TABLE I

Bacteriophage	Streptococcus strain	Inactivation dose in r*	Sensitive volume diameter mμ
B	563	200,000	26
C	594	110,000	33
D	693	60,000	43

* Dose giving inactivation ratio $\frac{N}{N_0} = 1/e$.

to influence the present results, since particle diameters depend (through volumes) on cube roots of inactivation rates.

A comparison with previously measured phages[5] shows that phage D is a medium size phage; phage C falls among small phages (like C13 Burnet); and phage B is still smaller.

The classification of these three phages as separate entities, based up to now on their different biological properties,[4] finds further justification in their different particle sizes.

A general relationship of inverse proportionality has been shown to exist between the size of phage particles and the size of the plaques they produce on agar (Elford, Burnet). Such comparisons are definite only for plaques produced in presence of the same host strain of bacteria, which was not the case in our experiments. Nevertheless, it is probably not meaningless that the smallest phage B is a "large plaque forming" strain, whereas the larger phage D produces very small plaques.

We hope soon to check the particle size values as given here by other methods (ultra-centrifugation, electron microscope).

We are indebted to Dr. A. C. Evans for supplying the strains of bacteriophages and of host bacteria. Only three out of four strains were studied, owing to difficulty in obtaining reproducible counts of the plaques formed by phage A.

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THE DETECTION OF POLIOMYELITIS VIRUS IN FLIES[1]

THE present note describes two instances in which the virus of poliomyelitis has been detected in collections of flies made in the field during epidemics of this disease. The first positive test was obtained from a summer camp (Camp S.) in Connecticut, where at least three frank cases of poliomyelitis occurred during the latter half of July, 1941, among a varying population of about 100 children, and where during the first half of August, two other proven, convalescent (intestinal) carriers were present among the campers. A sample of roughly 1,000–1,200 flies, caught out of doors in the vicinity of the camp kitchen on August 6th and 8th, was stored in the refrigerator until ready for inoculation. The bulk of this sample was made up of several varieties, including in particular, three types of green-bottle flies or *Lucilia* (*viz.: sericata, caesar* and *sylvarum*) and one variety of blow-fly, *Phormia regina.* In lesser numbers there were also representatives of the common house fly (*Musca domestica*) and flies of the following species: *Muscina stabulans, Sarcophaga haemorrhoidalis, Ophyra leucostoma, Protocalliphora,* and some questionable examples of *Stomoxys.*[2]

Two types of inocula were prepared: (*a*) an emulsion of 100–300 flies macerated in 200 cc of sterile water; and (*b*) washings from 400–600 flies in 50 cc of water. Sample *a* was centrifuged and from the mid-layer a 30 cc portion was frozen and set aside for nasal instillation, while to another 20 cc mid-layer portion, 15 per cent. ether was added (for bactericidal purposes) and it was allowed to stand in the ice box overnight before being injected intraperitoneally. Sample *b* was filtered through gauze and used for nasal instillation. On August 12th, 10 cc of the etherized portion of sample *a* was injected intraperitoneally, and on 3 successive days 2 cc amounts of samples *a* and *b* were instilled intranasally into one cynomolgous monkey (No. 1676). This animal developed poliomyelitis after an incubation period of 15 days.

The second specimen of flies to yield the virus was obtained in the vicinity of Jasper, Alabama, where poliomyelitis was epidemic during July and August, 1941. On August 20th, a fly trap was placed near a privy used by three households where cases of poliomyelitis had recently occurred. On August 24th, a sample of flies, representing about 200 specimens (unidentified as to species, except for the presence of green-bottle flies, blow-flies and probably house flies) were removed from the trap, packed in dry ice and mailed to New Haven, where they were prepared and inoculated into one cynomolgous monkey (No. 1840) which developed poliomyelitis after an incubation period of 9 days. The methods used were essentially the same as those described in the first animal.

Criteria for the identification of the virus in these two instances have been that the monkey developed signs and symptoms of the experimental disease; that typical histological lesions were found in the cervical

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

[2] We are indebted to Dr. R. B. Friend, of the Connecticut Agricultural Experiment Station of New Haven, for the identification of the specimens.

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.