Table 1. Effect of 5 g of L-tryptophan on the rate of urinary excretion of 5-HIAA (micrograms per hour).

Subject	No.	Rate		Increase
		Before tryptophan	After tryptophan	
National Institutes of Health				
Normal	6	229 ± 68	448 ± 53	219 ± 110
Schizophrenic	16	190 ± 91	373 ± 164	183 ± 93
Spring Grove State Hospital				
Schizophrenic*	8			
Before pyridoxine		128 ± 85	300 ± 159	172 ± 104
After pyridoxine		143 ± 106	299 ± 161	156 ± 132

* Feeding problem patients.

pound in schizophrenia. L-Tryptophan is the normal dietary precursor of serotonin, and 5-hydroxyindoleacetic acid is the major urinary metabolite of this amine. Zeller *et al.* (*1*) have reported that schizophrenic patients differ from normal control subjects in failing to excrete an increased amount of 5-HIAA after oral administration of large doses of L-tryptophan. Layton (*3*) found that 20 percent of a hospitalized group of schizophrenics excreted less 5-HIAA than any of the normal subjects he encountered. He suggested that 5-HIAA excretion might be used as a biochemical basis for separating a subgroup of schizophrenics.

We have attempted to repeat these findings of Zeller (*1*) and Layton (*3*) in 16 healthy male schizophrenic patients who had been living in the wards of the National Institutes of Health for at least 2 months under the same conditions and on approximately the same diet as six nonschizophrenic volunteers who served as controls. Five grams of L-tryptophan suspended in 200 ml of orange juice were administered orally. The amounts of 5-HIAA excreted in the urine (*4*) during the following three 2-hour intervals (total of 6 hours) were compared with the amounts excreted over the same time intervals during the previous day, when only orange juice had been fed. No significant differences in the rate of 5-HIAA excretion were noted between the schizophrenic and control groups before or after administration of tryptophan. Both groups showed similar increases in the rate of 5-HIAA excretion after administration of tryptophan (Table 1).

Weissbach *et al.* (*5*) demonstrated that serotonin levels in tissue are decreased in pyridoxine-deficient chicks. 5-Hydroxytryptophan decarboxylase activity is reduced in the vitamin B_6 deficient rat (*6*). An attempt was therefore made to relate pyridoxine deficiency to 5-HIAA excretion. Eight male schizophrenic patients who had been long-term feeding problems were studied at the Spring Grove State Hospital (*7*). The rate of 5-HIAA excretion was determined before and after tryptophan loading in the same manner as described for the first study. Following this the patients received 50 mg of pyridoxine hydrochloride orally, three times daily for 5 days. The rate of 5-HIAA excretion before and after tryptophan loading was again determined (Table 1). The rate of excretion of 5-HIAA before tryptophan loading in the feeding-problem group seemed lower than that in the well-fed group of patients and was possibly related to protein and tryptophan ingestion ($p = .10$). Dietary deficiency of protein or tryptophan may therefore explain the findings of Layton (*3*). The increase in excretion of xanthurenic acid following the tryptophan load was used in the estimate of the degree of pyridoxine deficiency (*8*). This was not related ($r = .144$) to the rate of 5-HIAA excretion, and pyridoxine administration did not affect this rate. After administration of tryptophan, the rate of excretion of 5-HIAA by the feeding-problem group of schizophrenic patients increased, just as it did in the well-fed schizophrenic and control groups.

IRWIN J. KOPIN
Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland

References and Notes

1. E. A. Zeller, J. Bernsohn, W. M. Inskip, J. W. Lauer, *Naturwissenschaften* 15, 427 (1957); J. W. Lauer, W. M. Inskip, J. Bernsohn, E. A. Zeller, *A.M.A. Arch. Neurol. Psychiat.* 80, 122 (1958).
2. D. W. Woolley and E. Shaw, *Brit. Med. J.* 2, 122 (1954); *Proc. Natl. Acad. Sci. U.S.* 40, 228 (1954).
3. G. B. Layton, *Brit. Med. J.* 2, 1136 (1958).
4. S. Udenfriend, E. Titus, H. Weissbach, *J. Biol. Chem.* 216, 499 (1955).
5. H. Weissbach, D. F. Bogdanski, B. G. Redfield, S. Udenfriend, *ibid.* 227, 617 (1957).
6. J. Buxton and H. M. Sinclair, *Biochem. J.* 62, 27P (1956).
7. These patients were made available for study through the courtesy of Dr. A. A. Kurland.
8. H. S. Glazer, J. F. Mueller, C. Thompson, V. R. Hawkins, R. W. Viller, *Arch. Biochem. Biophys.* 33, 243 (1951).

26 January 1959

Seasonal Growth Periodicity of Tissue Explants from Woody Perennial Plants in vitro

Abstract. Secondary phloem explants removed to standard aseptic culture in the spring proliferate most extensively. Explants taken successively through the growing season proliferate less. The decreasing growth trend reverses some months before bud break, and the increasing growth in winter is not dependent on the breaking of dormancy in the terminal buds.

The growth of perennial plants is markedly periodic. Growth activity in the meristems of the shoot, which commences in the spring with the onset of warmer weather, ceases in the summer while external conditions are still favorable for growth. Developmental periodicity is accompanied by changes in auxin or growth-inhibitor content and can be regulated to some extent by variations of day length. Explanations of periodic growth activity have noted such changes (*1*). However, the physiological basis of growth periodicity remains obscure.

Sterile culture techniques which have been applied so successfully to problems of tissue and organ growth have scarcely been used in the study of growth periodicity (*2*), and the study described in this report was an attempt to apply such methods to this problem (*3*). The approach adopted was that differences in growth potential of tissues excised from donor plants at different times of the year should be reflected either as quantitative or qualitative differences of growth in sterile culture.

Data on the growth of tissue explants in culture are being accumulated for 11 species. These are: *Ginkgo biloba,* four diffuse-porous dicotyledons (*Salix babylonica, Populus nigra* var. *italica, Syringa vulgaris,* and *Acer rubrum*), and six ring-porous dicotyledons (*Ailanthus glandulosa, Fraxinus americana, Catalpa bignonioides, Robinia pseudoacacia, Quercus alba,* and *Q. borealis* var. *maxima*). Secondary phloem was selected as the test tissue because of its functional and morphological uniformity and its known ability to proliferate in sterile culture.

The experimental procedure was as follows. At intervals of about 6 weeks a branch 2 to 3 cm in diameter was collected from each species. Short pieces of the branches were surface sterilized, an aseptic surface of secondary phloem was exposed, and rectangular explants were removed. The average-sized explant was 4 by 12 mm, and the fresh weight was between 30 and 100 mg for most species. Twelve explants from each species were inoculated apical end down, to take ad-

vantage of the polar movement of auxin, into each of three media. The simplest medium contained inorganic salts (*4*), trace elements (*4*), and 4 percent sucrose and was solidified with 0.8 percent agar. The second medium contained in addition to these constituents a mixture of vitamins (*5*) and naphthalene acetic acid (NAA) (0.5 mg/lit.). A third medium contained all of these constituents with the NAA concentration reduced to 0.05 mg/lit. and 15 percent autoclaved green coconut milk. The explants were grown in culture for 5 weeks in 12-hour days at 25°C. Approximately 3 percent of the cultures were discarded as contaminated by microorganisms.

Explants of all species proliferated in culture. However, the ability of the explant cells to divide at different times of the year, the amount and morphology of the callus formed, and the relative growth made on the different media varied considerably from species to species.

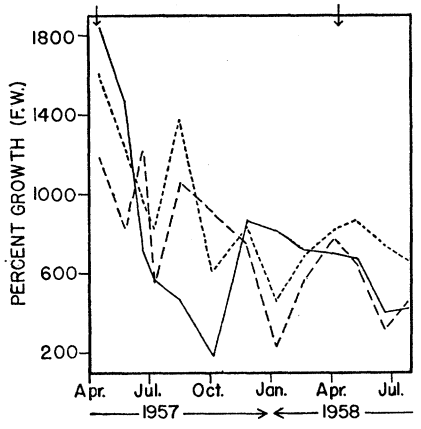

Fig. 1. Final fresh weight of lilac tissue explants cultured at different times of the year calculated as percentage of growth. The dotted line represents the average weight of explants cultured on the simplest medium, the dashed line the weight on the vitamin medium, the solid line the weight on the coconut milk medium. The arrows at the top indicate the approximate date of bud break in the donor plant.

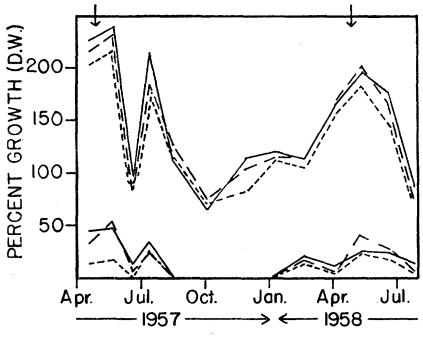

Fig. 2. Final dry weight of maple tissue explants cultured at different times of the year calculated as percentage of growth. The three upper curves represent the average weight of the entire explants, the three lower curves the average weight of proliferated callus. The identification of the lines and arrows is the same as that in Fig. 1.

Explants of lilac proliferated throughout the year and most extensively of the species studied (Fig. 1). Maximum growth on all media, as indicated by increase of fresh weight, dry weight, and size, was obtained with explants removed from the donor plant near the time of bud break in the spring; tissues removed progressively later in the summer proliferated less, but there are unexplained fluctuations in the data for 1957. The coconut milk medium which had supported the highest level of growth in the spring was highly inhibitory to the growth of late-summer explants. On all media the decreasing growth trend was reversed several months before bud break so that there was a gradual increase toward the spring peak. The final fresh weight of the tissues for the spring of 1958 was approximately half that for the spring of 1957, but the dry weights were almost identical. At present there is no adequate explanation for the variation in growth for these 2 years. It may be suggested, however, that weather differences between the years 1957 and 1958 underlie the growth variations, and it is hoped that data to be collected during 1959 will aid in the interpretation of these apparently conflicting results. Other differences in the callus formed at different times of the year included the ratio fresh weight: dry weight and xylem formation, which decreased through the growing season.

Seven other species proliferated through all or most of the year and showed growth periodicity like that of lilac. Explants of the species *Acer* and *Quercus* proliferated during shorter periods. Only those explants of maple removed between February and August proliferated in culture, most callus being formed near bud break time, and for several months before proliferation occurred successive explants showed increases in weight (Fig. 2). To determine whether this winter growth trend was related to the breaking of physiological dormancy in the terminal buds, shoots were brought indoors each time cultures were established. No vegetative buds opened in any of these tests.

These results indicate a periodicity in the growth potential of tissues from perennial species cultured in vitro. In all species most growth occurs near the time the vegetative buds open and diminishes through the summer. Developmental differences in explants removed during the growing season may account for some part of the observed growth trend. However, from the time the vascular tissues mature in late summer successive explants are anatomically identical. Growth differences in culture would then indicate physiological differences in the tissues. Of particular interest is the winter period of increasing growth which occurs at a time when the terminal buds are still in a state of physiological dormancy. Secondary phloem tissue does not exhibit a period of dormancy like that of the buds, but it does exhibit growth periodicity. Tissue-culture methods appear to be well suited to the study of this problem.

IAN M. SUSSEX
MARY E. CLUTTER
Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania

References and Notes

1. R. M. Smith, *Ann. Rev. Plant Physiol.* 5, 183 (1954).
2. D. Siminovitch and A. P. J. Chater, *The Physiology of Forest Trees* (Ronald Press, New York, 1958), p. 219.
3. This study was supported by a grant from the National Science Foundation.
4. T. A. Steeves and I. M. Sussex, *Am. J. Botany* 44, 665 (1957).
5. P. R. White, *The Cultivation of Animal and Plant Cells* (Ronald Press, New York, 1954), p. 74.

13 October 1958

Parthenogenesis in Culex fatigans

Abstract. Parthenogenesis occurs in the mosquito *Culex fatigans*. Three larvae were hatched from 85,851 eggs in tests of eggs from 1001 females. There were 618 egg rafts; the three larvae were from different rafts.

Parthenogenesis in mosquitoes has been recently reported in two species, *Culex molestus* (*1*) and *Aëdes aegypti* (*2*). Some puzzling results of species crosses in the genus *Aëdes* may possibly be explained by parthenogenesis (see *3*), and it is possible that this phenomenon may be more widespread than is presently suspected.

This paper reports parthenogenesis in a strain of *Culex fatigans*, derived from the Galveston strain in February 1948 and maintained by inbreeding since that date.

During 1957 and 1958 females of this species were tested for parthenogenesis. Single pupae were sexed and then isolated in shell vials. After hatching, the females were checked visually, then etherized and examined under a dissecting microscope. Thus triply checked for sex, the females were placed in a cage (40 cm on a side) and allowed to feed on pigeons. After a blood meal some *Culex* females will lay unfertilized eggs,