produced aerobically and anaerobically by mating types varies with the individual presumed unisexual polyploid. Both sexes 7 and 13 gave high yields, indicating that invertase is not the specific protein reported by Brock to take part in the sexual agglutination reaction of *Hansenula wingei*. It is to be expected that in addition to the extracellular enzyme, much invertase is bound to the surface of the cells (*6, 9*).

The isolates designated as code 3 and code 26, of opposite sex, are available from this laboratory. They may be used to demonstrate sexual agglutination and consequent easy harvesting of yeast cells from liquid media. The mating types are grown separately for a few days on YM slants, being transferred daily, and then grown for 48 to 96 hours in shaken flasks at about 28°C in 3 percent glucose or sucrose YM medium, or other liquid media suitable for yeast. The cultures are mixed and sexual agglutination immediately occurs, with rapid settling of the large clumps of agglutinated cells. Codes 3 and 26 are stable. They were transferred daily on YM slants, except for weekends, for 2 and 3 months, respectively, without monitoring, and at the end of this time they gave high yields of invertase (Table 1).

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16 November 1959

Mayaro Virus Isolated from a Trinidadian Mosquito, Mansonia venezuelensis

Abstract. A strain of Mayaro virus has been isolated in Trinidad from the mosquito *Mansonia venezuelensis*. This is the first record of isolation of this agent from naturally infected mosquitoes, caught in the wild.

Mayaro virus was first isolated in 1954 from the blood of a human being working in a forested area of southeastern Trinidad (*1*). The original report recorded the finding of this new agent during August and September in five persons widely scattered over the island. With the exception of the isolation reported here, there had been no further recoveries of this virus in Trinidad through September 1959. However, a survey conducted in 23 representative localities throughout the island has shown that 11 percent of a human population of 615 possess neutralizing antibodies for Mayaro, with localization in southeastern Trinidad, where rates as high as 48 percent were encountered (*2*).

Table 1. Biological properties of two strains of Mayaro virus and of Semliki Forest virus.

Pathogenicity for adult mice	Hemagglutinating antigen from mouse brain	Growth in chick embryo tissue culture
	Semliki Forest	
Highly pathogenic by intracerebral route	Yes	Multiplication and cytopathogenic changes
	Mayaro TRVL 4675	
Not pathogenic by intracerebral route	No*	No multiplication or cytopathogenic changes
	Mayaro TRVL 15537	
Moderately pathogenic by intracerebral route	Yes	Multiplication but no cytopathogenic change

* No hemagglutinating antigen results from acetone-ether extraction of baby mouse brain, but an antigen can be prepared by the sucrose-acetone method.

Mayaro virus is also found in the Amazon valley of Brazil, where it is associated with human illness (*3*), and immunity surveys indicate its presence in the Rupununi savannah and Mazaruni River regions of British Guiana (*2*). We present in this report the first record of the occurrence of this agent in a naturally infected arthropod.

Limited laboratory evidence indicates that mosquitoes are capable of harboring the virus for at least 12 days, and that on one occasion virus was transmitted by the bite of *Aëdes scapularis* (*4*).

Mayaro virus was not recovered from arthropods in this laboratory during 1955 and 1956, although well over 200,000 specimens were ground and inoculated into baby mice. Prior to 1955 this agent would have escaped attention in the entomological work, since only adult mice were then used to receive the original arthropod inocula. Not until March 1957 were we successful in isolating this virus from naturally infected forest mosquitoes. The isolation reported here is the sole Mayaro isolation from mosquitoes, despite the fact that 401,578 mosquitoes were examined in the interval from March 1957 to October 1959.

Mayaro virus was isolated in baby mice inoculated on 28 March 1957 with a suspension from a pool of 49 *Mansonia venezuelensis* (TRVL 15537). These mosquitoes had been taken over a period of 12 working days, between 11 and 27 March, and stored daily as whole insects in sealed ampules at −60°C. They were collected while attempting to bite human beings on the forest floor at our Rio Grande Forest tree station about 7 miles north of Sangre Grande in northeastern Trinidad.

The virus was isolated from the brains of baby mice inoculated intracerebrally with a suspension of this *Mansonia* pool; the agent was established in baby mice and was shown to be filtrable. The virus was reisolated from the original mosquito suspension, both in baby mice and in hamster-kidney tissue culture.

This virus from *Mansonia* is indistinguishable from Mayaro virus (TRVL 4675) by complement-fixation, hemagglutination-inhibition, and neutralization test techniques. Several interesting biological differences among TRVL 4675, TRVL 15537, and Semliki Forest viruses are presented in Table 1 (*6*).

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18 November 1959

Effect of Kinetin on Paramecium caudatum under Varying Culture Conditions

Abstract. When kinetin (1 mg/liter) is added to hay infusion medium, the generation time of *Paramecium caudatum* is shortened immediately upon transfer of the protozoa from stock to isolation culture. Kinetin is particularly effective when culture conditions are suboptimal, perhaps because it substitutes for or supplies some factor which becomes limiting after transfer.

In a previous report, increased rates of cell division in *Paramecium caudatum* were reported after addition of low doses of kinetin (6-furfuryl amino purine) to the culture medium (*1*). In subsequent tests with a new clone of *Paramecium* and new preparations of

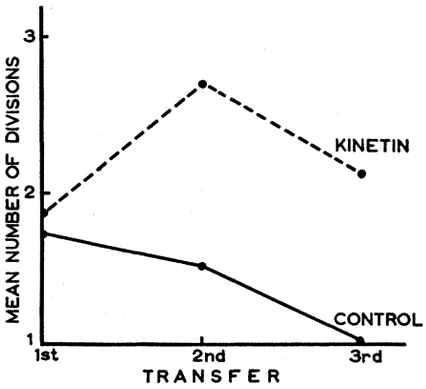

Fig. 1. Cell division in *Paramecium caudatum* under suboptimal culture conditions. The influence of kinetin on division frequencies after each of three daily transfers to new medium.

kinetin, cell division frequencies were also increased, but on a consistently lower level. Because of this lack of reproducibility, our earlier data have been reanalyzed, and these, together with the preliminary results of additional experiments, are presented here.

The results reported in the earlier paper (*1*) were of daily division rates based on the mean of three successive transfers. With 1 mg of kinetin per liter, the ratio of mean divisions in kinetin: mean divisions in control medium ($\bar{k}/\bar{c}$) was 1.45 for the 3-day period, which is highly significant. But when, subsequently, division frequency was recorded for each day separately, an entirely different picture was obtained. Figure 1 shows the original results, separated into the three daily transfers. Kinetin significantly increased the division rate even after the first transfer, but only by 10 percent. The high $\bar{k}/\bar{c}$'s of the following two days (1.78 on the second and 2.19 on the third) were due to two factors: (i) a rising division frequency in the kinetin-containing medium over the 3-day period and (ii) a drop in cell multiplication in the control medium, particularly after the second transfer.

Division of the protozoa after transfer is known to depend on such variables as the amount of food in the new medium, the condition of the transferred animal, the number and kind of bacteria transferred along with the paramecium, and other factors. Depression of division after transfer from stock solution was discussed in an early paper by Chejfec (*2*), while a detailed analysis of the effects of varying culture conditions may be found in a book by Wichterman (*3*).

Multiplication frequencies in our original experiments were low—never more than two divisions in 24 hours. One may assume that some factor necessary for division was suboptimal in these cultures and that this factor was increasingly diluted during the first three transfers. Unfortunately all experiments were terminated after 3 days, and no data are available on whether or when an equilibrium may have been reached.

The following series of tests were conducted a year later, with a new clone of *Paramecium caudatum*, on infusion made from a new batch of hay, and with two new samples of kinetin (California Biochemicals and Waldhof). In these cultures, division frequency per 24 hours was always above 2 and sometimes as high as 5. The experimental procedure was the same as in the previous tests, but daily transfers were made for 6 days. The data given in Fig. 2 are the means of results from ten separate series of tests. In these experiments there was no significant drop in division frequency in the control group. The daily k/c's varied from 1.07 to 1.11, each k being significantly above c at the 5 percent level. Thus even under near-optimal conditions of culture, kinetin could shorten the interdivision period—in these experiments from an average of 8.4 hours in controls to an average of 7.7 hours in kinetin. The constancy of the k/c's over the 6-day period indicates that kinetin does not accumulate in the protozoan, either to increase its stimulatory effect with time or, conversely, to reach an inhibitory level.

A subsequent experimental series was designed to ascertain the presence—or absence—of a stimulatory effect of 1 mg of kinetin per liter during the latter half of the interdivision period. Sixty paramecia were isolated from stock solution, randomized, and divided into groups of 30. One group was placed (singly) in control medium, the other in kinetin-containing medium. After 5 hours' incubation, the number of individuals in each group was recorded. This series was replicated five times. Generation time in these cultures (as ascertained by routine examinations) was approximately 12 hours. However, at the end of the 5-hour period only 5.33 percent of the controls had divided, while 12.67 percent of the paramecia transferred to kinetin-containing medium had completed cell division. The t-value for the difference between divisions in control and divisions in kinetin medium was found to be significant at the 0.01 level. These data indicate that kinetin may be of aid in overcoming the lag period following transfer from stock culture to depression slide, perhaps again by providing or substituting for some factor that is limiting under these conditions.

Finally, it should be pointed out that several samples of kinetin have been found to become toxic after about a year, even though they have been tightly closed and refrigerated. Also, bioassays of equal doses of California Biochemicals and Waldhof kinetin have given dissimilar results, the German preparation having a lower stimulatory action. Lack of uniformity in the effects of kinetin on microorganisms has also been reported by Braun (*4*), who ascribes some of the variability to an influence of high levels of amino acid and trace metal on the action of kinetin (*5*).

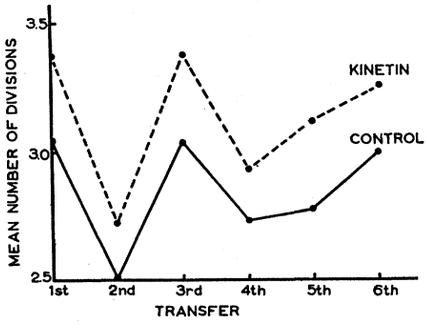

Fig. 2. Cell division in *Paramecium caudatum* under favorable culture conditions. The influence of kinetin on division frequencies after each of six daily transfers.

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Detection of an Anaplasma marginale Antibody Complex Formed in vivo

Abstract. A naturally occurring anaplasma-antibody complex was detected by exposing erythrocytes of infected cattle to fluorescein-labeled bovine antiglobulin. This technique revealed not only the classical marginal bodies but also the initial *Anaplasma* bodies in the erythrocytes of acutely infected animals. Singly occurring initial bodies were observed in the erythrocytes of healthy carriers.

Detection of an antigen in Weller and Coons' indirect fluorescent antibody method is accomplished by exposure in vitro to unlabeled antiserum derived from naturally infected animals or to antiserum produced by rabbits after inoculation with the antigen, and subsequent treatment with fluorescein-labeled antiglobulin (*1*). A modification of this technique was described by Mellors (*2*), who demonstrated histological