

AN IMPROVED VOLUMETRIC IMPINGER FOR POLLEN COUNTING

HENRY P. MARX, M.S., JAY SPIEGELMAN, M.D., AND GEORGE I. BLUMSTEIN, M.D.,
PHILADELPHIA, PA.

THE first studies of atmospheric pollen concentrations were conducted by Scheppegrél¹ in 1917. He attempted to derive a formula for converting the number of pollens on a square centimeter of slide to a volumetric equivalent by applying Stokes's law for small falling bodies.² Using this formula, Scheppegrél arrived at a factor of 1.8 for converting the number of ragweed pollens on a square centimeter of slide to a cubic yard equivalent. This remained the basis for counting and converting atmospheric equivalents until 1937, when Cocke^{3, 4} pointed out some difficulties in these formulas. In the first place, Stokes's law pertained to smooth, spherical bodies in still air; pollens are, for the most part, spiculated and aspherical, and the air is usually in motion. Second, it is necessary to determine accurately the rate of fall, the specific gravity, and the size of each pollen before one is able to apply Stokes's law. One would then obtain a separate factor for each pollen. Third, Cocke pointed out that Scheppegrél had erred in his original calculations and had used the diameter and not the radius of the pollen in calculating his factor for ragweed.

Dahl and Ellis⁵ reported on pollen concentrations of the atmosphere in 1942. Durham⁶⁻¹² did monumental work in conducting pollen surveys in this country and in developing a standard method of pollen counting. The major portion of this work was done by the gravity-slide method, utilizing pollen shelters of varying construction. The advantages of this method were its simplicity and the rapidity with which one could obtain the desired information. No further refinements were made in the procedure of pollen counting until 1946 when Durham,¹¹ dissatisfied with Scheppegrél's formula, ran parallel gravity-slide and volumetric studies. This work formed the basis for the adoption of standard apparatus, counting procedure, and conversion factor by the Pollen Survey Committee of the American Academy of Allergy¹³ in 1947. The conversion factor adopted for ragweed was 3.6, just double the previous one. At that time it was the feeling of the members of the Committee that the gravimetric method, even with the revised conversion factor, was unsatisfactory and that a volumetric method, if available, would be more accurate and preferable. Among the factors which prevent an accurate conversion from the gravity slide to the volumetric equivalent are such variables as the location of the shelter, wind velocity, wind direction, wind direction in relation to the source of the pollen, and convection and deflection currents.

From the Albert Einstein Medical Center.
Received for publication Aug. 15, 1958.

The standard gravity method of the American Academy of Allergy had the advantage of being simple, inexpensive, and devoid of moving parts. Its widespread use had given us much valuable information concerning the distribution of pollen in this country. For the reasons previously cited, however, the gravity method cannot be converted to volumetric figures with any real degree of accuracy. In addition, one is incapable of determining either pollen concentrations for any specified period of the day or the exact amount of pollen that a patient might be inhaling in a given period of time.

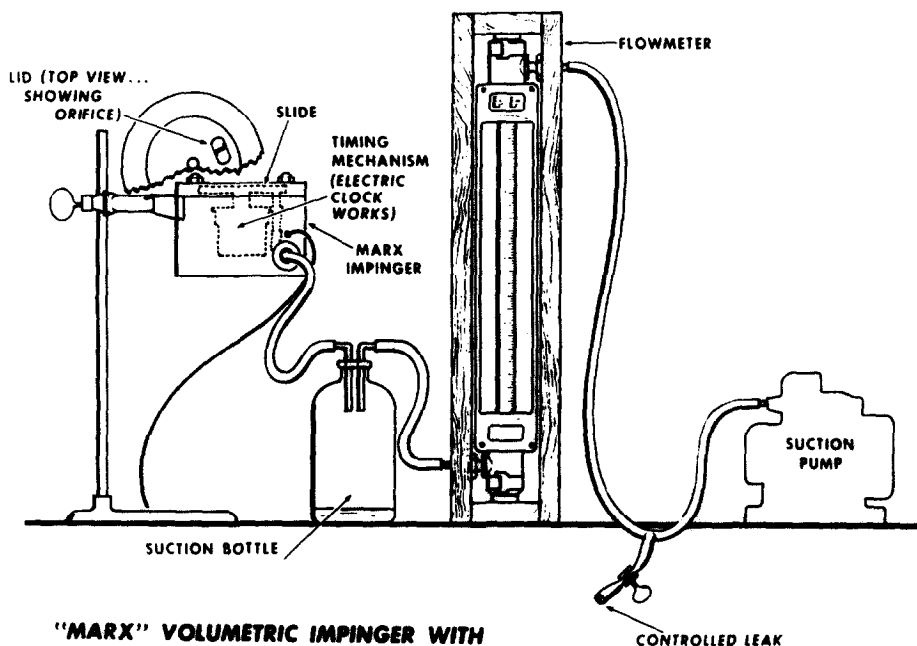
While most investigators were using the gravimetric method, several have attempted to use volumetric impingers. Duke¹⁴ used a motor-driven hair drier which delivered a stream of air at constant velocity. This device, however, lacked volumetric control or calibration. Keitt and Jones¹⁵ used a vacuum pump which pulled air through a nitrocellulose filter at the rate of 60 cubic feet per hour. Erdtman¹⁶ tested the air over the Atlantic Ocean, using a vacuum cleaner and filter papers. In 1942, Hawes, Small, and Miller¹⁷ devised an instrument which drew a known volume of air over a petrolatum-coated slide via a suction pump. The slide moved horizontally by means of a timing device at a rate that required frequent slide changes during a twenty-four-hour interval. This apparatus was used by Durham⁹ in his parallel volumetric and gravimetric studies. Stenburg and Hall¹⁸ devised a very ingenious volumetric sampler in which a given volume of air was impinged on the adhesive side of a moving transparent cellulose tape. After exposure, this was passed through a staining bath and the stained surface was covered with a facing piece of tape. This apparatus was used by Smith and Rooks¹⁹ in their studies of the diurnal variation of pollen grains. Sheldon and Hewson,²⁰ at the University of Michigan, have used still another volumetric device in their studies of atmospheric pollution with aeroallergens. Their device consists of a millipore filter through which a known volume of air is drawn. In their preliminary studies they ran the apparatus for thirty minutes every four hours, which gave them six samples a day.

It is the purpose of this article to present a new and improved volumetric impinger, which is simple, needs attention only once in twenty-four hours, and can be run for weeks at a time or through an entire pollen season without any mechanical difficulty. In addition, we have calibrated this device to sample 10 liters of air per minute, which we believe is a significant volume since it approximates human tidal respiratory volume. The pollen count obtained by this method would then represent the approximate number of pollen grains inhaled by the average patient on the day in question.

The present apparatus was devised by Marx²¹ in 1955, in connection with his work for a Master of Science Degree at Pennsylvania State University, and is a modification of an impinger used by Elder.²² The apparatus (Fig. 1) consists of the impinger with its spindle or slide and timing mechanism, a flowmeter to measure the volume sampled, and a suction pump.* A moisture

*This apparatus was part of the scientific exhibit at the fourteenth annual meeting of the American Academy of Allergy in Philadelphia, Pennsylvania, Feb. 3 to 5, 1958.

trap is also pictured, but this is superfluous since no difficulty was presented by rainfall entering the machine during three years of experience. The Marx impinger is a cylinder of stainless steel, 6 inches in diameter, that contains an air-tight chamber which houses the spindle or slide. The lid is of clear plastic and is held to the body by means of six thumb screws. In order to create a tight seal, a rubber gasket and silicone grease are used between the



**"MARX" VOLUMETRIC IMPINGER WITH
FLOWMETER & SUCTION PUMP**

Fig. 1.

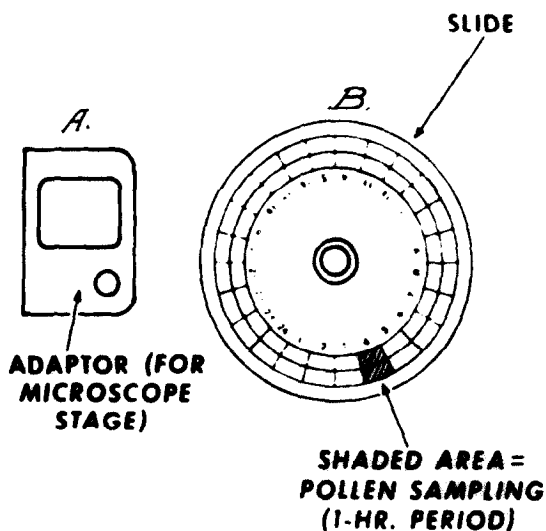


Fig. 2.

lid and the body. Within the apparatus itself is an electric timing mechanism that makes one complete revolution each twenty-four hours and upon which the spindle is mounted. The spindle is made of $7/32$ inch clear plastic, 4 inches in diameter, and is engraved as shown in the drawing (Fig. 2, *B*). The circular markings are 10 mm. wide and are so described as to be directly beneath the 1 by 10 mm. orifice of the lid through which the air is drawn. The circle is divided into twenty-four equal parts, each representing an hour of the day. The shafts of the spindles are uniformly arranged to correspond to the hours of noon and midnight and to be interchangeable from a time standpoint. The lid and slide are so engineered that the drawn air will be impinged only on the engraved area of the slide, as pictured (Fig. 2, *B*). The efficiency of the impingement was demonstrated on numerous occasions by our inability to find pollen on any area other than those described by our slide markings and on petrolatum-coated slides that were placed beneath the spindles.

The suction source was a DeVilbiss portable suction-pressure pump. We found it capable of drawing up to 14 liters of air per minute, and it was efficient and trouble free when run continuously through an entire pollen season. It is important that this pump have its own electrical line to avoid interference by other equipment, which might tend to overload the circuit and thereby decrease the efficiency of the pump. A controlled leak, as shown in Fig. 1, was created in the circuit in order to maintain the flow of air at 10 liters per minute as measured by a Fischer-Porter flowmeter. For reasons stated previously, we chose this figure because it represents the average tidal volume and because a direct and meaningful pollen count is obtained. This is equal to 14,400 liters of air per twenty-four hours. This figure may be converted to cubic yard values by multiplying by the factor 1.308×10^{-3} , which gives its equivalent as 18.8 cubic yards of air per day.

The operation of the apparatus is simple. The circular plastic spindle is covered with a thin layer of petrolatum in the usual manner and then placed in the apparatus. Since all of our spindles are identical and time factors on each correspond to one another, it is a simple matter to remove the exposed spindle and to replace it with another at the same time each day. After making one complete revolution in twenty-four hours, the spindle is removed and taken to the laboratory where the actual counting is done. In order to make pollen identification clearer and easier, the slide was stained with Calberla's solution and then covered with a circular cover slip of $1/16$ inch clear plastic which was fabricated especially for this purpose. We found that thinner plastic was unsatisfactory, for it tended to curl when in contact with the stain. At the present time we are experimenting with a specially prepared cover slip of glass, $1/32$ inch thick, fabricated to fit the spindle. The entire assembly, spindle and cover slip, was then held on the microscope stage by mounting it on a small plastic adaptor as illustrated (Fig. 2, *A*). This permitted the operator to rotate the spindle manually through 360 degrees without deviating from the fixed circular field under scrutiny. The microscope employed was a binocular type with a $10\times$ ocular and $10\times$ objective. On days of

low pollen concentration, the entire ruled area for each hour was easily counted. On days of high concentration, however, this proved to be a monumental task. In an attempt to simplify the process, it became necessary to test the accuracy and validity of counting part of a single field and multiplying it by a factor, the product of which would be representative of the whole field. Over the period of observation twenty such areas have been counted. The average factor was 5.3. When subjected to statistical analysis, a standard deviation of 0.46 and a coefficient of variation of 8.6 per cent were obtained, showing a high degree of correlation. It must be made clear that this factor will vary from microscope to microscope, depending on the strength of its contained lenses and on the part of the ruled area which is represented by a single strip observation.

TABLE I

NUMBER OF POLLEN GRAINS ON A SINGLE STRIP	NUMBER OF POLLEN GRAINS FOR THE ENTIRE HOUR	FACTOR
12	62	5.12
33	201	6.00
27	141	5.22
6	33	5.50
7	36	5.14
17	103	6.05
19	71	3.72
17	75	4.41
15	78	5.20
5	25	5.00
26	144	5.53
37	196	5.60
16	100	5.55
10	49	4.90
23	124	5.42
6	33	5.33
34	186	5.47
29	154	5.31
7	38	5.43
3	16	5.33

Average factor = 5.3

DISCUSSION

Using the apparatus as described, we obtained counts for forty-five consecutive days during each of the 1956 and 1957 ragweed seasons. We encountered no difficulty with the impinger, pump, or flowmeter. After a short period of practice we found it no more difficult to count the pollen on our circular spindle than on a conventional gravity slide, although it was a bit more time-consuming because of the increased area covered. This apparatus has the added advantage of supplying information regarding the pollen concentration for each hourly period of the day. It is, therefore, useful in studying the diurnal pollen variations and the effects of wind, rainfall, and humidity on pollen counts. In addition, it could supply data that might correlate the relationship between clinical hay fever symptoms and pollen concentration. The apparatus may also be used to test the efficiency of air filtering and air conditioning equipment in removing pollen from the air. For such purposes, the timing mechanism may be set to turn the spindle at a more rapid rate.

The behavior of particulate substances in air varies with their size and shape and with the direction and velocity of the wind.²³ A perfect sampling device would have to be oriented to wind flow and be isokinetic, that is, draw air through the sampling device at the same rate as the wind velocity.²⁴ This would avoid disturbing or altering the arrangement of particulate agents suspended in the air. It would require a variable source of suction that would adjust to each change in wind velocity. A weather vane would compensate for alterations in wind direction. A perfect sampler of this type could accurately measure the pollen content of the air, but it could not estimate the average daily exposure by the human being, for the respiratory apparatus is not a perfect sampling device. The volumetric impinger described has the same deviations from the perfect atmospheric sampler as the human respiratory system. Just how far they deviate from one another is conjectural at this time. Further refinements in our sampling instruments and techniques are needed to answer these questions.

CONCLUSIONS

A simple, uncomplicated volumetric apparatus, which will run unattended for twenty-four hours a day for months at a time and furnish an unbroken series of hourly air samples, has been described in detail. It offers the following advantages over previous methods:

1. Pollen concentrations for the entire day, or for any hour of the day, are easily determined.
2. The apparatus is simple and requires change of slides only once in twenty-four hours.
3. The volume of air sampled is easily controlled.
4. A conversion factor for pollens of varying size is unnecessary.
5. The relationship of pollen concentration to meteorologic phenomena is easily and accurately determined.
6. This apparatus can serve as a useful research tool.

In addition to the numerous advantages cited, the impinger that we have described overcomes the many defects inherent in our present gravimetric method. We feel that the apparatus and procedures herein described meet all the requirements of the Committee on Aeroallergens of the Research Committee of the American Academy of Allergy for a standard volumetric method of pollen sampling, and we herewith submit them for consideration.

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