

is given by J. W. Mellor [*Comprehensive Treatise on Inorganic and Theoretical Chemistry*, vol. III, p. 276]. A product closely similar in chemical composition, particle characteristics, and optical properties was made in this laboratory simply by grinding in a mortar copper acetate with a saturated solution of sodium carbonate, filtering, washing, and drying. A fuller account is in preparation.

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Response of Nasal Epithelium to Odor Stimulation

An *in vitro* preparation of nasal epithelium has been developed in order to study its response to odors in a most direct manner. (This work was supported by a grant from the Armour Research Foundation and by the Research Council of Florida State University.) The opossum contains a large yellow olfactory area. A 2-cm² sheet of this tissue was carefully removed and placed in a flat lucite chamber that allowed one to dissect, under high magnification, the nerves from the richly innervated epithelium. The underside was supported by lucite except for a 1-cm² surface that could be exposed to either purified room air or one of a number of different odors.

Recordings were made from very small branches of nerves. Mechanical stimulation of the tissue resulted in very large spikes, whereas only small spikes were recorded in response to odors. These small nerves conducted spikes at about 0.4 m/sec to 1.0 m/sec. Single fibers were rarely seen to respond at a frequency greater than 8 to 15 per second.

Odors were obtained by allowing an air stream to pass over the surface of 5 ml of odorous liquid contained in a 50-ml erlenmeyer flask at the rate of 30 ml/min. A portion of this stream was allowed to diffuse into the air 1 cm below the surface of the epithelium.

Most preparations that contained a number of active fibers showed resting activity. Odors such as amyl acetate, benzene, cajeput, eucalyptus, leaf cloves, spruce, Florida orange, asafetida, 2-furaldehyde, and freshly ground coffee beans stimulated a number of such preparations, and the nerve activity increased markedly.

The percentage increase in the number of spikes per second above resting activity of a given preparation as various odors were presented for 30 sec is as fol-

lows: amyl acetate, 110; cajeput, 42; spruce, 27; Florida orange, 21; leaf cloves, 0; musk xylol, 0. A 5-min rest in purified air was given after each odor stimulation. High concentrations of amyl acetate may have a detrimental effect.

A nerve bundle containing only a few active fibers reveals that one fiber may not respond to a given odor as well as another fiber, although this relationship may be reversed if a different odor is chosen. Figure 1 shows the response of a few-fiber preparation to various odors. Note that the same fibers do not respond to all the odors.

The nasal epithelium in the olfactory region is innervated by both olfactory and trigeminal nerve fibers. The type of stimuli to which the preparation responds and the slow conduction velocity of the nerves suggest that the recordings are from olfactory nerves. On the other hand, some nerve twigs contain a few large fibers that respond to mechanical stimulation, which suggests that trigeminal nerves may be involved. For these reasons, a live-rabbit preparation was devised so that the responses to odors could be recorded from the olfactory

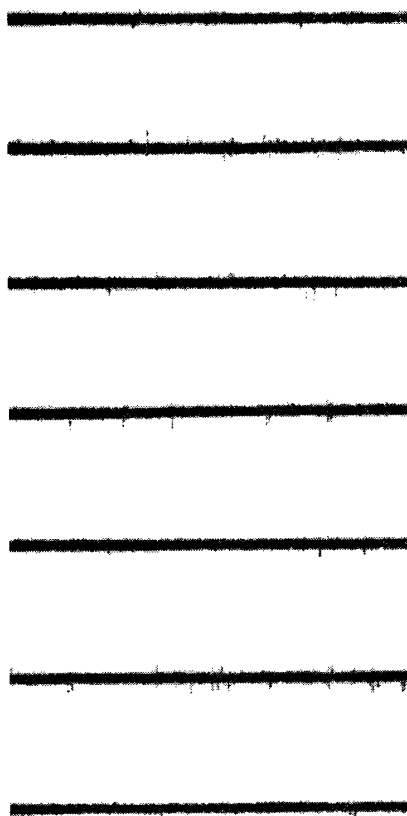


Fig. 1. The response of a few-fiber preparation to various odors. Several minutes elapse between each record. Top to bottom: air, 2-furaldehyde, air, heptane, air, coffee, air. One-half-inch horizontal space represents 1 sec.

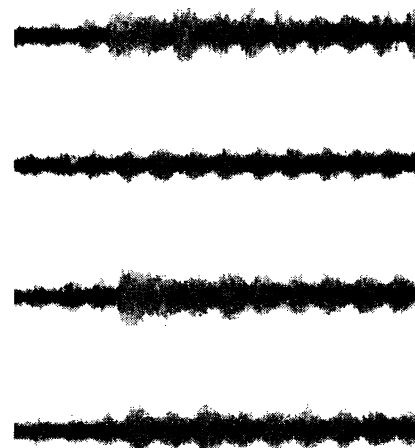


Fig. 2. Response of olfactory nerve twig to odors inhaled during breathing under anesthesia. Top to bottom: amyl acetate, benzyl benzoate, *n*-heptaldehyde, eucalyptus. One-half-inch horizontal space represents 1 sec.

nerves as they passed through the cribriform plate (Fig. 2). Such results were compared with those recorded from known trigeminal nerves of the same rabbit. Both of these nerves responded to most of the stimuli chosen. Therefore, the olfactory and trigeminal systems are much more similar in the type of stimuli to which they respond than was previously realized.

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Manometer Flask for Measuring Respiratory Quotients

Although the methods presently available for measuring respiratory quotients (RQ) provide insight into patterns of metabolic changes, they leave much to be desired, both in ease of manipulation and validity of the results (1, 2). With these methods the measurement of changes in the RQ over extended and varying periods of time on the same tissue sample is usually impossible (3).

The techniques that permit the measurement of RQ rates at different or continuous intervals, such as those described by Laser and Rothschild (4), Noyons (5), Prop (6), Asprey (7), Wolf *et al.* (8), and Gaffron (9), require elaborate equipment and considerable manipulation. Other manometer flask modifications that are used for RQ determinations measure the net oxygen uptake and carbon dioxide evolution over a period

of time. At the end of the study, the amount of carbon dioxide produced is determined by admitting alkali into the vessel (10). In these latter methods, the assumption is made that the RQ is constant during the interval of measurement, a condition that is often not the fact (1, 4). To obviate these difficulties and to permit a rapid, valid measurement of the RQ, a modification of the standard Warburg flask has been developed for use with a constant-volume respirometer.

The RQ measuring flask (11) permits the determination of the carbon dioxide production and the oxygen uptake of a single tissue sample during immediately adjacent intervals, thus eliminating the need for duplicate samples in two separate flasks. The flask (Fig. 1) has one or two hollow stopcock side arms into which folded filter paper and alkali are placed. These side arms replace the usual center well. The hollow stopcock has a large hole (bore) in one side that opens into the central chamber and exposes the absorption surface of the filter paper to the vessel atmosphere. When the stopcocks are in the open positions, the flask measures oxygen uptake in the same way that a standard flask with alkali in the center well does. When the stopcocks are closed, the flask serves as the pair member without alkali in the Warburg direct method (12).

The procedure for determining the RQ involves the use of three different time intervals, the first two of which are equal in length. In the first interval, the stopcocks that contain KOH are opened into the chamber, and the change in pressure is proportional to the oxygen uptake; during the second interval, the stopcocks are closed off, and the change in pressure is proportional to the net effect of the carbon dioxide produced and the oxygen uptake. The change in pressure recorded on the manometer during these two intervals, together with the known vessel constants, permits the computation of the RQ (13). In the third interval, the stopcock is again opened in

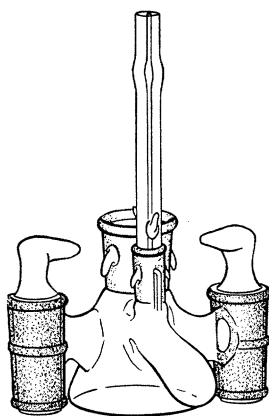


Fig. 1. Manometer flask for measuring respiratory quotients.

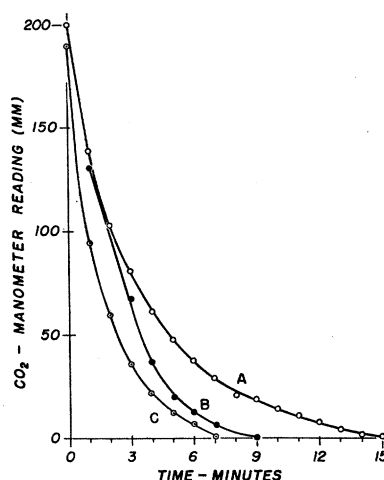


Fig. 2. Effectiveness of RQ flask in absorbing carbon dioxide. (A) RQ flask with one alkali-containing stopcock; (B) standard flask with center well; (C) RQ flask with two alkali-containing stopcocks. The vessels were of 6- to 7-ml volume; similar results were obtained with vessels of 20-ml volume. The main compartment contained 0.5 mg of Na_2CO_3 ; 0.25 ml of 10-percent oxalic acid was added from the side arm at zero time. Stopcocks contained 0.15 ml of 20-percent KOH and filter paper. The center well contained 0.05 ml of 20-percent KOH and filter paper. Bath temperature was 30°C ; Lardy-type Warburg, shaking at 112 oscillations/min.

order to permit the absorption of the carbon dioxide released during the second and third intervals; this sequence, starting with the first interval, may then be repeated. In principle, this procedure is similar to the one recommended for a flask by Gaffron (9).

One recognized limitation of the standard flask is the rate at which the alkali in the center well absorbs carbon dioxide. Figure 2 compares the rate of carbon dioxide absorption in the RQ flasks with that in the standard Warburg flasks. It is apparent that the RQ flask with both stopcocks open is as efficient in carbon dioxide absorption as is the standard Warburg flask with KOH and filter paper in the center well. By using the procedure of Dixon and Elliott (14), the time required to absorb a given volume of carbon dioxide may be computed. Thus, the shortest time interval can be determined for which each flask may successfully be used with a given rate of carbon dioxide evolution. In studies conducted upon germinating seeds at this laboratory, the rate of evolution of carbon dioxide and of change in RQ has been well within the absorption capacity of all flasks in use.

A more serious limitation of the standard Warburg flasks, which is in part circumvented by the use of these RQ flasks, is that certain tissues and cells give significantly different respiratory patterns

and rates when they are in an atmosphere in which the carbon dioxide tension is reduced to zero (3). In the RQ flask the tissue is respiring in the absence of carbon dioxide for only the minimal amount of time required to measure the oxygen uptake; this is closer to the ideal situation than is the case in the usual Warburg direct method.

Other suggested uses of the RQ flask are for the measurement or absorption of metabolically produced hydrogen (15) and for the measurement of ethylene production (16).

Precautions must be taken to acquire the technique of opening and closing the stopcocks without disturbing the positions of the flasks on the manometers. But, as with other manipulative manometric procedures, only time, patience, and an awareness of the potential defects will permit the successful application of the instrument (17).

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Geologic Application of a Test for Citrate-Soluble Metals in Alluvium

One of us has described a procedure for determining the content of citrate-soluble heavy metals, principally zinc, copper, and lead, in geologic materials (1). This is a rapid and simple procedure readily adaptable to use in the field at the collecting site.

Experiments with this test in 1953 in New Brunswick and the Gaspé Penin-