

comparing the fusion point of the crystals with that of a known standard, in this case fumaric acid (M. P. 287° C.).

Several attempts were made to determine the pattern of the crystals by means of x-rays, the "scatter" method being used. Three early attempts were entirely negative. A fourth test, made with some 15,000 amoebae massed into a clump the size of a pin-head and exposed for fifty hours, resulted in a series of vague lines which are of doubtful value. An attempt to produce a single large crystal for such analysis failed.

Certain chemical tests have been carried out and are being continued. At present, taking into consideration the physical constants of the crystals and the results of the chemical tests already performed, it seems possible that these crystals may be composed of calcium chlorophosphate. We shall report further upon the subject.

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PLANT PIGMENTS AND REPRODUCTION¹

THE suggestion of Murneek² that the amounts of carotinoid pigments in the plant may bear a significant relation to reproduction recalled to mind some unpublished measurements of the pigments in apple leaves in 1933 at the time of blossom bud differentiation which were given a different interpretation. A second series of samples were collected in October, 1934, from Wealthy trees. The results follow (Method of Schertz³):

TABLE I
PIGMENTS IN WEALTHY APPLE LEAVES, 1934

Vegetative condition	Fruiting condition	Milligrams per 100 sq. in. leaves		
		Chlorophyll	Carotene	Xanthophyll
"Under-vegetative"	Non-fruitful	6.08	0.225	0.545
Moderately vegetative	Fruitful	12.30	0.483	0.396
Same, girdled	Very fruitful	4.21	0.355	0.426
Strongly vegetative	Slightly fruitful	38.56	1.388	0.896
Same, girdled	Very fruitful	17.19	0.868	0.817
"Over-vegetative"	Non-fruitful	34.55	1.451	0.962

These data are similar to those of 1933 in that the presence of the three pigments measured is in general directly proportional. Also the carotinoid content is not related to fruitfulness, unless it be the intermediate contents. The samples were not collected at

¹ Published with the permission of the director of the Agricultural Experiment Station.

² A. E. Murneek, *SCIENCE*, 79: 528, 1934.

³ F. M. Schertz, *Plant Phys.*, 3: 211-216, 1928.

the period of blossom bud formation, but the relative colors in the early season remained the same in the fall except in the case of girdled branches.

The pigment content of leaves of beet, *Datura* and Maryland Mammoth tobacco plants in which fruitfulness was regulated by photoperiod treatments is given in Table II. These data further fail to indicate a

TABLE II
PIGMENT CONTENT OF FRUITING AND NON-FRUITING PLANTS, APRIL, 1935

Plant	Condition	Milligrams per 100 sq. in. leaf area		
		Chlorophyll	Carotene	Xanthophyll
Tobacco (in sand)	Fruiting	18.4	0.55	1.29
	Vegetative	25.3	0.81	1.41
Tobacco (in soil)	Fruiting	31.8	1.03	2.14
	Vegetative	32.5	1.19	1.85
Beet	Fruiting	27.4	0.78	2.50
	Vegetative	31.5	0.82	...
<i>Datura</i>	Fruiting	21.5	0.78	1.41
	Vegetative	22.9	0.80	1.71

correlation between the carotinoid pigments and fruitfulness.

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THE CULTIVATION OF THE VIRUS OF ST. LOUIS ENCEPHALITIS

RECENT experimental work^{1,2,4} has established a specific filterable virus as the etiological agent responsible for human epidemic encephalitis of the type encountered in St. Louis during 1933. Further, the susceptibility of the white mouse to the virus following its introduction by intracerebral or intranasal routes has been demonstrated.^{2,3,4}

The object of this communication is to present evidence of successful *in vitro* propagation of the virus in the presence of living cells.

The brains of mice, dead or moribund following the intranasal instillation of the "Daily" strain of St. Louis encephalitis virus,⁵ were used as a source of

¹ R. S. Muckenfuss, C. Armstrong and H. A. McCordock, *Pub. Health Repts.*, U. S. P. H. S., 48: 1341, 1933.

² L. T. Webster and G. L. Fite, *SCIENCE*, 78: 463, 1933.

³ C. Armstrong, *Pub. Health Rept.*, U. S. P. H. S., 49: 959, 1934.

⁴ L. T. Webster and G. L. Fite, *Jour. Exp. Med.*, 61: 103, 1935.

⁵ We are indebted to Dr. Ralph S. Muckenfuss, Washington University School of Medicine, St. Louis, for the "Daily" strain of virus. Dr. Muckenfuss has written us that he obtained this strain originally in 1933 by inoculating brain tissue from a patient into *Macacus rhesus* monkeys. After 3 monkey passages, he carried the virus in mice, sending it to us in glycerolated mouse brain suspension. We transferred the virus serially by intracerebral and intranasal inoculations into Swiss mice through 9 generations before undertaking the cultivation experiments.

virus. The brain tissue was ground without abrasive and diluted to make a 10 per cent. suspension in nutrient broth. After centrifugation at 2,500 r.p.m. for 15 minutes, the supernatant fluid was added in 0.3 cc amounts to 2.7 cc of culture fluid media consisting of from 1 to 2 drops of finely minced embryonic tissue suspended in 1 part of fresh mixed normal rabbit serum to 2 parts of Tyrode's solution in a 50 cc Erlenmeyer flask. The inoculated flasks were incubated at 37° C. with successive passages or generations made every fourth day by the direct transfer of 0.3 cc of the preceding passage flask to similarly prepared minced tissue Tyrode-serum mixture in 2.7 cc amounts. The absence of bacteria was established by direct smears from each flask and by subinoculations into Douglas' broth with subsequent aerobic and anaerobic incubation.

During this investigation many experiments were performed, of which two are reported herewith. In both embryonic mouse tissue was used.

Series A: Mouse brain tissue virus in the supernatant fluid of a bacteria-free centrifuged 10 per cent. suspension in broth, lethal for mice in a dilution of

10⁻⁴, was used. This was carried through 19 generations. Undiluted samples of each culture generation were tested for their lethal effect by intracerebral inoculation into mice, under light ether anesthesia. Titration of the sixth generation was lethal in a dilution of 10⁻²; of the twelfth 10⁻¹; and of the eighteenth 10⁻².

Series B: An inoculum similar in its preparation to that of Series A, but of a different mouse brain passage, was used to initiate the cultures. This series was carried through 20 generations. Titration of the sixth generation was lethal in a dilution of 10⁻²; of the twelfth 10⁻²; and the eighteenth 10⁻².

Thus it appears that the virus of St. Louis encephalitis is capable of multiplication *in vitro* by the simple method of cultivation described. Experiments on the serological specificity of the culture virus and its immunological relationships, following repeated cultivation, will soon be reported.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A GENERAL SOURCE OF RADIATION FOR THE VISIBLE AND INFRARED SPECTRUM

If a portion of Welsbach mantle be introduced into the glow or arc discharge produced by the secondary of a transformer the mantle may be caused to glow with intensities ranging from the barely perceptible to those rivaling a tungsten lamp. High and medium intensities are used in the visible and near infrared spectrum, *i.e.*, up to 15 μ , while the lower intensities are reserved for the far infrared, where advantage is taken of the unique properties of the Welsbach mantle.¹

The construction adopted is shown in Fig. 1, where A is a tube of pyrex glass, about 3 cm in diameter, supplied with two side-tubes B₁, B₂, over whose ends the water-jackets CC are slipped. Polished plates of rock-salt R and crystalline quartz Q are attached to the open ends of the side tubes with a mixture of beeswax and rosin. The luminous element is supported by two plates of asbestos board DD fitting diametrically into tube A and supplied with lateral openings for the escape of radiation. The plates are given a 2 mm separation by the copper electrodes EE, which are held in place by the small bolts FF. Each copper electrode is supplied with a tongue T, about 1 mm thick, the edge of which is lined with heavy plati-

num wire P. The length of the air-gap between the electrodes is about 15 mm.

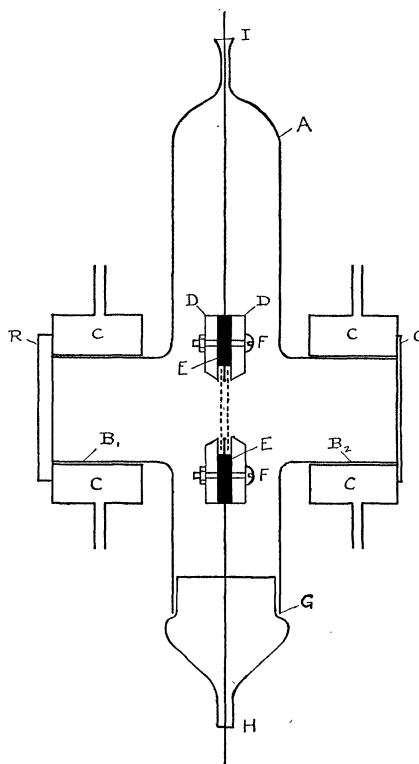


FIG. 1

¹ H. Rubens, *Ann. d. Phys.*, 18, 725, 1905.