stated that the flowering grasses, not hay, were the usual cause of the disease. According to him the plants belonging to the Gramineae have this property to a more marked degree than others; but there are plants belonging to other orders which have it in an almost equal and in some cases to an equal degree. According to his findings the toxicity of the different sorts does not depend upon the size or visible characteristics of the granules. Moisture prevents spread of pollen while heat, dryness, and wind all help to disseminate it.

He found that there is a threshold of immunity and that the amount necessary to bring on an attack is small. He surmises that an immunity can be developed in early life since fewest cases are seen in farmers who are exposed the most.

Blackley used a weather vane with slide attached to aid in obtaining a more accurate count.(8)

In 1925 Dr. William Scheppergrell of New Orleans confirmed Blackley's observations with a kite by exposing slides in an airplane at elevations over 10,000 feet.(9)

The first noteworthy American contribution to our knowledge of hay-fever was made by Morrill Wyman, of Cambridge. With some other members of his family he had been a life long sufferer of an autumnal form of the disease. Besides Dr. J.A. Swett of New York, who in his treatise on "Diseases of the Chest" published in 1852(10) mentions a periodical catarrh occurring in the autumn, no physician had called attention to this form of the affection. In 1854 Wyman described the disease in his lectures at the Medical School of Harvard University, and in 1866 he read a paper on the late form of hay-fever at the meeting of the Massachusetts Medical Society, giving it the name of "Autumnal Catarrh!"

Wyman was one of the first to recognize in the pollen of ragweed(*Ambrosia artemesia folia*, Roman wormwood) one of the causative

agents of autumnal hay-fever. Of his experience with ragweed he writes:

"Early in September 1870, I gathered in my grounds at Cambridge, some Roman wormwood in full flower, covered with pollen, taking the whole plant, stalks and roots. This was carried to the White Mountain Glen, about 1200 feet above tide, where we remained until September 23rd in the afternoon. The parcel containing it was then opened and freely sniffed by myself and son. We were both seized with sneezing and itching of the nose, eyes, and throat, with a limpid discharge. My nostrils were stuffed and my uvula swollen, without cough, but with the other usual symptoms of autumnal catarrh. These troubles continued through the night and did not disappear until the afternoon following. Dr. Jeffries Wyman, who was of the same party, but did not sniff the plant, had none of the symptoms described. (11)

Similar experiments with ragweed were carried out on himself by Dr. Elias J. Marsh of Patterson, New Jersey in 1876 and reported in a paper read before the New Jersey Medical Society in 1877. He not only confirmed Wyman's observations but supplemented these with counts of the number of *Ambrosia* pollens in the atmosphere before and after the appearance of symptoms. In summing up his conclusions Marsh writes:

"From these investigations the writer has formed the opinion that autumnal catarrh, like the English hay-fever, is caused by the presence of pollen of flowering plants in the atmosphere, and its irritant action on the respiratory mucous membrane of susceptible persons".(12)

The pollen etiology of hayfever so ably established by the crucial experiment of Blackley and Wyman, was by no means generally accepted in the U.S. and Europe. Their work was published at the beginning of the bacteriologic era. Under the influence of the research of Koch and Pasteur, hayfever began to be considered an infectious disease. Hermann Von Helmholtz, who was a sufferer of the disease described vibrio-like organisms which he considered the causative agents

of hay-fever. The bacterial of the disease found many adherents, although not one of Koch's postulates had been fulfilled in any of the experiments described.

In 1876, another monograph on hay-fever appeared, written by Dr. George M. Beard, a neurologist of New York City (13), who concluded that the whole question of the origin and nature of hay-fever is yet an open one and advances the view that the disease is primarily a neurosis.

More than a quarter of a century elapsed before Dunbar (14), also a victim of the disease, repeated the main experiments of Blackley, elaborating them by new ones, and establishing beyond a doubt the role of pollen as the cause of hay-fever. In 1902, the investigations on hay-fever undertaken by him many years previously were brought to a temporary conclusion and their results published. He advanced the theory that hay-fever is a disease caused by vegetable poisons contained in the pollen of certain plants. These substances were connected with the proteid of the pollen grain and of a highly specific character. (15). Applying the modern methods of Immunology to the problem, Dunbar and his pupils laid the scientific foundations which have made possible the evolution of methods for the specific diagnosis and treatment of hay-fever during the past twenty-five years.

In summing up this historical sketch I might say that as far as air study itself is concerned, it began with Blackley's experiments launched in 1859 as the reader no doubt has perceived, and has been and is being carried on by a great many capable men through the world who have come to realize the great importance of air study in the control and prevention of hay-fever and asthma.

A REVIEW OF AIR STUDIES IN THEIR RELATION TO HAY-FEVER AND
ASTHMA.

If one can feel certain of the numerical strength of his enemy he has already laid the groundwork of his defense. Therefore the need for quantitative data . Certainly in a task which is all defense, the advantage of accurate statistics can hardly be over estimated. (16).

In order to correctly interpret the data which is obtained through air study a more or less detailed knowledge of pollen is necessary.

We have seen that the pollens of rose and of the grasses were long suspected of being allergenic. Until recently the brightly flowered plants, such as the rose and the goldenrod, were popularly considered the cause of hay-fever and asthma. One may be actually sensitive to these pollens as well as to many other beautiful flowering plants, but they are not the usual offenders.

The reason is interesting . Beautiful flowers, in fact all brightly colored flowers, are insect pollinated as contrasted with those plants without color, which are wind pollinated. The pollen of ~~th~~ the former is heavy and sticky and will not be carried long distances through the air. The sweet odor and brilliant colors exist for the purpose of attracting bees, butterflies, and other insects which come to suck the honey and nectar. The sticky pollen adheres to the body of the insect and is thus transported from one plant to another.

Close contact, within a hundred feet or so, may produce symptoms in one who is allergic to some particular flowering plant. This is especially the case when flowers are used for purposes of decoration in houses.

The drab, and uncolored plants which the sufferer had passed by un-noticed, on the other hand, produce heavy amounts of light dry pollen which is carried from plant to plant on the wind. Such a haphazard method of distribution requires its production in enormous quantities.

Ragweed pollen will be carried as far as 15 miles through the air. It is almost as abundant 4000 feet up in the air as just above the ground. (17)(18). Pollen granules have been found up to a height of 15,000 feet. It is almost as abundant down town in the average large city as in the outskirts.

Some of the tree pollens are carried much farther. A particular variety of fall-blooming elm pollen has been found in Indiana, over a hundred miles from its nearest tree. (19).

John P. Henry of Memphis, Tenn., (20) says: " An interesting feature about tree pollen is the height from which it takes the air. In the same wind, a pollen 10 microns, which goes 184 feet, when the plant is two feet high, will go two hundred and sixty-eight feet when the plant reaches five feet. How much farther a tree pollen of similar size would travel you can readily see. Increase that wind from two to twenty miles per hour, and you can readily see how much farther it will travel."

The male and female trees may be miles apart, and large amounts of pollen must therefore be shed by the male. Many varieties of trees have been found responsible for symptoms.

The grasses are so common and so much a matter of every day life that most people do not think of them as possible sources of trouble. It is not alone the field grasses, such as timothy, orchard-grass, June grass or Blue grass, red top and sweet vernal grass, but also the lawn grasses, Bermuda grass, wire grass, barn-yard grass, etc., which may be responsible for symptoms.

Pollen granules are formed in the anthers, the male sexual organs, of plants. They correspond to the sperm cells of animals. Their function is to fertilize the ovum, in the seed. This is essential to reproduction.

The different pollen granules vary considerably in size, shape and general conformation.

A flower consists of a group of specialized and highly differentiated leaves arranged in four series. The firm greenish leaves covering the bud and found at the base of the flower are called "sepals" and are grouped in a circle to form the calyx. The calyx exists to give protection and support to the flower.

The next inner group of differentiated leaves is the corolla made up of the petals of the flower. This circle may consist of but a single layer or it may have several layers according to the type of flower. These brightly colored petals attract insects. The honey and the nectar are found at their bases.

On pulling back the petals of most flowers, such as the buttercup for example, one will see a circle of thread like structures standing erect, with nodular tips, surrounding a larger central organ with a clustered head. The former represent the third series of modified leaves and are the stamens, carrying on their tips the anthers in which the pollen granules are formed. The central structure, the fourth of the modified leaf system, is the pistil with its terminal carpel which contains the ovaries of the flower.

In such a flower as the butter cup there should be no difficulty in the migration of the pollen granules for the short distance from anther to carpel. But Nature has observed that plant which fertilize themselves often do not flourish. Many plants are self-sterile. Even though they have stamens and pistils, they do not fertilize themselves. This is established in a variety of ways. Some times the pollen grains

mature at different times from the structure of the carpel so that the grains do not find lodgement on the stigma but must be carried to other similar plants which have matured somewhat earlier. Sometimes the male and female reproductive structures are found on different parts of the plant and not in the same flower, as in the buttercup, and sometimes they are entirely separated, being found on different plants.

So, as a rule, even when the male and female element are present in the one flower, facilities must be at hand for fertilization from one flower to another. This can be accomplished through the intermediary of insects or by the wind. As has been said, the brightly flowering plants usually have heavy sticky pollen and depend upon the insects for its transport.

Bees are interested in gathering pollen almost to the same extent as honey. They use it as a food and store it in large quantities in the open cells of the brood combs. It is the source of protein food for the larvae of the growing bees. This may explain some of the cases of allergy from the eating of honey. While the severe illness of certain persons resulting from the sting of a bee might possibly be due to pollen introduced with the sting, there is evidence that one may be specifically allergic to the bee protein itself and ~~and~~ this appears to be the more logical explanation.(21).

Where wind pollination occurs it is obvious that tremendous amounts of pollen must be produced in order that a very minute fraction will reach its destination on the pistils of the female flower. One who has walked through a field of grass or weeds at the right time will find his trouser legs quite covered with a yellowish coating of pollen dust.

Even the trees shed tremendous amounts. A tree in the process of pollination when shaken may emit clouds of dust almost as thick as smoke from a fire.

One should not confuse the pollen with the seed. The pin-head size seed of the dandelion attached by a rather long stalk to its white cottony parachute which carries it on the wind is not the pollen. Pollination occurred long before the dandelion went to seed and is the yellow material which as a child you rubbed on your chin.

Some pollens have wings, stationary wings like on an air glider which carry them many miles; but these are microscopic and are not to be confused with the winged seeds of trees, such as the maple, elm, ash, or linden.

Small as it is, the pollen grain is not a single cell but usually contains from two to four cells and is both vitalistically and chemically a most complex substance. It is a spore, and capable of continuing to live as such for a remarkably long period and under adverse circumstances. It contains the elements of life and when joined with the reproductive cell of the ovum, will reproduce in all of its entirety the plant from which it came. (23).

The chemical analysis of pollens shows that they invert cane sugar, invertin being present in the grain previous to germination. They also contain diastase and are capable of dissolving starch paste if present in culture fluid. The intine, or inner coat, is usually rich in cellulose, and also in pectin. Most pollen grains contain starch, this being especially the case with the grass pollens. Protein is present, this being about 25% in the ragweed pollen, and is of special importance from a hay-fever standpoint.

When stained with chloro-zinc-iodine, the extine is stained brown and the intine blue. Many pollen grains contain oil. In sulphuric acid the intine immediately dissolves but the extine remains. The contents are colored rose-red. In a solution of chloral hydrate most pollens become transparent, so that the nuclei may be observed. In Lugol's solution, the grass pollens quickly show the characteristic blue reaction of starch, which is a useful means for their different-

iation from other pollen. In the pollen of certain conifers, such as the cedars, the addition of a saline solution, or of glycerine, causes swelling of the inner coat(intine), which results in the shedding of the outer coat(extine). As a result of this, they present a characteristic appearance when found in the glycerin of the atmospheric-pollen plates, which simplifies their identification.

The following is an analysis of the ragweed pollen by Frederick W. Heyl.(22).

Alcohol soluble - - - - -	42.9%
Moisture - - - - -	5.3%
Crude fiber - - - - -	12.2%
Pentanes - - - - -	7.3%
Ash- - - - -	5.4%
Dextrin - - - - -	2.1%
Protein - - - - -	24.4%

Of the protein, about 7.5 % could not be extracted, while 6.75 % was extracted with diluted alkali, and only about 5 % using 10 % salt solution. The albumin and globulin fraction is, therefore, quite small. The analytical figures indicate the presence of proteoses. The nitrogen in the alcoholic extract (1.08 %) is probably a base, and the nitrogen in the saline extract after alcohol had precipitated the proteins (1.9 %) probably contains this base, and also some proteose.

The alcoholic extract (42.9%) contains:

Fat - - - - -	10.8%
Lecithin - - - - -	0.75%
Ether soluble but not	
ligroin soluble - - - - -	1.75%
Sucrose - - - - -	0.4%
Glucose - - - - -	1.6%
Resin - - - - -	17.4%

And a nitrogenous base.

Pollengrains are of different sizes, shapes and colors. The size varies from small pollens such as that of the trailing mimosa (*Mimosa trigolosa*), which measures only 6 microns* in diameter, to that of the marshmallows (*Hibiscus*), which are so large (180 microns) that they can easily be seen with the unaided eye. The color is most frequently yellowish, but may be white, red or other colors.

The shape is usually spherical or ovoid, which is the case with the ragweeds, the grasses and many of the tree pollens. Sometimes it is prismatic as with some of the sedges, and occasionally there are other shapes. The shape is not only similar with the various species, but is usually characteristic of the whole genus, so that it is a valuable assistance in their identification. In the wormwoods (*Artemisias*) for instance, which has a three-lobed pollen all the species examined have this characteristic shape. The pine pollens, which fortunately are harmless in hay-fever, are provided with wings or double parachutes which enables them to traverse distances out of proportion to their size.

The appearance of many pollens vary under atmospheric conditions. This refers especially to the spherical pollens of the grasses and trees. When exposed to the drying effects of the air, they lose a portion of their moisture, and present a shrivelled appearance under the microscope. The addition of a normal saline solution, or other liquid, quickly restores the spherical form. The pollen of the ragweeds and similar spiculated pollens, are not noticeably affected by this exposure. (24).

Vaughn (25), describes grass pollens, as a class, as being smooth-walled, spherical or somewhat pear-shaped, often somewhat irregular in shape due to indentations on the surface. They remind one somewhat of a round clay ball into which a child had pressed his fingers at various places. The indentations are sometimes fairly round and

* (MICRON is 1/1000 millimeter or 1/25,400 inch).

sometimes long enough to be described as infoldings. The fresh grass pollen is often smooth and spherical without the indentations or infoldings, which presumably make their appearance in the course of drying. While the grass pollens have smooth surfaces, the pollens of the Ambrosiaceae which include the ragweed, false ragweed, marsh elder, sunflower, aster, daisy, and goldenrod all present rough or spiny surfaces reminding one of a cocklebur with very short spines. The chenopods, such as lamb'squarters and thistle, and the amaranths produce pollen which is spherical with small round indentations giving somewhat the appearance of a dimpled golf ball. Tree pollens vary quite definitely among themselves and are in most instances different enough to be closely identified. Thus the pollens of the willow group, the oak group, and the maple group are oval, smooth and distinctly larger than the ragweed pollens. Most oval pollens have a longitudinal indentation or line somewhat resembling that of a grain of wheat. Sometimes the ends are flattened, especially in the oak and red sorrel. Here again the flattening is probably associated with drying.

TESTING THE WIND-POLLINATION OF HAYFEVER PLANTS.

While pollens are generated by all the members of the flowering plants, only those which are wind-pollinated need to be considered in connection with hay-fever. While there are many plants whose pollens may cause the hay-fever reaction when applied to the nostrils, only pollens which float in the air and can reach the nostrils in the course of normal respiration are responsible for true hay-fever. In the selection of hay-fever plants it is, therefore, important first to decide that they are wind pollinated, as the pollen of these only are found in the air.

An apparatus to determine this feature was devised in the biological laboratory of the American Hayfever Prevention Association by means of which this test may be made without the necessity of visiting the growing plant. It consists of a hollow cylinder with a fan attached at one end, which is operated by a set of pulleys by means of which the current of air may be given any velocity from one to fifteen miles per hour. The flowers to be tested are held in place by means of an attachment in the center of the tube, at the outer end of which there is a receptacle, at an angle of 45 degrees, for holding the glass slides on which the pollen is deposited.

The slide is given a thin coat of glycerin, as in the case of the atmospheric pollen plates, so that the pollens which come in contact with it are collected. The wind velocity is noted, and the length of time, so that a quantitative count of the pollens may be made.

When the slide is removed a glass cover is placed over the glycerin, and a stain of Lugol's solution is added to facilitate the count. This is made with a mechanical stage by means of which every part of the glass slide is passed across the field of the microscope.

The count is then calculated per square centimeter of the exposed glycerin.

The hay-fever pollens which have given the highest average in these tests are those of the common ragweed, which is closely followed by the giant ragweed and the marsh elder. The grasses also, which are the principle cause of spring hay-fever, distribute their pollen in enormous quantities, but their potential area is more restricted on account of the greater size of these pollens (average diameter 40 microns). This is especially the case with the pollen of corn. This pollen gives a positive hay-fever reaction similar to the grass pollens, but its size (80 microns in diameter) limits its potential area. Under the same wind conditions ~~the~~ the common ragweed pollen would traverse $\frac{1}{2}$ mile (it being 15 microns in diameter), while the corn pollen would only traverse 43 feet. On account of this, hay-fever from corn is rare, as a patient sensitive to its pollen, must be in the cornfield or within a short distance of it, during its pollinating season, in order to be affected. (25).

THE COLLECTION AND IDENTIFICATION OF ATMOSPHERIC-POLLENS.

The knowledge of the exact pollens which cause hayfever is important from an etiological as well as from a prophylactic and therapeutic standpoint. In prevention we must know the plants that produce the pollen in order to eradicate them if possible and if not, to have the patient avoid localities infested with such plants. As for therapeutics, the modern treatment gives special prominence to methods, the correct application of which requires the knowledge of the proper hayfever pollens. (26).

Hayfever pollens are not deleterious to health per se, as the great majority of persons (99%) are not inconvenienced by them. Those suffering from hayfever have a special sensitivity to these pollens, the aim of modern methods being to correct this by immunizing the patient against these pollens.

The usual method of testing the susceptibility of the patient by applying an extract of various pollens to the conjunctival sac or scarified skin, does not always give reliable results, as the question is not whether a patient reacts to various pollens, but whether he is sensitive to the pollen to which he is exposed.

A person in New York may be sensitive to the pollen of the Western mugwort but therapeutic use of this extract is not indicated as this plant is not found in the eastern or southern states and therefore the patient is not exposed to it. Thus we see a striking example of ^{the use of} atmospheric pollen plates to determine just which pollens are in the air in given localities. The information from these plates should form the basis of applied pollen therapy.

The atmospheric-pollen plates are ordinary glass slides used for the microscope, the central square inch being covered with a uniform coating of glycerin. Scheppegrell having tested various combin-

ations, found the pure glycerine, thoroughly applied to a glass which has been previously cleansed with alcohol, to be the most practical. In extremely moist weather, when the glycerine deliquesces too rapidly, he substitutes a layer of boiled linseed oil. (24).

Scheppegrell has tried various forms of apparatus for the purpose of having these plates always at right angles to the direction of the wind. He states that, while this furnishes useful statistical data, the complicated construction gives it a limited range. He says that for practical purposes the pollen plate may simply be exposed to the wind at the residence of the patient or at special stations. (24).

A. P. Hitchens suggests a little device (27), which he thinks might be of practical assistance in ascertaining the varieties of pollen with which the patient comes in contact. This device is in the form of a button or brooch consisting of a circular metal frame with prongs attached to the back edge. An ordinary round cover slip is placed in the frame, then a pad of some kind to prevent fracture and finally the solid metal back with the pin attached which is held in place by the prongs mentioned above. After the cover-slip is coated with the glycerine mixture (7), suggested by Blackley, the brooch may be worn on the coat or waist for a certain number of hours and then sent to the laboratory, where the cover glass is removed, mounted, and examined under the microscope. If a patient notes that in a certain locality his symptoms become exceptionally severe, the brooch may be removed and left in a properly protected place for a sufficient period to collect the pollens in that particular neighborhood.

W. T. Penfound and G. B. Efron suggest a standardized method for pollen air study. (28). They state that this study has come to play an important role in the diagnosis and treatment of allergic diseases caused by weeds. They further say that the usual method of determining the amount of wind-borne pollen is as follows: A portion of a slide is marked off near the center and thinly coated with vaseline, glycerin

or corn oil. It is then placed in a horizontal position or at an angle in some exposed situation for 24 hours. The slide is then taken to the laboratory in a closed container and the pollens on the ruled portion are identified and counted with the aid of a mechanical stage and a micrometer ocular.

Comparisons of slides placed in various sections and situations in New Orleans throughout 1929, revealed a great difference in the numbers and percentage composition of pollen of various species. Very high counts were often obtained in the heart of the business district and at the same time low counts prevailed in the residential and suburban districts. Slides placed on different sides of the same building at the same time, showed marked discrepancies in both numbers and species of pollen. In addition relatively low counts were obtained during periods of marked hayfever, and vice versa. These results were, no doubt, directly related to the degree of exposure.

This diversity of findings raised the question as to whether one might not get a great difference on slides placed in one location simultaneously, and exposed in positions varying from the horizontal to the vertical. Accordingly, slides were exposed at various inclinations with the horizontal and counts were made. The vertical slides showed more pollen on windy days, provided they were exposed perpendicular to the direction of the wind. The horizontal slides revealed more pollen on calm days. These results are explained by the fact that pollen s are continually settling out of the air unless they are supported or carried in the reverse direction by the air currents. Duke (29) has demonstrated the pollen count of still and moving air. (This will be discussed in detail later on). Scheppegrell (24) has determined the distance traversed by pollen of different size. He showed that the smaller, lighter and more spiculated the pollen, the greater distance it would be borne by a wind of a given velocity. He also showed that the length of time a given pollen grain remained in the

air was directly related to the rate of air movement. On calm days the pollens are continually falling whereas in moving air they may be moving horizontally or upward.

Accordingly, an apparatus was designed to determine more accurately the relative percentage of pollens falling or being supported by the air at a given time.

This apparatus consisted essentially of a weather vane on which were mounted 2 slots (one horizontal and one vertical) into which microscopic slides could be inserted. The base consisted of an inverted pan 8 inches in diameter filled with concrete into which was secured one upright support 3 feet 6 inches high. A sensitive weather vane, made of sheet metal was mounted on a roller skate bearing at a point 9 inches from the top of the upright rod. The slots were $1\frac{1}{4}$ x $3\frac{1}{2}$ inches. The flange of sheet metal holding the slides being $\frac{1}{8}$ inch wide all the way around except for the slide opening. In the case of the horizontal slot, a key was necessary to keep the slides from being thrown out by the wind. The vertical slot was soldered in the metal strip just in front of the roller bearing support and the horizontal slot was mounted just in front of the vertical.

The apparatus was placed on top of the Tulane University Gymnasium, which is several hundred feet from any effective obstruction, and counts were made over an extensive period. A few typical examples are here presented. On November 6th, 7th, and 8th, the vertical slides showed 17, 1, and 4 ragweed pollens per. square centimeter respectively, whereas the horizontal showed 7, 2, and 1 pollens for similar areas. On January 14th. and 20th., the vertical slide showed for American Elm 102 and 3, and the horizontal 20 and 2. On the same dates 92 and 3 red cedar pollens were formed on the vertical, and 41 and 7 on the horizontal. In comparing these data with the weather charts, a relatively greater number of pollens were found on the vertical slides on windy days, and on the horizontal slides on calm days. The counts of the horizont-

al slide, therefore, are not indices of the amount of pollen which is carried in the air. It is believed that pollen counts on vertical slides are essential if one is to obtain a good index of pollen abundance in the air.

The ratio of the number of pollens on the vertical slide to that on the horizontal was noted and called the "buoyancy index." If the ratios are calculated over a considerable period of time, they should indicate the relative buoyancy of various pollens. The buoyancy indices for those pollens studied in detail were found to be as follows:- Ragweed, 4; elm, 5; and red cedar, 2.

W. W. Duke, of Kansas City, in an article published in 1928, (29) after a study of the pollen content of still and moving air, state that still air, so far as its content of pollen is concerned, is a great deal like still water, so far as its content of mud is concerned. Muddy water becomes clear, through settling, if it remains still long enough, but is likely to continue muddy as long as it is in motion. Pollen settles out of the air in the same way that mud settles out of the water, so that air which has been still for a long time is practically free from pollen.

The correctness of this statement can be observed in the accompanying tables, in which studies of the pollen content of the air are reported. Duke used slides greased with white petrolatum and exposed them to the air for varying periods of time. He advocates the use of a shelter with the top 6 inches above the slide and open on the sides for outside exposure. The slides were exposed under different conditions of air stillness, natural air motion and air put in motion.

The total number of pollen granules found microscopically on 0.55 square inch (1 square centimeter) of surface were counted and compared.

He mentions in passing that counts made each day from slides exposed to the outside air for 24 hours, during a period of two years,

in Kansas City, varied from nothing, during the winter days, to as high as 4,500 during some of the fall days.

TABLE ONE.

	Sum Total of daily counts.	Calculated daily average.
Room closed constantly 1 yr. ---	185	0.5
Room well ventilated 1 yr. -----	20,576	56.0
Outside air 1 yr. -----	41,643	114.0

TABLE TWO.

Room closed $1\frac{1}{2}$ yrs.-----	0.0
Room closed 1 day -----	3.0
Room closed 1 day (with fan going 12 hrs.) ----	8.0
Ventilated room, same day (window open 6 in.) --	20.0
Outside air count, same day -----	191.0

Slides exposed for 24 hours.

Therefore we see that if room is kept closed for as short a time as one day it is a good place for care of a pollen sufferer on a day when the outside air is heavy with pollen. Furthermore we see that if the room is clean there is no great contra-indication to the use of a fan.

TABLE THREE.

On the ground at Aviation Field - - - - -	0.6
Airplane propeller moving about $\frac{1}{2}$ speed - - - -	82.0
Airplane rising to 200 ft. ,prop. full speed - -	289.0
Airplane at 400 ft.,prop.at full speed - - - - -	335.0
" " 600 " " " " " - - - - -	328.0
" " 1100 " " " " " - - - - -	319.0
" " 1600 " " " " " - - - - -	341.0
" " 3000 " " " " " - - - - -	329.0
" " 4000 " " " " " - - - - -	10.0
" " 5000 " " " " " - - - - -	0.0
" " 6000 " " " " " - - - - -	0.0
" " 7000 " " " " " - - - - -	0.0
" " 8000 " " " " " - - - - -	0.0

Table three shows a comparison of pollen counts made after exposure of plates for 30 seconds on an aviation field (Calculated from $1\frac{1}{2}$ hours exposure) to exposure made behind the propeller of a plane with propeller moving slowly and rapidly and with plane rising to height of 8000 feet.

This shows in outside air rapid motion of air caused by the propeller of the plane on ground causes a deposit of more than a 100 times as much pollen as still outside air, whereas; with propeller moving rapidly enough to lift the plane, the deposit is nearly 1500 times as great and at a still higher level, even up to a height of 3000 feet, the amount of pollen deposit is in excess of 1500 times the amount deposited by still outside air on the ground.

The foregoing statements serve to demonstrate the intense exposure encountered by pollen sufferers on trips by auto or train on days when the air is filled with pollen.

Rackmann and Smith (30) use ordinary glass slides bearing a

thin coat of glycerine or vaseline and expose them for 24 hours, out of doors, under a small shelter to keep off rain and dew. They state that these slides studied daily yield important information. They provide an index of the concentration of pollen in the air which after all is the important factor in the onset and severity of hayfever. They make possible a qualitative study of several pollens to indicate which ones are probably of greater clinical importance.

Karl K. Koessler and O. C. Durham, of Chicago, (32) during the course of their air studies, found that corn oil or cottonseed oil was better than glycerine for use in coating the atmospheric-pollen plates, because they did not absorb moisture, and in this way, distort the pollen grains.

Herbert Detweiler and Helena Hurst, of Toronto, (33) coated their slides with a thin layer of glycerin jelly prepared according to Kaiser's formula which follows:

Finest French gelatin - - - - -	40 grams
Distilled water - - - - -	2 c.c.
Glycerine - - - - -	325 c.c.
Carbolic acid crystals - - - - -	5 grams

The gelatin was soaked in the water for two hours. Then the glycerine and carbolic acid crystals were added and the mixture was heated in a water bath for fifteen minutes and then stirred until cool. Filtered in paraffin oven and then put into tubes and corked. Before being used the jelly was melted in a water bath and smeared on the slide with a glass rod using just enough for surface area of the cover slip.

NUMBER OF POLLENS IN THE AIR.(31)

After the pollens have been counted they are proportioned to the number per square centimeter, as Scheppegrell's unit is the number of pollens per square centimeter in twenty-four hours.

As the object of the pollinometric records is to determine the amount of pollen in the air, a working formula has been prepared by means of which the number of pollen per cubic yard or kiloliter of air may be determined from these plates.

The number of pollen dropping on a given surface, e.g., a plate, depends only on the number of pollen per unit volume of the air, and the velocity of fall of the pollen grains.

This means that unless the plate is inclined at an appreciable angle, e.g., 15 or 20 degrees, with the direction of the wind near the plate, the number of pollens dropping on the plate is independent of the wind velocity, other factors such as the number of pollens per unit volume of air, being constant. The number of pollen in the air, however, bears a direct relation to the velocity of the wind.

If the pollen grains fall with a velocity "v" feet per second, and there are in the air "n" per cubic yard, the total number "N" falling on a square centimeter in "t" hours is given by the formula:

$$N = 0.143 \times "n" \times "v" \times "t"$$

$$"n" = \frac{7}{"v"} \times \frac{N}{"t"}$$

For example, if the diameter of the grain is .02 mm., "v" 0.16 feet per second. If "N" pollens are gathered in twenty-four hours:

$$"n" = \frac{7 \times "N"}{.16 \times 24} = 1.8 N \#$$

If $N\#$ represents the number of pollen grains gathered per square centimeter on an atmospheric-pollen plate in twenty-four hours, and the number of pollen per cubic yard is represented by "n", the following are the numbers by which $N\#$ is to be multiplied to give "n" for the pollen of different diameters:

Diameters - 10 microns,	"n" equals	$7.30N\#$
" - 15 "	" "	$3.20 N\#$
" - 20 "	" "	$1.80 N\#$
" - 25 "	" "	$1.20 N\#$
" - 30 "	" "	$.80 N\#$
" - 40 "	" "	$.45 N\#$
" - 50 "	" "	$.30 N\#$
" - 60 "	" "	$.20 N\#$
" - 70 "	" "	$.15 N\#$
" - 80 "	" "	$.12 N\#$

To obtain the number of pollens per kiloliter, divide the number $N\#$ per cubic yard by 1.3.

On October 10, 1920, the pollenometric records indicated nineteen common ragweed pollens for twenty-four hours. As this pollen has a diameter of 15 microns we multiply the number of pollens by 3.2 of the formula, which shows that there were 61 pollens in each square yard of air on that date.

Durham (34) (35) found that this calculated number for the number of pollen grains in a cubic yard of air is the same as the actual number found in an area of 1.8 square centimeters of slide.

Rackmann and Smith (30) say that the number of pollen grains which fall on a square centimeter of slide when multiplied by 1.80 represents the number of grains per cubic yard of air.

CONVECTION OF HAYFEVER POLLENS. (31).

Any small particles which will appear to float in the air like a cloud of dust, fog, pollen, etc., are really going through a process of falling with a uniform velocity. The rate of falling is under these independent of the density of the particle. If the surface of the particle is rough, the velocity is less than if it was smooth.

If the particles have the form of smooth spheres of a diameter not less than 0.001 mm., it has been shown both from theory and by actual experiment that the velocity with which such spheres fall, is given by the formula:

$$"v" \text{ equals } \frac{2 "g" "r"{}^2}{9 "k"} \quad (\text{Stokes' law})$$

where "v" equals the velocity in centimeters per second, "g" equals 980 cm., the acceleration of gravity; "r" equals the radius of the sphere and "k" equals the coefficient of viscosity for air equals .00018.

By substituting the different values for the radius of spheres and the known constants in the above formula, the velocity of pollen grains of any size can be calculated. The calculation gives exact values for the smooth pollen grains only. The values will be considerably higher in the case of the pollen with spicules, the velocity being considerably less than the calculated value.

When the velocity has been calculated, the time of fall of a pollen grain can be calculated for any distance by dividing the distance by the velocity. If a wind is carrying the pollen horizontally while it is thus falling, the distance it will be carried is equal to the product of the velocity of the wind multiplied by the time of fall. In this way the distance which grains of different size will be carried, can be calculated.

The above formula has been used extensively in physical invest-

igations and there are numerous proofs of its correctness. The accepted value of the electric charge of an electron or cathode particle depends on the accuracy of this formula.

MICROSCOPIC STUDY OF POLLENS.

(Counting methods, ways of identifying, and classification,)

George Piness and H. E. Mc Minn, of Los Angeles, (36) examined pollens fresh from flowers, several days after falling, and after being treated with ether. They observed these under the microscope using the 4 mm. and oil immersion objectives with the pollen mounted dry, in water, and in balsam.

They found that the dry and ether-dry pollens, when mounted dry, were similar in all visible characteristics with the exception of color, whereas the fresh pollen mounted dry varied much in shape and other visible characteristics, this being due, maybe, to the amount of dessication occurring before and during shedding. Water and balsam mounts caused most dry and ether-dry pollen to become spherical, exceptions being noticed in a few species with oval, elliptical and pyradimal pollen grains. These simply swelled slightly, retaining their characteristic shape. when dry.

Water mounts proved the most valuable in studying the germinal pores and certain features of the exine, while dry mounts were used for study of size, shape and furrows caused by dessication. They have considered the dry condition the natural condition of pollen for their studies because the pollen when wind-blown soon becomes dessicated and shrunken.

Photomicrographs were made of all pollens and these aided materially in supplementing the microscopic observations.

Duke and Durham (37) in an air study of Kansas City state that made their counts by examining the slide with a low power objective (16 mm.) and ocular elevated to such an extent that passing over the

slide from side to side ten times covered an area 55/100 inch square. This count represents the number of pollen granules that fall in such an area. They state that this method may not be over accurate but it is certainly accurate enough for practical purposes. According to them the identification of the pollen granules found is not difficult. One should know in advance the relative time at which the more important of the flora of a district pollinate. Pollen collected from such flora can be examined under the microscope and compared with granules found on the slide. Their identity is then simple and easy

Noessler and Durham, (32) in a report of an air study of Chicago, say that the distinction between pollens of the various groups and species was based largely on the shape and surface marking. The size was not found to be a safe differential point as it varies too much in pure specimens. Except in the case of corn it was not found practical to distinguish between the various grasses. Short and giant ragweed pollens could not be differentiated, their average size and general appearance being much the same. Hop and hemp could not be differentiated. The docks and plantains, though not related botanically, were also counted together.

Paul Patterson and Leslie N. Gay, of Baltimore, (38) in a report on a study of the pollen content of the air of that city state that "check" slides of pollen grains were made from plants as they came into bloom and identification "keys" were constructed for the various pollen granules occurring during the various seasons. They found that one of the most helpful diagnostic characters besides conspicuous pores, large differences in size, surface markings, and differences in shape and transparency was the appearance of the pollen grains in a dry condition. Many of them when dry are curled, wrinkled, indented or folded in a characteristic manner and counts were made when such criteria were sought using 95% alcohol as a mounting medium.

Paul Acquarone and Leslie Gay, (39), reporting on a study made

of Baltimore, in 1929, formerly used the number of pollen grains falling on an area 0.55 square inch. This is hard to visualize. They say that regardless of how the counts are made they should be reported as , the number of pollen grains per square centimeter per day.

They used a Bausch and Lomb microscope with standard 16 and 4 mm. objectives and a 10x ocular equipped with a mechanical stage.

While observing a stage micrometer through the 10x ocular and 16 mm. objective, the draw tube was extended until the diameter of the field was 1.515 mm. The draw tube length was then noted and counts were always made with the tube set at this length. With most microscopes this tube length is about 160 mm., the length for which microscopic lens are corrected. This is advantageous in observing the finer details of pollen grain walls. With a field of this diameter (1.515mm.), three sweeps from a square or oblong cover-glass 22 mm. wide, cover an area of almost exactly 1 square centimeter, as the following shows:

" 1.515 (Diam. of field) x 22 (Width of cover-glass) x 3 (Sweeps) equals 99.999 square millimeters."

A 4 mm. objective, parfocal with the 16 mm., was used for examining grains of doubtful identity.

The figures recorded were three times the average count of 6 or 8 sweeps across each preparation.

Their method of identifying pollen grains was based almost wholly on morphological features since chemical tests are hardly applicable to preparation undergoing careful counting.

Identification was based on such criteria as size and shape of cells, thickness and sculpturing of walls, and number, size, character and locations of pores. These pores are a valuable aid but are hard to see and often the presence or absence of cover or plug in the pore cannot be determined without the use of stain.

For staining they used a modification of Moore and La Garde's

Acetonigrosine stain. This acetonigrosine is a solution of nigrosine in 2 % acetic acid. The acetic acid penetrates the pollen grain rapidly causing the dry grain to swell to its normal size and shape. The nigrosine diffuses more slowly, in a cone, through the pores, and the number of pores and their relative size is determined by counting the cones and noting their size. If covers or plugs are present they filter out the nigrosine and become stained a jet black making them readily visible. It was found that a solution of nigrosine in dilute lactophenol (which is made up of lactic acid, phenol, glycerine and distilled water; used commonly in botanical laboratories as a temporary clearing and mounting medium) possessed the staining properties of acetonigrosine and, in addition, "cleared" the grains somewhat, which helped in seeing details. The glycerine content prevented dessication of the preparation so that a slide could be kept several weeks. The "lactophenol-nigrosine" solution consisted of 0.1 gm. of nigrosine (Gruber) dissolved in 100 c.c. of 20 % lactophenol. This was allowed to stand 24 hours, with occasional shaking, and then filtered through paper. It was kept in a dropper bottle ready for instant use. When ready to examine a slide a large drop was placed on the slide and a clean cover-slip floated on it.

They used "keys" made up by Moore and La Garde which helped in identification but they verified each by tracing the pollen to its source (possible in all cases except some of the conifers) and comparing the grains on the slides with grains obtained by dissection from mature but unopened anthers. It is obvious that pollen grains which appear on the slides in considerable numbers must come from plants which are abundant and are flowering freely. Thus one notes what species are pollenating abundantly and secures pollen of each directly from the anthers in order to obtain standards for comparison with the unknowns on the slides.

Pollen grains, the microspores of seed plants, possess in common with plant spores in general, a high degree of specificity.

That is, each species possesses distinctive spores. Color, shape, size, thickness of wall, pores in the walls, plugs in the pores, cell content, reactions to reagents or to organic dyes or combination of dyes are features which are only slightly variable in the spores of any given species, and by noting some or all of these features in pollen grains their identity may be established.

In general there are generic and family resemblances in pollen grains but there are also specific differences. These differences are often so slight that they are noticeable only by statistical methods. These are often used in a routine way in mycologic taxonomy, where spore characteristics are sometimes the only features sufficiently fixed to be of diagnostic value in determining species. Pollen grain characteristics can be used in identifying and differentiating species of flowering plants, and are occasionally so used.

Since the characteristics of spores are so definitely fixed in each species, it is clear that it should be possible to identify pollen grains with reasonable certainty. Positive identification, to the genus, is usually made with little difficulty, except in some of the grasses. Specific identification can usually be made, but is often more difficult, especially in the case of trees, as is discussed below.

Acquarone and Gay were interested in checking the accuracy of the description of pollens by Moore and La Garde and found them on the whole quite accurate and trustworthy, with the exception of those of some of the tree pollens, especially the oak. Without implying that Moore and La Garde's description of the latter are actually erroneous they never-the-less found it difficult, and sometimes impossible, to identify some of the oak pollens by means of this "key". While oak pollens are fairly distinct generically, specific differences seem inconstant. To a lesser degree this was also true of pollen of other arboreal genera, such as ACER (the maples and box elder) and

POPULUS (poplars, cottonwoods and aspens). The difficulty of identifying oak pollens positively is due perhaps to the fact that the species of oaks, especially the red and black oaks, are poorly defined, and hybridize freely, producing many intermediate types with intermediate and aberrant types of pollen. This is also true of poplars. For example, the "carolina poplars" (POPULUS EUGENI) so widely used as shade trees are hybrids derived by the crossing of at least three different species of POPULUS, and at least four morphologically distinct types of pollen could sometimes be obtained from a single anther. Positive identification of pollen, to the species, is in such cases impractical, although not necessarily impossible.

Fortunately for the allergist and for his patients, this is not an insuperable difficulty. Pollen can usually be traced with certainty to the genus, and it seems to be generally true that a person sensitive to pollen of one species in a genus is sensitive to that of other species in the same genus, though, perhaps, not to the same degree. Thus, a person who reacts to pollen of QUERCUS RUBRA (red oak) is almost certain to react to that of QUERCUS VELUTINA (black oak) or of many other oaks. Hence an idea of the abundance of pollen from a genus is as significant as data on pollen produced by various species in the genus.

Detweiler and Hurst, of Toronto, (33) add drop of glycerine jelly to slide and cover-slip is mounted on this. They find that in this way pollen granules can usually be made out by the low dry power of the microscope but, however, it is usually necessary to use the high dry lens for definite identification of the type of pollen. If the total number of pollens caught is small the whole slide is counted while if it is large, certain definite proportion is counted. The mechanical stage is used of course.

They give the following descriptions of pollens:

MAPLE - (Ceases to bloom about the middle of May).

Size - 26 to 29 microns.

Color - Very pale yellow.

Shape - Round.

Extine is clear and moderately thick.

Perin is coarsly granular.

OAK - (Season- Last 3 weeks of May. and 1st week of June).

Size - 26 to 28 microns.

Color - Yellow (pale).

Shape - Round.

Extine is thick and clear.

Perin is coarsly granular.

BIRCH - (Season - 1st week of May to about June 1st).

Size - 24 to 28 microns.

Color - Definatly yellow.

Narrow yellow extine.

Very finely granulated perin. Always occurs with three distinct germinal pores, two of which are often in focus at the same time. In general this pollen has a very definate, regular and clear cut appearance.

ELM - (Shorter season than Maple).

Size - 24 to 28 microns

Color - Very pale yellow.

Extine is clear and thick.

Perin is coarsly granulated.

Hard to tell from Maple. Perin is thicker and the size, on the whole, is slightly smaller but these feature are not sufficient to differentiate. The shorter season is helpful.

POPLAR - (Season - Last week in April to 1st or 2nd week in May).

Size - 24 to 26 microns.

Color - Pale yellow.

Shape - Round.

Extine is clear and thick.

Perin is coarsely granular.

Confused with Maple which has a longer season.

WILLOW - (Season - Latter part of April and early in May).

Size - 14 to 17 microns.

Color - Fairly pale yellow.

Shape - Round.

Extine is fairly thick and clear.

Perin is homogenous.

CHESTNUT - (Season - Last two weeks of May and first week in June.)

Size - 17 to 21 microns.

Color - Very pale.

Shape - Slightly oval.

Extine is thin and smooth.

Perin is homogenous.

These pollen granules are easily recognized. They may have brick red color but they are usually colorless.

JUNE GRASS - (

Size - 27 to 30 microns.

Colorless.

Shape - Round with smooth refractile outline.

Extine is very narrow, clear and colorless.

Perin is finely granulated.

ORCHARD GRASS

Size - 25 to 27 microns.

Colorless.

Shape - Perfectly round and smooth.

Extine is narrow, clear and refractile.

Perin is finely granulated.

TIMOTHY

Size - 35 to 38 microns.

Colorless.

Shape - Round and smooth with refractile surface.

Extine is narrow, clear and colorless.

Perin is finely granulated.

RED TOP

Size - 24 to 27 microns.

Almost colorless.

Shape - Round and smooth.

Extine is clear, thin and highly refractile.

Perin is finely granulated.

RAGWEED

Size - 17 to 20 microns.

Pale yellow.

Shape - Round.

Extine is narrow. The surface is covered with short, blunt spicules, causing the pollen grain to resemble a golf ball in appearance.

GOLDENROD

Size - 17 to 20 microns.

Pale yellow.

Shape - Round.

Extine is narrow. The surface is covered with sharp spicules which are slenderer and longer than those of ragweed.

Scheppegrell (26) conducted a careful series of investigations with a view of dividing all the most common hayfever pollens into groups which should be morphologically alike, and the number of which should be as few as possible, in order to simplify the question of pollen therapy. Thus far, he states that he has succeeded in reducing the number to four groups. He says further that, should he succeed in finding a pollen which would morphologically represent these four groups, it would still further simplify the application of pollen therapy. The four groups are as follows: Ambrosiaceae; Gramineae; Artemiseae; and Chenopodiaceae.

AMBROSIACEAE

An extended series of tests showed that members of this family have a marked resemblance morphologically and in their hayfever reactions. They vary only in size. They give the same reaction to Lugol's solution.

The common ragweed (*Ambrosia elatior*) causes 35% of fall hayfever in the Eastern and Southern states. It is spiculated and measures 15 microns in diameter. The giant ragweed (*Ambrosia trifida*) which replaces the common ragweed in moist sections and which in general appearance bears no resemblance to common ragweed, has a pollen with the same hayfever reaction and differs only in size (20 microns). The Western ragweed (*Ambrosia psilostachya*) also has the same hayfever reaction and differs only in size. (25 microns).

The Gaertneria, sometimes called "false ragweed", also belongs to the Ambrosia family. Its pollen gives hayfever reaction similar to common ragweed and the pollens resemble them very much.

The marsh elder (*Ivas*), also a genus of Ambrosia family, gives a reaction similar to ragweed but less marked. Spicules are less prominent but more numerous. They equal the Ambrosias in amount of pollen generated.

The cocklebur (*Xanthum*) belongs to this family and resembles the

other species in microscopic appearance and biological reaction. The spicules, however, are even less prominent than those in the Ivas and are more numerous. The reaction also is less marked.

GRAMINEAE

In this grass group there also is similarity of reaction. There are about five thousand species of grasses. The pollens are relatively large (30 to 80 microns), spherical and are stained blue-black by Lugol's solution because of the high proportion of starch. The variety of grasses varies greatly in different localities but the reaction varies only in degree. This includes also the cereals, such as corn, rye, wheat and oats. The pollens of these, however, are relatively large so they are not responsible for hayfever except in limited areas. The grasses with small pollen such as Bermuda grass and June grass are responsible for more cases than the grasses with the large pollen, such as foxtail grass, bull grass, cockspur grass, etc., on account of their greater buoyancy which enables them to traverse a great territory.

ARTEMISIA

The wormwoods also bear a marked resemblance to one another in their microscopic appearance (except *Artemisia biennis* which has spiculated pollens but otherwise is similar in appearance and reaction) and chemical and biological reaction. The three lobed form is characteristic of all the Artemiseae which we have examined, viz: Mugwort, wormwood sage, sage brush, absinthe wormwood, darkleaved mugwort, biennial wormwood, California old man, Indian hair tonic and bad-bush. The wormwoods are the most important hayfever plants of the Pacific and Rocky mountain states and the hayfever reaction of all these varieties is marked (ten times more active than the ragweeds).

CHENOPODIACEAE

Among the Plants which rank next to the Ambrosiaceae, Artemisiae

and Gramineae are the members of the amaranthaceae, chenopodiaceae, and the Rumex families, which are both numerous in their varieties and general in the geographical distribution, over fifty of each having been described, most of them being typical hayfever weeds and wind pollinated.

The members of the amaranthaceae, chenopodiaceae, and Rumex generate pollens which are smooth and spherical and have a general resemblance to one another. They also have a similar reaction to the Lugol's solution indicating a low percentage of starch. All the varieties tested gave a mild hayfever reaction and, with few exception persons susceptible to one species react also to other members of the group.

The chenopodiaceae are a large family which includes, in addition to the true chenopods, several other varieties, such as the orache, the grease weeds and salt wort. They all give a similar mild hayfever reaction.

The branch of the amaranthaceae family which has several members of interest in hayfever is the Aconida or water-hemp. Some of these, such as the Western water-hemp, generate a large amount of pollen. Most of these are limited to swamps and moist land. The reaction is similar to that of the Amaranthus Rumex and Chenopodium which they also resemble in the general appearance of the pollen.

While the members of this group give a mild hayfever reaction they are sometimes of importance by causing hayfever at seasons when the grasses, ragweeds and wormwoods are not pollenating.

The similarity of the Amaranthes, Chenopods, water hemp and docks, both as regards the character of the hayfever reaction and the individual susceptibility to these pollens, demonstrates a similarity in their toxic principle. The indications are, therefore, that a pollen extract of any of these pollens will be applicable to all the members of this group.

In addition to the members of these groups there are local and special cases of hayfever due to the pollen of plants not included in them. This is the case with the Western cottonwood to which a number of persons are susceptible. Another is the pollen of the Mountain cedar, found in certain parts of Texas. These pollens, for instance bear no resemblance in their appearance, chemical or biological reaction, or individual susceptibility. Such cases of hayfever must for the present, be given individual consideration.

Scheppegrell concludes by saying that the four groups he has described, however, include most of the pollens that are responsible for hayfever. As his studies were carefully conducted he believes that the findings will not only simplify pollen therapy but will put it on a more scientific basis.

Scheppegrell (24) examines the slides under the microscope with the aid of a mechanical stage so that the whole surface can be traversed. A low power (125 diameters) is sufficient for traversing the slide and when a pollen is found, a high power (500 diameters) is used for its identification. As the glycerine on the slide usually leaves a surface of uneven refraction, it is necessary to inject a solution that will correct this. He uses the ordinary Lugol's solution of iodine and iodide of potash, which not only corrects the refraction but simplifies the identification of the pollens.

All the grass pollens, on account of the high percentage of starch, are stained blue-black, which easily distinguishes them from all other pollens. The members of the Ambrosiaceae group, including ragweeds, marsh elders, gaertnerias and cockle burs, are stained brown by the iodine and are recognized by the spicules. The amar-anths, chenopods, docks and other members of the Chenopodiaceae group, have a low percentage of starch, and are partially stained by the iodine. These pollens are spherical and smooth.

The wormwoods (Artemesias) are stained brown and are recognized by their three-lobed appearance when seen from the end, other-

wise they appear ovoid with one of the lines of the lobes showing longitudinally.

Balyeat,(40) in examining slides,uses a 10x ocular with the barrel pulled up as high as possible. The low power is used and ten trips across the slide are made counting all within the field of vision.

FACTORS DETERMINING THE POLLEN CONTENT OF THE AIR.(40).

1. Methods of study.

Various methods have been mentioned and there is a real need for a standard so that the results of air studies can be correctly interpreted by their readers.

2. Precipitation.

In the first place the amount of precipitation determines the number of plants that will be present and then, after pollination, it has its effect on the number of pollens that will be in the air. A short rain immediately followed by sunshine and wind will not do much towards decreasing the number but cloudy weather with a slow rain for two or three days cleans the air quite thoroughly.

3. Wind.

The velocity of the wind determines the amount of pollens going into the air and how far they will be carried.

4. Time of day.

Pollen is ripened in the mature anthers early each morning.

5. Sunshine.

There is poor production of pollen on cloudy days.

6. Abundance of plant life.

This is the most important. The actual location of the majority of plants is not an important factor if they are within three or four miles. This is true except where pollen is very heavy.

Balyeat says, that, if other factors are equal, the number of pollens on the slide is in direct ratio to the velocity of the wind.

SURVEYS.

O. C. Durham, of Chicago, (41) is conducting a five year air study of pollens in the United States, Canada, and Mexico. Slides are exposed under uniform conditions and apparatus at stations of the U. S. Weather Bureau, the Canadian Meteorological Service and the Mexican Meteorological Service. His reasons for this study are as follows:

1. To establish boundaries of the effective ragweed district of North America.

2. To compare the severity of pollen incidence in various sections.

3. To determine the average of local dates of onset, crisis and termination of the ragweed season.

4. To learn as much as possible about the local and general factors affecting the production and distribution of ragweed pollen.

Duke and Durham (37) observed the pollen content of the air daily for one year in Kansas City. Slides were placed in two localities; one in the suburbs and the other on a window ledge on the eighteenth of a building in the business district. They compared these counts with similar ones made last year and at the same time in Chicago (Koessler and Durham) and in Oklahoma City (Balyeat).
1925 1926

As a result they say that it is easy to account for differences in the severity of the symptoms of surface allergies such as hayfever, asthma and eczema at different seasons and on different days in the same season and in different localities on the same day. These studies also explained why treatment of a given symptom, which may be successful in one city or in one year, may be a total failure in another city or in the same city in another year.

W. W. Duke (42) in a survey over a four year period found that only one season was normal with all the trees, grasses and weeds pollinating luxuriously. The other years saw one or more of all the trees, grasses and weeds greatly reduced. This was due, in the spring season, to frosts; and in the summer and fall seasons to draughts. During the four years, the pollination of each pollinating plant, except the ragweed, was almost completely inhibited once, or for several seasons. During one year ragweed was reduced to a small proportion of normal.

Scheppergrell (18) studied hay fever pollens in the upper air by means of an airplane. Exposure of slides for twenty minutes gave best results as a shorter exposure did not collect enough pollen and a longer time caused the film on the slide to dry and the pollen was lost. Tests were made at various altitudes from hedge-hopping to 19,000 feet in the air. At 4,000 feet there were large numbers of pollens, at 6,000 feet they were diminished and at 15,000 feet the pollens were comparatively few in number. He found that unless they were precipitated by rain, clouds of ragweed remain in the air, practically during the whole pollinating season of these weeds, the fallen pollen, being replaced by new, brought up by ascending air currents. The existence of these pollen clouds and their gradual descent due to cessation of ascending currents, explain the increase of hay fever pollen after sunset and during the prevalence of cold winds, both of which are associated with aggravation of hay fever attacks. The studies demonstrated that hay fever pollens are commonly found in large numbers up to high altitudes during the pollinating season of plants and trees that produce them. The distance that they traverse in their descent may therefore be from 5 to 10 miles, according to the variety of pollen in the air. The studies also show that, because of the extensive potential area of hay fever pollens, the

effective control of hay fever by municipal anti-weed ordinances is insufficient. The various states and the national government must work together on this phase.

Acquarone and Gay (39) made a survey of Baltimore and their pollen counts indicate four subseasons:

1. Tree season- this began March 12th with the anthesis of elms and reached its culmination on April 9th when a total of 700 pollen grains, chiefly from oaks, settled on one square centimeter of slide. After this tree pollens dwindled to almost nothing. As a rule the period of pollen dissemination for a given tree species in a locality is short but intense, hence spring hay fever is likely to be of short duration unless the patient is subject to the pollen of a number of different species of trees.

The following species contributed the greatest amount of pollen during the tree season:

CONIFERAE - (pines, spruces and hemlocks)- Pollen extremely light. Appeared as early as March 1st.

QUERCUS - (the oaks (the red, scarlet and black). Reached a peak April 8th.

ULMUS - (the elm). The first native pollen appeared March 14th.

POPULUS - (the poplars). In the air from March 19th. to April 1st. Because of the free hybridization these pollens could not be identified specifically.

ACER - (the maples). Pollen appeared March 20th.

BETULA - (birch). Pollens appeared on slides between March 20th. and April 10th.

2. Grass season - Pollens appeared about May 3rd. and reached a peak May 31st. Pollens chiefly from Orchard grass. The season ended about June 25th.

ORCHARD GRASS - This is possibly the greatest source of

grass pollen in the East and Middle West.

TIMOTHY - not very much of this pollen in the East but more people are sensitive to this than Orchard grass. The pollens are not carried far. They reached a maximum on June 19th.

JUNE GRASS- (Kentucky Blue Grass). These pollens appeared in small amounts between April 5th. and September 15th. reaching a maximum April 8th. This is the most widely used lawn grass. Close clipping and mowing generally keeps it from flowering abundantly. Sensitivity to it may be high.

The following grasses produce small amounts of pollen during May, June and July: Bent grass, redtop, sweet vernal grass, oats, rye grass, darnel, Italian rye grass, corn and maize. The last two mentioned appeared during July and August.

Hay is commonly cut at or very near the time of Anthesis and the mowing, raking and stacking may produce very dense local clouds of pollen.

3. Plantain season - Pollen appeared on the slides between the middle of May and the middle of August. This is the mildest season and it overlaps considerably on the grass season. The most abundant pollen was produced by rib-grass, buckhorn and English plantain. Lesser amounts were produced by common plantain, pig ear and sheep sorrell. Other contributors were white sweet clover, yellow sweet clover, alfalfa, common daisy, sunflower, amaranth, Mexican tea, wormseed, lamb's - quarters, pigweed and dwarf pigweed.

4. Ragweed season - Pollens first appeared on slides on April 8th. (Common ragweed). Maximum reached on September 18th. Pollens appeared until the middle of October. The ragweed counts were uniformly high.

SPORES - These were found on the slides in numbers between 20 and 30 usually. As high as 100 were counted at times. They were

mostly fungus. There were a few moss and fern spores. A great many of them were teliospores of rust fungi. No attempt was made to identify the spores. They have not been studied enough to determine their importance in hayfever and asthma.

PLANT HAIRS - These were found at all times in winter and summer. The number appearing varied between 30 and 50 grains as a rule. They are smaller than pollens. The protein content of most plant hairs is very low, probably much less than pollens. They deserve investigation because of sheer abundance.

Having mentioned spores and plant hairs it seems appropriate to speak of the studies of S. J. Parlato, of Buffalo, N. Y. This man (43) proved definitely that the emanations of sand-flies are present in the air and cause allergic symptoms. Having found a close biological relationship between the sand-fly and the moths and butterflies, (44)(45)(46)(47) it occurred to him that the hairs and scales of the moths and butterflies would show the same thing to be true. The abundance and ease with which desquamations are thrown off by these flies has long been recognized.

Parlato (48) exposed and studied a series of slides in 1925, 1926 and 1927, and compared the distribution of the common pollens in the air with the emanations of the flies mentioned above.

Emanations were found in the air during June, July, August and September. The amount of hair and scales on some days corresponded closely to the amount of pollen on the slide. These emanations were found in the air at various places where people usually assemble. The sand-flies predominated in the city while the scales of moths and butterflies were more abundant in the suburbs and rural districts.

A single moth, like the butterfly or sand-fly, is capable of losing a large amount of scales and Parlato believes that

more scales and hair are cast into the air by a single moth than the amount of pollen which is blown from the stamen of a timothy grass over a similar period of time.

Figley (49) reported four cases of asthma due to the pellicle shed by the May fly which is found in swarms in and about Toledo, Ohio.

It is possible that hypersensitiveness to other common flies may be demonstrated in the near future.

Parlato believes that there is ample justification for delving into this great field of flies for the exciting cause of hayfever and asthma which occurs during the summer months, especially for those cases which do not respond so well to our usual forms of treatment.

POLLEN - STATISTICS : A NEW METHOD OF RESEARCH IN PALEO - ECOLOGY.

G. Erdtman (50) says that in Europe no other branch of Paleo-ecology has been more popular during the last few years than pollen-statistics, or the method of tracing the history of forests from the occurrence of the fossil tree pollen grains in peats and sediments, which was elaborated some twenty years ago by the Swedes, G. Lagerheim and L. von Post. The field work is chiefly confined to the summer. During winter, however, when the lakes are frozen over, bottoms sediments could be obtained by means of borings through the ice.

Samples are taken at different levels beneath the surface, and are studied under the microscope. In examining the slides the height and depth as well as the breadth of the pollen grain proper should be measured due to extreme distortion in some cases.

Erdtman says that about 150 grains should be counted to get trustworthy percentages. The frequency of the pollen grains of hazel, the willows and other species which are more or less confined to the under growth of the forests, is calculated separately and expressed as a percentage of the sum of the pollen grains of the forest trees proper. Thus, a willow pollen frequency of 138 percent indicates that the number of willow pollen grains in that preparation was bigger than the sum of the pollen grains of the forest trees. The frequency of Sphagnum spores, tetrads of Ericaceae, etc, is expressed in the same way.

A pollen diagram is constructed by means of percentage figures. The relative frequency numbers, which are produced for the pollen species found in a sample, constitute the "pollen spectrum" of the sample.

As a help in identifying pollen grains, stamens of herbarium specimens could be boiled with 10% caustic potash and mounted in glycerin jelly.

After the study of recent pollen grains, it would be advisable to search samples from the surface of the peat for pollen grains. The pollen grains found among the branches of living Sphagnum and in the moss cover of stumps and fallen trees, give a picture of the composition of the contemporaneous pollen grains. That would give a key to the conclusions which can be drawn from fossil pollen in general, but in this respect, too much care cannot be exercised. We know, for instance, that pollen grains can be carried by wind for very long distances, so that coniferous pollen might be encountered in the peats of Greenland, and further, that the pollen grains of some trees might be under-represented in the pollen spectra due to their being distributed at a time when the lakes and the peat surfaces are still frozen, or from other causes, are not as fit for catching and preserving the pollen grains as at a later season. Because the delicate *Populus* pollen grain may not be preserved in peat, it is understandable, too, that a virgin Cordilleran coniferous forest would produce somewhat the same pollen spectra in the mountain bogs as do some of the popular forests, with scattered conifers, in the muskegs of the Great West Plains of the U.S.

CASE REPORTS.

Case 1. (51)

A physician suffered for a number of years and was tested by specialists with negative results. Thirty-five different pollens were used but they had been gathered, in making up the extracts, one thousand miles away. His hayfever developed at the usual time and at that time a survey of his neighborhood. Russian thistles and Lambsquarters were found in great abundance in a nearby lot. Pollen plates set on his bedroom window ledge showed granules from pine, lambsquarters and Russian thistle. Immediately it was evidenced that ~~pine that~~ pine was not the offender. Tests of the other two showed a 4 plus reaction to the lambsquarter and a negative reaction to the Russian thistle. Immediate benefit was obtained by the use of the appropriate extract and the man free of symptoms the following season.

Case 2. (51)

A woman in Colorado Springs had been suffering with hayfever regularly each season for twenty years. She had been given a full course of immunizing injections while in the East. She had been found to react to ragweed. She spent the next season in Colorado Springs and there was no improvement. The next two seasons were spent in Philadelphia, at a summer home, where she experienced relief from symptoms. The next season she was in Colorado Springs and her hayfever appeared. The date of her first attack coincided with the appearance of sage pollen so she was tested and gave a very severe reaction. But following this test she obtained complete relief for two days. The use of weak solutions every few days kept her practically free from irritation for the balance of the season.

Case 3. (51)

A business man, sensitive to ragweed, was thoroughly immunized and had no hayfever for two seasons. His business called him to Pueblo, 45 miles distant. Soon after going there he called to say that his hayfever had returned in a severe form. A survey made of weeds surrounding his house in Pueblo showed an unknown weed to be present. Pollen from this caused a strong reaction. After study it was found to be the ordinary summer cypress, often grown in gardens, which had, in this case, escaped and was growing wild.

Case 4. (49)

A young man consulted Parlato in July 1928 for treatment of hayfever which commenced in June and ended in September. He gave a positive skin reaction to pollens of grasses and ragweeds. The usual pollen injections gave only partial relief. In April 1929 he reported for preseasonal injections and at that time he was tested with sand-fly extract and gave a stronger reaction than he gave for the grasses and ragweeds in the skin and conjunctiva. Therefore, sand-fly extract was given along with the pollen injections and complete relief from symptoms resulted.

Case 5. (48)

Mrs. C.P.P. consulted Parlato in the summer of 1928. She had been suffering with typical hayfever symptoms of moderate severity since 1924, experiencing the same symptoms while living in her summer home located near Lake Ontario. She really felt better when she stayed in the city. Repeated tests with routine extracts proved negative. Even raw grass and ragweed pollen when dropped into eyes and nose failed to elicit a reaction. She had a large garden with about 35 varieties of flowers and she spent much of her time in this garden.

Slides were exposed about her home and pollen of only three species were found upon them. These were uprooted with no improvement. In March 1931 slides were again examined and a large amount of scales and hairs belonging to moths and butterflies were found. In view of this positive finding, the negative reactions to the routine tests, her work in the large garden where moths and butterflies could be found and her feeling better in the city, it is reasonable to assume that the emanations of these flies could be the cause of her hayfever symptoms.

Case 6.

There was an outbreak of hayfever in Austin, Texas. Atmospheric-pollen plates were exposed by Dr. S.N.Key and then sent to Scheppegrells laboratory. The examination of these showed that the air contained an average per kilometer of 88 pollens of the mountain cedar, the biological test of which showed a marked hayfever reaction. As the cedar, theretofore, had not been identified with hayfever, the importance of the atmospheric- pollen plates in such a case is clearly demonstrated.

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