Attachment of cells in mitosis has been particularly tenuous. Colchicine solutions (0.4 μg/ml) appeared to intensify dislodging and this necessitated modified methods of chromosome counting to prevent loss of mitotic cells. Colchicine was added to media in tubes showing active mitosis; they were incubated for 2.5 hours, then each received an equal volume of NaCl-free Earle's BSS.

After 15 minutes at 25°C, the cells were scraped free and concentrated by centrifugation at 200*g* at 4°C for 10 minutes. The medium was smoothly decanted, two drops of aceto-orcein were added with mixing, and squash preparations were made immediately. This procedure prevented loss of dislodged mitotic cells.

With colchicine, a modal number of 42 chromosomes was found among 65 cells in 46th to 51st passage. The range of chromosome numbers was 24 to 79, the mean being 44, and 70 percent of the counts being in the group 44 ± 3. Since the diploid number for this species is 26, the cells are near-triploid (Fig. 2). The mean for 15 counts made without colchicine was 42, and this was considered ample evidence for intrinsic heterploidy, the usual condition found in established cell lines.

The FT cells are large, having a mean diameter of 25 microns for spherical cells, with the range being 10 to 42 microns. They do not support growth of IPN virus, the agent of infectious pancreatic necrosis of trouts (*7*), and this feature has been used as part of a method for presumptive identification of this virus.

The FT cells are readily preserved by freezing at −80°C, but they have not been cloned.

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Plasmodium berghei: Cyclical Transmissions by Experimentally Infected Anopheles quadrimaculatus

Abstract. *A number of strains of* Plasmodium berghei *were isolated from sporozoites of* Anopheles dureni. *Laboratory-bred* Anopheles quadrimaculatus *fed on carriers of the newly isolated strains showed overwhelming midgut infections and moderate or mild salivary gland infections. Successive cyclic transmissions by the bite of experimentally infected* A. quadrimaculatus *in laboratory-bred tree rats* (Thamnomys surdaster) *were carried out.*

Within the biocene of certain forest galleries of Upper Katanga in the Congo, a sylvatic mosquito, *Anopheles dureni* Edwards, transmits *Plasmodium berghei* (*1*) among tree rats (*Thamnomys surdaster*).

Sporozoites in the salivary glands of wild-caught *A. dureni* were recorded by Vincke and Lips (*1*), and Vincke (*2*) in 1946. These sporozoites obtained in mosquito dissections during the rainy season (December to March) served for the isolation of a number of strains of *P. berghei* in the last 15 years. However, all these plasmodial strains were induced by syringe inoculation of harvested sporozoites. No cyclical transmission by the direct bite of a naturally or experimentally infected *A. dureni* has ever been recorded. The few successful transmissions of *P. berghei* with other unrelated anopheline species originated from sporozoites of crushed mature oocysts inoculated into experimental rodent hosts (*3*).

We now report successive cylical transmission of *P. berghei* in the laboratory by the bite of experimentally infected *Anopheles quadrimaculatus.*

During the period from the 20 December 1963 to 3 January 1964, 2300 engorged *A. dureni* were caught in the forest gallery of Kisanga in the vicinity of Elisabethville. The mosquitoes were kept at 22°C in small cages within cooling containers with the relative humidity at 90 percent. These climatic conditions resemble those of the natural environment at the height of the transmission season and of mosquito infections in nature. The *A. dureni* were brought by plane to our laboratories in New York and kept under similar conditions in an insectary (*4*). Dissection of the weak and dying mosquitoes revealed a high incidence of infection, there being 24 percent oocysts in the midguts (81 positive of 334 dissected) and 10.5 percent sporozoites in the salivary gland (35 positive of 334 dissected).

Twelve new strains of *P. berghei* were isolated from these sporozoites by intraperitoneal inoculations into laboratory-bred *Thamnomys* (*5*), Syrian golden hamsters, young albino rats, and white mice. The plasmodial strains, each derived from different naturally infected *A. dureni,* were designated as N.K. (New York-Kisanga) strains, maintained by blood transfer and preserved at low temperatures (*6*). Incubation periods, from sporozoite inoculation to the appearance of parasites in the blood, varied from 72 hours to 8 days in the infected animals.

Batches of laboratory-bred *A. quadrimaculatus* were allowed to feed on mouse number M2293 on the third day of its patent blood infection after inoculation of the sporozoites from *A. dureni* (N.K.$_1$). The engorged mosquitoes were kept at 22°C. Mature oocysts developed within 8 days after the infective blood meal, but sporozoites in the salivary glands were encountered only from the 13th day onward. Of 36 dissected *A. quadrimaculatus,* 28 (77 percent) showed very heavy (200 to 400) oocyst midgut infections, 12 (33 percent) showed light or medium salivary gland infections.

The remaining 21 mosquitoes were divided into two groups; they were allowed to engorge on two laboratory-bred *Thamnomys,* BTR-65 and BTR-67. Parasitemia ensued in both these animals, 4 and 7 days respectively, after the bite. Three further successive cyclical transmissions by the bite of infected *A. quadrimaculatus* in laboratory-bred tree rats have thus far been carried out. These cyclical passage strains were designated as N.K.$_4$BI, N.K.$_4$BII, N.K.$_4$BIII, and N.K.$_4$BIV.

Another sporozoite isolated strain (N.K.$_1$) has been successfully transmitted through three cyclic generations

by the bites of *A. quadrimaculatus* in Syrian golden hamsters (*Mesocricetus auratus*).

We are endeavoring to continue the successive cyclic transmission of these strains in an attempt to adapt *P. berghei* for regular sporozoite-induced infections under laboratory conditions.

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Interferons: Selectivity and Specificity of Action in Cell-Free Systems

Abstract. *The inhibition by interferons of viral replication was studied with cell-free preparations of the enzyme RNA synthetase derived from calf kidney cells infected with influenza virus and from chick embryo fibroblasts infected with Newcastle disease virus. Calf, mouse, and chicken interferons inhibited these enzymes with the same characteristic species specificity observed in tissue culture. A mechanism of action is proposed.*

Infection of an organism or tissue by a virus may be resisted in several ways; one of these is the production by the infected tissue of a substance that interferes with the multiplication of the virus. This substance, a protein of low molecular weight, is called interferon; it was discovered and purified by Isaacs (*1*), and other workers (*2*). Among its unusual properties is its species specificity (*3*), that is, interferon derived from a virus-infected tissue is more effective in protecting a homologous than a heterologous tissue. Accordingly there appear to be many interferons, the activity of each seeming to depend on the tissue from which it was derived rather than on the particular virus which elicits its production.

The mechanism of action of interferon has been studied to elucidate the site or sites at which viral multiplication is inhibited (*3*). Since interferon inhibits replication in cells exposed to either whole virus or infectious viral RNA (*4*), it appears that interferon does not block virus adsorption, penetration, or uncoating of the nucleic acid (*5*). That interferon does not merely inhibit the release of mature virus was demonstrated by the experiments of Ho and Enders (*6*). Infected tissue treated with interferon has been disrupted by various methods, and unlike the cases in control cultures, no excess intracellular infectious material has been found.

Cocito *et al.* (*7*) have shown that interferon can inhibit viral RNA synthesis in tissue culture cells infected with virus but does not affect the incorporation of radioactive orthophosphate into both DNA and RNA of uninfected control cells. Further, Lockart *et al.* (*8*) have shown that after treatment with interferon, the production in vivo of both virus and infectious RNA ceases abruptly, simultaneously, and to the same degree. The main action of interferon would, therefore, appear to be the inhibition of viral nucleic acid synthesis; however, it has not been possible to demonstrate without question whether interferon affects assembly of mature virus particles or virus-associated protein synthesis. Further, if there are effects, it is not known whether or not they are secondary to the nucleic acid response (*7–9*).

Infection of cells in tissue culture with an RNA-containing virus induces the formation of an RNA-synthesizing enzyme, RNA synthetase, not found in normal noninfected tissue. This enzyme has been isolated from calf kidney cells infected with influenza virus and from chick embryo fibroblasts infected with Newcastle disease virus (*10, 11*). Similar preparations have been isolated from various tissues infected with other RNA-containing viruses (*12*).

Since intracellular replication of viral nucleic acid has been implicated in the mechanism of action of interferon in tissue culture cells, it should be possible to demonstrate an effect of interferon on the synthesis of viral nucleic acid in a cell-free system. Further, if this effect is the mechanism of antiviral action of interferon, it should show the

Table 1. Properties of RNA synthetase isolated from calf kidney tissue infected with influenza virus. This incubation mixture contained: Tris buffer, *p*H 7.2, 20, μmole; $MgCl_2$, 1.5 μmole; mercaptoethylamine, 2.0 μmole; phosphoenolpyruvate, 1.0 μmole; pyruvate kinase, 35 μg; ATP, CTP, and UTP, 0.1 μmole each; GTP-8-C^{14} (specific activity, approximately 10^7 count/min μmole), 0.1 μmole; and enzyme, 350 μg in a final volume of 0.1 ml. The mixture was incubated at 37°C for 10 minutes, the reaction was terminated by the addition of 2 ml of 6 percent trichloroacetic acid, and the incorporation was determined in the usual manner (*11*). The activity is expressed as micromicromoles of GMP incorporated in 10 minutes per milligram of protein.

Prior treatment of enzyme (μg/ml)	Test system	Activity ($\mu\mu$mole/mg protein)	Inhibition (%)
	Microsomal fraction from infected cells (10)		
None	Complete	555.0	
Deoxyribonuclease, 20	Complete	392.0	29.0
Ribonuclease, 20	Complete	0.0	100.0
	Microsomal fraction from noninfected cells		
None	Complete	112.0	
Deoxyribonuclease, 20	Complete	25.2	77.5
Ribonuclease, 20	Complete	54.0	52.0
	Microsomal fraction from infected cells		
Deoxyribonuclease, 20	Complete	298.4	
	Minus Mg	236.0	20.9
	Minus ATP regenerating system	11.7	96.1
	Minus ATP	41.0	86.3
	Minus CTP	54.6	81.6
	Minus UTP	60.4	79.8
	Minus ATP, CTP, UTP	56.6	81.0