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.

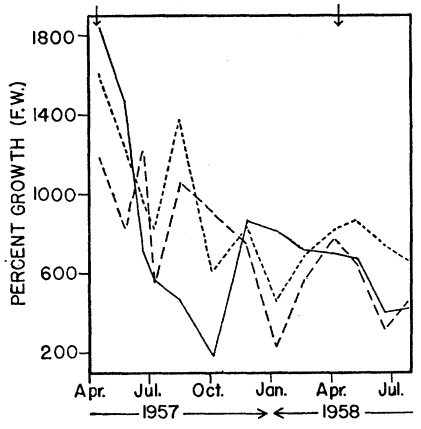

Fig. 1. Final fresh weight of lilac tissue explants cultured at different times of the year calculated as percentage of growth. The dotted line represents the average weight of explants cultured on the simplest medium, the dashed line the weight on the vitamin medium, the solid line the weight on the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

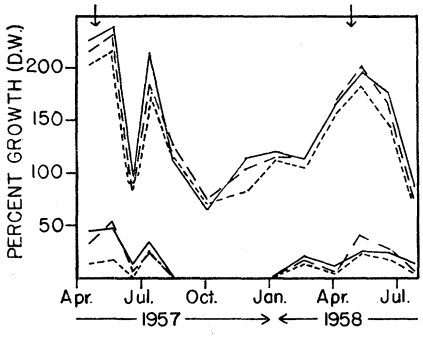

Fig. 2. Final dry weight of maple tissue explants cultured at different times of the year calculated as percentage of growth. The three upper curves represent the average weight of the entire explants, the three lower curves the average weight of proliferated callus. The identification of the lines and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

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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,

although not all females will do so. Eggs were collected daily, observed carefully for 4 days, then discarded. Fertilized eggs normally hatch, under laboratory conditions, 24 to 48 hours after deposition.

During these experiments 1001 females laid 618 egg rafts containing 85,851 eggs. Three first-stage larvae hatched. The three hatched larvae were in three different rafts; thus, in each case only one of approximately 140 eggs hatched. It cannot be argued, therefore, that a male had somehow escaped detection, for if fertilization had in fact occurred, all or nearly all of the eggs in the raft should have been fertile.

All three larvae were weak. One survived the first molt but died soon thereafter; the other two died a few hours after hatching.

Species crosses in *Culex* (*4*) usually produce some eggs which are fertile but which fail to hatch. Such eggs, if sufficiently developed, can be identified under the dissecting microscope. A sampling of 150 egg rafts (containing an average of 125 eggs per raft) from the parthenogenesis experiments failed to reveal any such fertile but unhatched eggs.

Parthenogenetic development, therefore, apparently does occur in *Culex fatigans,* but the frequency of such occurrences is low (*5*).

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16 October 1958

Selection of Auxotrophic Bacterial Mutants by Tritium-Labeled Thymidine

Abstract. A method is proposed for the efficient isolation of auxotrophic mutants of cells of diverse origin by the use of radioactive materials. An example is described in which mutants of *Escherichia coli* B were selected by growing irradiated cells in the presence of tritium-labeled thymidine.

Bacterial mutants with specific nutritional requirements have been used extensively in biochemical and genetic studies. These auxotrophic mutants are ordinarily selected with efficiency by incubation of a mixed culture in minimal medium with penicillin. The growing wild-type are killed, and the dormant mutant cells are spared (*1*).

Many cell species which can be grown in culture are not sensitive to penicillin. No convenient method exists, with few exceptions, for selecting auxotrophic mutants of these cell species (*2*). It seemed possible that an alternative approach might be provided by radioactive materials, such as tritium-labeled thymidine (thymidine-H^3) of high specific activity. Growing cells of both plants and animals incorporate thymidine into deoxyribonucleic acid (DNA) (*3*), and localization of thymidine in the nucleus has been demonstrated by autoradiographs of thymidine-H^3 (*4*). The addition of thymidine-H^3 to HeLa cells growing in tissue culture has recently been shown to decrease viability by as much as 50 percent (*5*).

This report (*6*) describes an initial test of the proposed method in a system which has been studied extensively. First a reconstruction experiment was done with wild-type *Escherichia coli* B and a histidine auxotroph. These were grown separately on *enriched* medium [medium A (*7*) supplemented with 0.2 percent Difco yeast extract and 0.2 percent Sheffield tryptic casein hydrolyzate]. After washing, a suspension of each was placed in 1 ml of *minimal* medium A to which 0.1 ml of thymidine-H^3 was added (*8*). After dilution, the activity of tritium was 40 μc/ml, and the concentration of carrier thymidine about 4 μg/ml (*9*). The cells were incubated at 35°C for 3.5 hours, by which time the count of viable wild-type cells per milliliter had increased from 10^6 to 10^7. Half of each sample was removed, and the remainder was reincubated. After an additional 2.5 hours, wild-type had increased to $5 \cdot 10^7$. The number of histidine auxotrophs was not significantly changed by incubation. A control without thymidine-H^3 was included. After incubation, all samples were stored at 5°C.

Table 1. Decrease in viability after growth in the presence of tritium-labeled thymidine.

Tube No.	Thymidine-H^3 (μc/ml)	Duration of incubation (min)		Viable bacteria after cold storage (No./ml)		
		Before addn. of thymidine-H^3	After addn. of thymidine-H^3	0 days	7 days	13 days*
1	45	0	130	$6.6 \cdot 10^7$	2,600	190
2	45	45	85	$7.5 \cdot 10^7$	1,800	340
3	45	90	40	$6.0 \cdot 10^7$	3,000	210
4	15	0	130	$16.0 \cdot 10^7$	14,000	5200
5	15	45	85	$9.6 \cdot 10^7$	6,600	2100
6	15	90	40	$9.0 \cdot 10^7$	17,000	2400
7	0	130		$11.0 \cdot 10^7$	$3.0 \cdot 10^6$	
8	0	130		$15.0 \cdot 10^7$	$1.4 \cdot 10^6$	

* In all samples at 13 days, the fraction of mutant colonies exceeded 50 percent.

Viability was determined at intervals by plating appropriate dilutions on enriched agar plates. In the absence of thymidine-H^3, the viability of both the wild-type and the mutant fell to approximately 10 percent during 6 days of cold storage. After incubation with thymidine-H^3 the mutant showed a similar decrease, but the count of wild-type fell much more sharply.

The fraction of mutants surviving, divided by the fraction of wild-type surviving, can be expressed as an enrichment ratio. Without thymidine-H^3 this ratio was approximately 1. For the tubes incubated with thymidine-H^3 for 3.5 hours, the enrichment ratio at the end of 6 days of storage was about 4000. In the samples incubated with thymidine-H^3 for 6 hours, the ratio was only 70.

In a mutant isolation experiment, wild-type *E. coli* B were treated with ultraviolet radiation to approximately 1 percent survival. One-half of one milliliter was added to 5 ml of enriched medium and incubated for 24 hours. The cells were washed, diluted by 10^3, and 2.5 ml of the suspension placed in each of eight tubes. The amount of thymidine-H^3 and the time of addition were systematically varied, as is shown in Table 1. During the incubation period of 130 minutes, two to three divisions occurred. The tubes were then stored at 5°.

In this experiment, survival of bacteria in the tubes lacking thymidine-H^3 was 1 to 3 percent after 7 days at 5°C. In the presence of thymidine-H^3, survival was about 1/100 to 1/1000 as great. Each tube was carefully kept cold when samples were removed. After 7 days a search by the replica plating technique (*10*) showed that mutants were present at a frequency of 5 to 10 percent. At 13 days, the yield of mutants was above 50 percent, and in a few cases as high as 90 percent. Although killing of bacteria was greater with 45 μc (tubes 1 to 3) than with 15 μc (tubes 4 to 6), the fractions