and lumbar levels of the spinal cord. Both strains of virus have been passed in other monkeys, and 6 mice inoculated intracerebrally with each strain have remained well.

During the summer of 1941 four other samples of flies from epidemic areas have been tested in eight cynomolgous and green African monkeys in our New Haven Laboratory with negative results. Previously we had also tested by various methods many samples of flies and many varieties of other insects collected from seven epidemics over a period of ten years. In the majority of these earlier tests rhesus monkeys were used; all these tests proved negative.

It is well known that house flies contaminated artificially will harbor or carry the virus of poliomyelitis for several days.[3] Furthermore, occasional attempts at transmission of the experimental disease through the agency of the stable fly (*Stomoxys calcitrans*) seem to have been successful.[4] To our knowledge, however, the only other report which might be construed as an example of a positive test from flies in nature, is that of Rosenow, *et al.*[5] In one of their monkeys (variety unspecified) inoculated with a filtrate of flies collected during the epidemic of poliomyelitis in Kentucky in 1935, poliomyelitis apparently developed.

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THE LOCALIZATION OF THE NICOTINE SYNTHETIC MECHANISM IN THE TOBACCO PLANT

EVIDENCE is to be presented elsewhere[1] that tobacco shoots grown as scions upon tomato roots contain only traces of nicotine and that tomato shoots grown as scions upon tobacco roots accumulate large quantities of the alkaloid. Analogous experiments have just been completed in which reciprocal grafts of *Datura Stramonium* L. and *Nicotiana tabacum* L. var. Turkish have been examined for their nicotine content. Essentially the same results have been obtained as have been reported for the tobacco and tomato graft hybrids. After approximately one month of growth the *Datura* scions had accumulated 10 mgms of the alkaloid each, which represents a concentration of 0.03 per cent. on the basis of fresh weight. The leaves of each tobacco scion contained on the average only 4.5 mgms of nicotine, a quantity which was not significantly greater than the amount present in the scion at the time of preparation of the graft. Increase in fresh weight during the thirty-day period of growth was 44-fold for the tobacco and 10-fold for the *Datura* scions. Since the above data obviously suggest the possibility that nicotine may be manufactured in the root system of the tobacco plant and not in the leaves, as has been believed heretofore, a number of experiments have been performed which contribute indirect evidence in support of such an interpretation.

A number of Turkish tobacco leaves were cut from the stalks and rooted in moist sand. In the beginning each leaf contained 0.96 mgm of nicotine, but after development for about two months the alkaloid content increased to 46 mgms per leaf. At the end of another 16 days this figure had again increased to 71.6 milligrams. The root system attached to each leaf of the last two collections contained 2.4 mgms and 2.7 mgms, respectively. In view of the constantly increasing amount of nicotine in the leaves, the relative constancy of the amount present in the root system appears to substantiate the idea that the seat of nicotine synthesis is the root and that the presence of the alkaloid in the leaf tissues may best be explained on the basis of translocation and accumulation.

If the presence of nicotine in tobacco leaves is to be regarded as a result of translocation from the roots and not as a synthesis *in situ,* then it should be possible to detect the alkaloid in appreciable amounts in either the xylem or the phloem of the tobacco stalk. Consequently, the stalks of four mature, field-grown Turkish tobacco plants were separated into three fractions which consisted almost entirely of (1) xylem, (2) pith and (3) phloem, pericycle, cortex and epidermis. The separation was easily effected, since the secondary xylem of the stalk forms a hard woody cylinder from which the more succulent tissues are readily peeled. The results of the analyses of these fractions are given in Table I. It is readily observed that nicotine was present in the xylem in sufficient amount to establish this tissue as a possible path for the movement of the alkaloid from root to leaf. In substantiation of this observation, the cut stumps of six Connecticut Broadleaf No. 38 tobacco plants were allowed to bleed into

[3] S. Flexner and P. F. Clark, *Jour. Am. Med. Assn.*, 56: 1717, 1911; C. W. Howard and P. F. Clark, *Jour. Exp. Med.*, 16: 850, 1912.

[4] M. J. Rosenau and C. T. Brues, *Trans. XV Internat. Cong. Hyg. and Demog.*, Washington, 1912, 1: 616, 1913; J. F. Anderson and W. H. Frost, *U. S. Pub. Health Rept.*, 27: 1733, 1912.

[5] E. C. Rosenow, L. H. South and A. T. McCormack, *Kentucky Med. Jour.*, 35: 437, 1937.

[6] National Research Council Fellow.

[1] R. F. Dawson, in press.

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.

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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

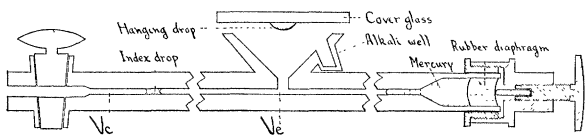

FIG. 1. Diagram of micro-respirometer. Alkali well is at right angles to position in diagram. A T-connection for introducing mercury is not shown. Overall length, 17 inches.

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.