Although spheres with an original coefficient of about unity (instead of about 0.78) might be produced (external diameter, about 4.4 cm. instead of 5 cm.), the many published evaporativity records based on the 5-cm. instrument render it undesirable to introduce a smaller sphere, exposure of which to sunshine and air currents would naturally differ from that of the 5-cm. sphere in several ways other than with respect to extent of exposed surface.

While the corrected rate of evaporation from a standardized atmometer depends upon prevailing vapor-pressure deficit, air movement, and radiation, rates at stations in calm and shade may be taken to be essentially proportional to the prevailing deficit; under these conditions air movement and radiation effects may be left out of consideration. The value of the deficit, D, at any time may be computed from the current relative humidity, H, and the standard vapor tension, T, for the current temperature, since $D = T\ (1-H)$. But the average value of D for a period is not to be satisfactorily derived from the corresponding average values of T and H unless at least one of them is maintained throughout the period. When both are maintained, the value of the deficit is naturally also constant throughout the period.

This paper reports the result of a 12-month series of weekly observations on the performance of a standardized 5-cm. white atmometer sphere (with coefficient of 0.78), freely exposed under conditions of shade and calm in an indoor storage space with air temperature and relative humidity (and also vapor-pressure deficit) automatically well maintained, at about 70° F., 50 per cent, and 9.29 mm. of mercury column, respectively. The almost constant daily corrected rate of evaporation from the instrument was found to be 15.5 ml., and the ratio of this value to the nearly constant value of the deficit is about 1.668. This new calibration characteristic of the standardized sphere should be essentially correct generally for air conditions of shade and calm, especially indoors, as in homes, offices, and workrooms without considerable radiation or air currents. It may be useful also in comparative studies on outdoor evaporativity.

Our observation period extended from October 1937 to September 1938. The instrument, with bottle reservoir and without nonabsorbing valve (*1*) stood in an air-conditioned storage room in Cleveland, Ohio, kindly placed at our disposal by the National Carbon Company. Each weekly reading was first multiplied by 0.78, the standardization coefficient of the sphere used. From the resulting corrected weekly rates the average corrected daily rate for each of the 12 months, October to September, was then computed as follows: 15.8, 15.7, 14.6, 15.9, 15.4, 16.3, 15.4, 15.0, 15.5, 15.5, 14.8, 16.1 ml./day. For the whole period, the average rate was 15.5 ml./day, or 0.646 ml./hour, and the range of fluctuation from month to month is seen to have been narrow—between extremes of −6 per cent (December) and +4 per cent (September).

Close agreement among the monthly averages constitutes clear evidence that the air-conditioning of this room was remarkably efficient. The automatic temperature and humidity controls were set to maintain air temperature of 70° F. (21.1° C.) and a relative humidity of 50 per cent. Doubtless there was some insensible air circulation at all times, but air movement was not noticeable. Standard vapor tension for 21.1° C. is 18.58 mm. and, since the room air was always about half saturated, the prevailing vapor-pressure deficit was about 9.29 mm. Consequently, the ratio of the value for average daily evaporation rate to that for average deficit is 15.5/9.29, or 1.668. Based on the average hourly rate, this ratio value is 0.0695.

The air temperature of the room used (70° F.) was lower than is usual for artificially heated homes and offices in this country, where 72° F. is often regarded as most satisfactory for the comfort of sedentary occupants. Many short-period tests, carried out at Baltimore with standardized 5-cm. spheres, have indicated that a high degree of winter comfort is often attained in homes and offices with calm air at about 72° F., corrected evaporation rates about 0.75–0.80 ml./hour, humidity about 45–42 per cent, and deficit about 10.8–11.5 mm. To secure such winter conditioning in the region of Maryland, Ohio, etc., artificial humidification of some kind is, of course, generally necessary, especially in periods of coldest weather.

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A Simple, Rapid Technique for the Study of the Action of Hydrolytic Enzymes on Insoluble Substrates

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For the study of the enzymatic digestion of catgut a very simple technique has been used (*1, 2, 4*) for determining the end point of the reaction as indicated by the complete loss of strength of the suture. In this procedure a suitable weight is attached to the suture, which is in turn immersed in the enzyme solution. The falling of the weight resulting from the

rupture of the suture is taken as the end point of digestion. This technique has been made quantitative by Lion and Sizer (*3*), who recorded automatically on telephone-message counters the digestion times of the sutures. The technique has been found to be sufficiently quantitative for use in the study of other fibrous material such as casein, soybean protein, and collagen filaments (*5*).

Since most substrates for enzyme action are not available in the form of filaments, it seemed desirable to adapt this technique to the study of insoluble solid substrates which become soluble upon digestion. It would then be possible to utilize this "breaking time" technique for the study of a wide variety of proteins, carbohydrates, lipids, and other organic compounds. In preliminary experiments this was done by coating a narrow strip (3 mm. wide) of lens paper (Cenco) with a suitable substrate which could be rendered insoluble by heat denaturation or by immersion in some suitable precipitating bath. For example, a lens-paper strip is dipped in 5 per cent egg albumin, dried, then dipped for a few seconds in boiling water to coagulate the protein. After drying the strip a suitable weight[1] is attached and the preparation immersed in a solution of trypsin. This technique was found satisfactory for the qualitative study of the digestion of a number of substrates but usually lacking in satisfactory reproducibility.

An entirely satisfactory method, which was finally devised, is the following: A sheet of lens paper (Cenco) is cut in half. A strip about 1.2 cm. wide along the edge of one piece is painted (using a camel's-hair brush) with a suitable solution or suspension of the desired substrate. The second piece is then placed over the previously painted portion, the edge overlapping by 1.2 cm. The substrate functions as an adhesive in sealing the two pieces together. After drying, the substrate is rendered insoluble by some appropriate technique and the preparation again dried. The lens paper preparation is then cut into 65 strips 2 mm. wide, with the overlap area in the middle of each strip. A colored glass bead is tied to one end of the strip with thread and the preparation immersed in the enzyme solution (see Fig. 1). For short digestion times the falling of the bead to the bottom of the test tube can be detected by eye or ear, but when digestion proceeds slowly, the automatic timing device is preferable.

This coated lens-paper technique is suitable for studying such properties of the enzyme as total activity and the effect of environmental factors such as pH, temperature, oxidation-reduction potential, activators, inhibitors, etc. On the other hand, this technique yields no information concerning the chemical transformation of the substrate induced by the enzyme, except to indicate that the substrate is eventually rendered soluble. In addition to its great simplicity and its reproducibility, the coated lens-paper technique has the advantage that relatively small amounts of substrate are required; with most substrates less than 1 mg. suffices for a single test.

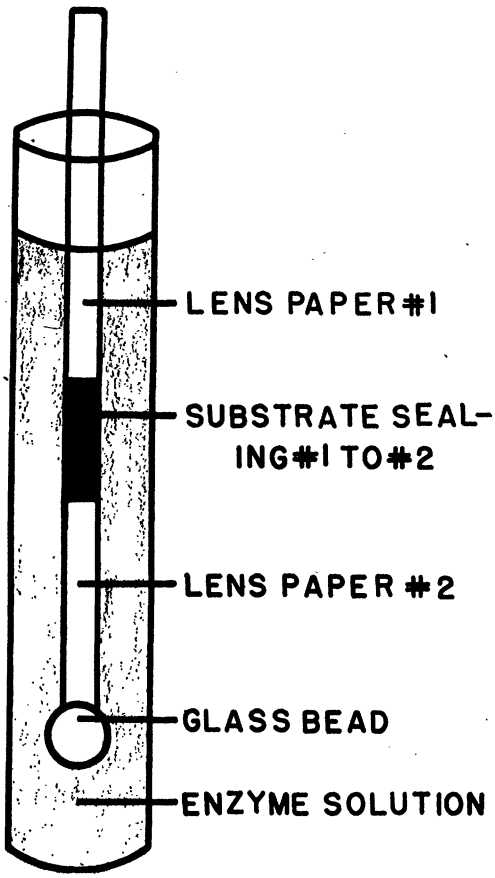

FIG. 1

METHODS AND RESULTS WITH TYPICAL SUBSTRATES

Proteins

Egg albumin. The edge of the lens paper was coated evenly with a saturated solution of egg albumin (Merck), using a camel's-hair brush. The second piece of paper was placed on top of the first with the overlap area 1.5 cm. wide. The coated paper was dipped for a few seconds in boiling water, dried, and then cut into strips. The overlap area of each strip was found to be coated with 0.71 mg. albumin. A 830-mg. glass bead was attached by a thread to each of six strips, which were then immersed in 5 ml. of 2 per cent filtered trypsin (Cenco, final pH 7.0) at

[1] It must be heavy enough to break an uncoated strip of lens paper but not so heavy as to break the coated strip until after digestion of the film has occurred.

37 ± 0.1° C. Several series were run; in a typical experiment the digestion times were 46, 46, 49, 50, 53, and 53 minutes (average, 49.5).

Globulin. A 5-per cent solution of bovine globulin (Armour) was prepared. The preparation and digestion of the coated lens-paper strips were the same as for albumin except that a 1-per cent trypsin solution was used. Each strip was found to be coated with 0.30 mg. globulin. In a typical series the digestion times were 8, 11, 11, 11, 12, and 15 minutes (average, 11.3).

Hemoglobin. A 2.5-per cent suspension of hemoglobin (Merck) was prepared. The preparation and digestion of the lens-paper strips were the same as for albumin except that 0.5 per cent trypsin was used. Each strip was found to be coated with 0.20 mg. hemoglobin. Digestion times in a typical series were 7, 7, 8, 8, 8, and 9 minutes (average, 7.8).

Casein. A 5-per cent solution of casein (technical) was prepared in 0.1 N NaOH. The lens paper was coated in the usual way and the casein rendered insoluble by washing with 0.1 N HCl followed by distilled water. A 1-per cent solution of trypsin was used for the digestion. Each strip contained 0.40 mg. casein. In a typical series the digestion times were 18, 18, 19, 22, and 23 minutes (average, 20).

Collagen. A dilute collagen gel (about 1 per cent) was prepared by blending with distilled water beef tendon, which had been swollen in 1 per cent acetic acid. After coating in the usual manner, the collagen was precipitated onto the lens paper by washing with saturated sodium bicarbonate followed by distilled water. An average of 0.015 mg. collagen was found on each strip. Typical digestion times by 2 per cent trypsin in a series were 9, 9, 10, 10, 10, and 13 minutes (average, 10.2).

Carbohydrates

Starch. The edge of the lens paper was coated with a boiling, 5-per cent suspension of potato starch (Baker's). Each strip was sealed with 0.43 mg. starch. The amylase used was 1.0 per cent taka-diastase (Parke, Davis, pH 6.9). Typical digestion times of a given series were 3, 3, 3, 4, 4, and 5 minutes (average, 3.7).

Pectinic acid (de-esterified). The coating agent was 2 per cent de-esterified pectinic acid.[2] It was precipitated with $CaCO_3$ followed by washing with distilled water. Each strip was sealed with 0.23 mg. pectinic acid. The digestion was carried out at pH 4.0 (acetate buffer) in 1 per cent pectinase.[3] The following digestion times characterized the series of coated lens-paper strips: 8, 10, 10, 11, 11, and 12 minutes (average, 10.3).

Lipids

Several fats were tried, but oils would not seal the two pieces of lens paper together. On the other hand, very hard fats like tristearin and diglycol stearate were found refractory to enzyme action. Soft fats such as the mixtures present in the commercial products, oleomargarine, Crisco, and Spry, were found very satisfactory.

Spry. Spry was melted and then spread on the overlapped area of the lens papers. Each strip contained 1.30 mg. Spry. Small beads, weighing 427 mg., were employed instead of the usual 830-mg. ones. The enzyme solution was a 10-per cent suspension of steapsin (Eimer and Amend) in phosphate buffer (pH 8.1) containing the detergent, Tween-20 (0.1 per cent). The digestion times were 10, 10, 11, 11, 17, and 17 minutes (average, 12.7).

Methyl stearate. The edges of the lens papers were sealed with hot, liquid methyl stearate, which solidified on cooling. Each strip contained 0.68 mg. stearate. Digestion was carried out in a 2.5-per cent filtered solution of the mold enzyme, keralin (American Cyanamid, pH 5.6). The digestion times were 34, 34, 34, 35, and 43 minutes (average, 36).

[2] Kindly furnished by Dr. Speiser, Eastern Regional Research Laboratory.

[3] Kindly furnished by the Food Technology Laboratory, M.I.T.

Summary

A rapid, simple, quantitative technique has been developed for the study of the action of hydrolytic enzymes on certain insoluble substrates. The end point of digestion is indicated by the release of a glass bead, when two pieces of lens paper sealed together by the substrate separate due to its digestion by the enzyme.

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Method for Making Filters Transmitting the Near Ultraviolet and Absorbing Visual Light

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The development of simple and inexpensive sources for the near ultraviolet light has resulted in great interest in that band of the spectrum that is transmitted by glass. Filters for absorbing the visual light either are of glass, both expensive and limited in size, or are precision gelatin filters that are also quite expensive. Preparation of filters that serve the usual experi-