TABLE I

THE DISTRIBUTION OF DRY WEIGHT AND OF NICOTINE IN TOBACCO STALKS

Tissue fraction	Dry weight per stalk gm.	Nicotine per stalk mgm	Per cent. of total nicotine in one stalk
Xylem	14.3	2.8	19.2
Pith	3.8	4.7	32.2
Cortical tissues	4.5	7.1	48.6

porcelain evaporating dishes, and the sap thus obtained was analyzed for nicotine. The results show that this material, which, incidentally, could be seen to exude from the xylem only, contained 0.24 mgm of nicotine per milliliter. The possibility is not to be excluded that the translocation of nicotine, if it actually occurs, may take place, in part at least, in the phloem. From the evidence at hand, however, the following tentative suggestions are advanced: (1) nicotine is apparently synthesized *in appreciable amounts* only in the roots of the tobacco plant; (2) the presence of the alkaloid in the leaves of the intact plant in higher concentrations than exist in either stalks or roots may be explainable on the basis of translocation and accumulation; and (3) the presence of the alkaloid in appreciable amounts in the xylem suggests that nicotine may move from root to leaf principally through this component of the vascular system.

RAY F. DAWSON

UNIVERSITY OF MISSOURI

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW TYPE OF MICRO-RESPIROMETER

THIS apparatus functions as a constant pressure volumeter, which is a type not hitherto described. It consists simply of a fine bore capillary tube with a mercury piston at one end, a stopcock at the other end and, near the middle, a T-connected conical tube that has a pocket for alkali and supports a coverglass on which the experimental material is placed as a hanging drop. An index drop (high boiling kerosene), placed between T-joint and stopcock, divides the gas space into an experimental volume (V_e) and a control volume (V_c). V_c is maintained constant by returning

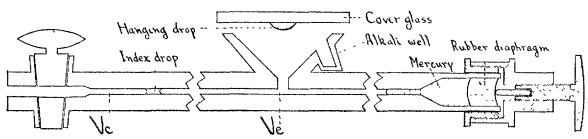

FIG. 1. Diagram of micro-respirometer. Alkali well is at right angles to position in diagram. A T-connection for introducing mercury is not shown. Overall length, 17 inches.

the index drop, at each reading, to its original position by means of the mercury piston. The distance that the mercury moves, multiplied by the cross-sectional area of the capillary, gives directly the volume of gas absorbed or produced. The respirometer is immersed in a water bath and readings are taken through microscopes with eyepiece micrometers. Some of the principal features of the apparatus are: (1) Construction is simple and inexpensive. (2) The readings are not affected by temperature change, since according to the gas laws a change from T to fT in the system is accompanied by a proportionate change in pressure to fP. Thus, if the absorption or production of x moles of gas gives a volume change, ΔV, at T and P, it will be $\frac{f}{f}\Delta V$ at fT and fP. In other words, the temperature may vary by any amount and displacements of the mercury meniscus will still measure the same amounts of gas change as at the temperature and pressure of the initial reading. Temperature control is therefore unnecessary, except to the extent that it is desirable to avoid large variations in the rate of metabolism of the tissues. It is, however, essential to avoid temperature differences between the two gas spaces. (3) By setting the drop in motion before each reading the mercury piston insures attainment of pressure equilibrium. It also simplifies the initial setting of the drop and wetting of the capillary. (4) Fluid and gas volumes need not be known. (5) Cells can be observed during the experiment. (6) Material can be added from an adjacent drop by tapping or tipping or the incorporation of a small piece of iron filing and the use of a magnet. (7) Since the cells lie on the bottom of the hanging drop, gas exchange is facilitated without the necessity of shaking the apparatus.

Using capillaries of 0.2 mm diameter and reading to the nearest 0.005 mm, a volume change of 0.003 cu mm (0.1 mm displacement) is measurable with a reading error of 10 percent. There is, however, a factor that has prevented the attainment of this sensitivity; namely, that the index drop drifts when the respirometer is empty. The drift is variable and has ranged in different tests from 0.05 to 0.2 mm per hour. It is always in the direction of V_e. Tests under various conditions have eliminated, as possible causes, such factors as non-uniform temperature change, leaks, gravity, non-uniformity of capillary bore, osmotic pressure of solutions, etc. Dr. Needham[1] suggested that it may be due to a slow oxidation of the petrolatum used for sealing on the coverglass, but other greases have not as yet eliminated the drift. In each

[1] Personal communication.

run it is fairly constant, so some correction for it may be made by taking blank readings. With sufficient quantities of material the drift is negligible. Five experiments with samples of 1,000 to 2,000 fertilized *Strongylocentrotus* eggs gave oxygen consumption values of 6.6 to 7.1×10^{-5} cu mm/hour/egg as compared with an average of 6.4×10^{-5} from Warburg manometer measurements on about 100,000 eggs. Four experiments on samples of 100 to 200 eggs gave, when corrected for the drift, values of 5.3 to 9.1×10^{-5}. While this variation would be greatly diminished by elimination of the drift, the apparatus is still usable with reasonably small amounts of material. It should be noted, too, that it offers advantages over other types of respirometers on a macro-scale as well.

ALBERT TYLER
WILLIAM E. BERG
CALIFORNIA INSTITUTE OF TECHNOLOGY

A QUALITATIVE TEST FOR BILE IN THE URINE

DURING the course of an investigation involving frequent determinations of sulfonamide drugs in urine by the Maher-Camp modification of the Marshall method,[1] several samples of urine were obtained which developed a marked green color upon the addition of the sodium nitrite reagent used in the determination. Subsequent investigation indicated that bile, present in these urines, was responsible for the green color, and led to the following qualitative test for the presence of bile in urine:

Ten cc of suspected urine are placed in a test-tube and acidified by the addition of 1 cc of a 20 per cent. solution of para-toluenesulfonic acid. Ten per cent. hydrochloric acid may also be used, although better results have in our hands followed the use of the organic acid. Two minutes later, 1 cc of a 0.1 per cent. freshly prepared solution of sodium nitrite is added, and the contents of the tube are mixed well. The development of a green color indicates the presence of bile, presumably by the oxidation of bilirubin to a green derivative. So far, we have found no substances which produce a similar reaction.

Further studies have indicated a marked difference in the reaction of various types of bile to this test. We have been able to detect the presence of dog bile (gall-bladder) in aqueous dilutions of as high as 1:1,000, and the presence of this concentration of dog bile diluted in normal human urine can be demonstrated by this test. However, rabbit bile (gall-bladder) is usually not demonstrable in aqueous dilutions greater than 1:50 to 1:100. Human biles have varied rather widely, with the sensitivity in aqueous dilution ranging from 1:60 to 1:500.

[1] F. T. Maher and W. J. R. Camp, *Jour. Lab. and Clin. Med.*, 24: 1198, 1939.

In three samples of pathological urines, obtained from jaundiced subjects, the sensitivity and convenience of the above method were compared with results obtainable by the usual Gmelin and Huppert techniques. Using these three urine specimens, little difference in sensitivity could be demonstrated—in each case positive tests for bile were demonstrable in aqueous dilutions of 1:50 to 1:60, and results were unconvincing in higher dilution. However, the nitrous acid oxidation method was more rapid and convenient of application, avoided the use of the nitric acid or the shaking out with calcium hydroxide, and afforded results comparable with the best results obtainable with the Gmelin or Huppert techniques. Results are more easily read than those by Gmelin's test, due to the diffusion of the green color and the avoidance of the ring formation.

Attempts to establish a quantitative analysis based upon the above procedure have not been successful due to the difficulty in preparing a stable and utilizable solution of bilirubin.

Obviously, the presence of bile in the urine of jaundiced subjects may interfere with the determination of sulfonamide drugs in such samples.

FRANK T. MAHER
DEPARTMENT OF PHARMACOLOGY,
UNIVERSITY OF ILLINOIS,
COLLEGE OF MEDICINE

BOOKS RECEIVED

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