to develop decreased with increasing temperature. It is noticeable that the same kind of interaction between light and temperature was observed during fruiting of *Coprinus congregatus.*

Many photoresponses to threshold light levels are known for fungi[14] and plants[15]. Many of the requirements are less than the flux provided by moonlight. According to Tansey and Jack[14] and Thorington[16] and using the conversion factor of 1 lx = 4 mW m^{-2} to convert photometric units into units of flux density, the radiant flux density calculated for moonlight was respectively 1.5 and 2.7 mW m^{-2}. It was clear from the present results that at a temperature higher than 20 °C, a radiant flux density far lower than the flux provided by moonlight elicited an inhibitory fruiting response in *Coprinus congregatus.*

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An increase in Sendai virus-induced cell fusion of erythrocytes infected with *Plasmodium chabaudi*

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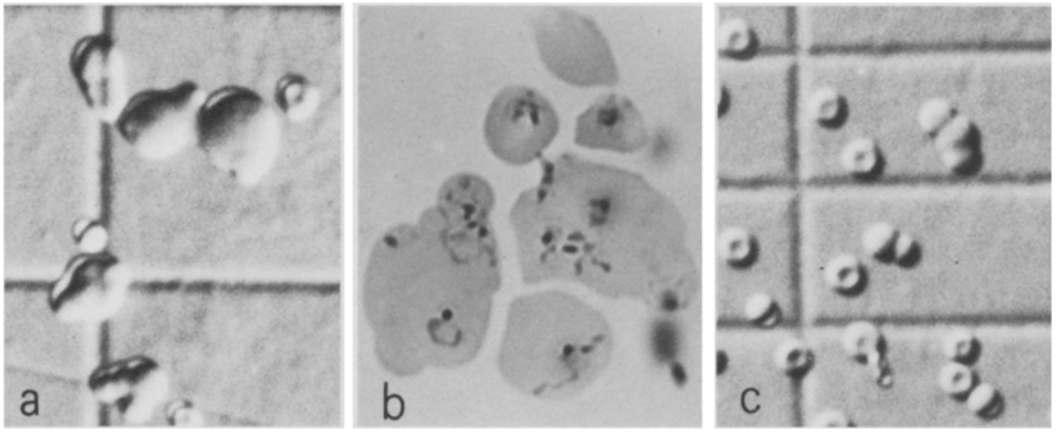
Summary. The extent of cell fusion induced by Sendai virus was examined in erythrocytes infected with *Plasmodium chabaudi.* An increase in cell fusion of erythrocytes with Ehrlich tumor cells and of erythrocytes with erythrocytes was observed with the infected erythrocytes. However, agglutination by the virus was not changed between erythrocytes of normal and malarial mice. These results indicate that the increase in cell fusion occurred in the process of membrane fusion, suggesting that some membrane property of *Plasmodium*-parasitized erythrocytes is changed in terms of Sendai virus-induced cell fusion.

Erythrocytes infected with mammalian malarial parasites have been shown to have a changed membrane structure[2]. Alteration of spectrin and other membrane proteins[3-8] and the appearance in the erythrocyte membrane of parasite-derived proteins[9] have been reported. Changes in protein architecture in the parasitized erythrocytes (PE) are thought to result in the aberrant cell morphology commonly seen in malaria infected blood[3], and in abnormal membrane properties[2]. We investigated whether PE respond differently from normal erythrocytes to an induction of cell fusion by Sendai virus, because it is known that temporal re-organization of membrane architecture is a prerequisite for the virus-induced cell fusion[10-14]. The results showed that PE are more prone to cell-cell fusion.

Materials and methods. Malarial infections were initiated by i.p. injection of 7-week-old male C57Bl/6 mice with 10^6–10^8 *Plasmodium chabaudi* PE. 5 or 7 days after the injection, the mice were anesthetized with chloroform and their blood collected by cardiac puncture with a heparinized syringe (10 units/ml). The plasma of normal or malarial mice was separated after centrifugation of the blood at 650 × g for 5 min and stored at −80 °C. The pellet was suspended in balanced salt solution (BSS: 140 mM NaCl, 5.4 mM KCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4,



Polyerythrocytes formed after induction of cell fusion by Sendai virus at 1000 HAU/ml (a and b) and 250 HAU/ml (c). Nomarski differential interference micrographs (a and c, × 570), and light micrograph (b, × 1300) of Giemsa-stained erythrocytes in which many plasmodial parasites are found.

Sendai virus-induced fusion of erythrocytes infected with *Plasmodium chabaudi**

Source of erythrocytes	No. of mice examined	Parasitemia (%)	Reticulocytosis (%)	Hemolysis (%, ± SE)	Erythrocytes fused (%, ± SE)	p-value
Normal mice	7	-	1.4	40.6 ± 1.0	4.08 ± 0.27	
Malarial mice infected with						
10^6 PE*2	5	14.7	1.5	45.2 ± 2.2	5.34 ± 0.41	< 0.005
10^7 PE	5	40.8	1.1	47.7 ± 3.1	8.65 ± 0.95	< 0.01
10^8 PE	5	58.1	1.8	43.1 ± 2.4	19.63 ± 2.94	< 0.005

*At a virus concentration of 250 HAU/ml. **Parasitized erythrocytes.

buffered with 10 mM Tris-HCl at pH 7.6) containing 2 mM $CaCl_2$ (BSS-Ca^{2+}), and then passed through a cellulose powder column (Whatman, CF11)[15] to remove leukocytes and platelets. The erythrocyte suspension was eluted and then washed 3 times with BSS-Ca^{2+} at 650 × g for 5 min, and the packed cells were diluted to make a 2–50% suspension in BSS-Ca^{2+}. The number of PE, parasitemia, was determined by counting 500 erythrocytes in Giemsa-stained blood smears. Ehrlich ascites tumor cells (ETC) were collected by abdominal puncture of Balb/c mice 7 days after inoculation (i.p.) with 0.2 ml of a 10% suspension of the cells. The cells were washed 3 times and adjusted to a 10% suspension with BSS-Ca^{2+}. The Z strain of Sendai virus[16] was suspended in BSS, and diluted with BSS to various hemagglutination unit (HAU) concentrations. A mixture of 0.1 ml of a 50% suspension of erythrocytes, 0.1 ml of a 10% suspension of ETC, and 0.5 ml of the virus suspension was incubated at 4 °C for 20 min and then at 37 °C for 30 min. Fusion of ETC with erythrocytes was determined by counting the number of ETC containing hemoglobin by the wet benzidine method[17]. To induce fusion between erythrocytes, 0.2 ml of a 10% erythrocyte suspension was mixed with 0.2 ml of the virus suspension, and then incubated as above. The extent of erythrocyte fusion was expressed as the number of erythrocytes fused per 1000 erythrocytes observed with a Nomarski differential interference microscope (× 600, Olympus, Japan). The rate of hemolysis was expressed as the percentage of lysed erythrocytes by counting the number of erythrocytes with a hemocytometer before and after the incubation. The effect of the plasma of infected mice on the fusion was also examined by incubating 0.18 ml of plasma with 0.02 ml of packed erythrocytes for 2 h at 37 °C. The cells were then washed 3 times with BSS-Ca^{2+}, suspended in 0.2 ml of BSS-Ca^{2+}, and cell fusion by the virus induced as described above. Agglutination of erythrocytes by the virus was tested by incubating 0.025 ml of a 2% cell suspension from normal or malarial mice with 0.025 ml of the virus suspension in a microtiter tray for 2 and 24 h at 4 °C, when agglutination titers were read.

Results. PE with 50–70% parasitemia collected 7 days after injection of 10^6 PE were induced to fuse with ETC. Representative results of 5 experiments showed that erythrocytes from malarial mice fused with 63.0% ETC at 100 HAU/ml and 48.3% at 50 HAU/ml, whereas erythrocytes from normal mice fused with 45.3% and 25.6% at these HAU levels. On the other hand, it was found that erythrocytes from malarial mice fused easily to each other at the high virus concentrations (2000–1000 HAU/ml), resulting in large polyerythrocytes (fig. a and b); this was not seen with erythrocytes from normal mice. At low concentrations (250–125 HAU/ml), it was possible to count the number of erythrocytes fused (fig. c), because large polyerythrocytes were rarely seen. Then, the extent of fusion was expressed as the number of erythrocytes fused. PE were harvested from the infected mice 5 days after the inoculation of 10^6, 10^7 or 10^8 PE. By changing the inoculation dose of PE, high or low parasitemia was obtained (table). However, reticulocytosis, a high level of which was seen in the later stage of *P. chabaudi* infection in C57Bl/6 mice[18] and might affect the rate of cell fusion, was not yet elevated on day 5 of the infection. The table shows that an increase in the number of erythrocytes fused was noticed even in erythrocytes with low parasitemia (14.7%), and further enhancement of fusion was observed in erythrocytes with higher parasitemias. The rate of hemolysis after the incubation was at nearly the same level between erythrocytes of normal and malarial mice. Neither was a difference in the agglutination titer of erythrocytes noticed. Since it is known that membrane lipid level, especially that of cholesterol, affects the extent of Sendai virus-induced cell fusion[19], the effect of plasma, whose lipids can be readily exchanged with erythrocyte membrane lipids[20], from normal or malarial mice was compared. No effect of malarial plasma was found on fusion of normal erythrocytes. Normal plasma also had no effect on fusion of erythrocytes of malarial mice.

Discussion. Sendai virus-induced cell fusion involves sequential events at the cell membrane, i.e. agglutination of cells by the virus and membrane fusion. The present experiments showed that erythrocytes parasitized with *Plasmodium chabaudi* were markedly susceptible to induction of cell fusion by Sendai virus. However, agglutination of erythrocytes by the virus did not differ between erythrocytes of normal and malarial mice. We also found no difference in erythrocyte agglutination with Limulin, a lectin which binds specifically to sialic acids, the receptor of Sendai virus (not shown). Thus, it follows that an increase in the fusion of *P. chabaudi* parasitized erythrocytes occurred in the process of membrane fusion.

Recent studies with freeze-fracture electronmicroscopy on membranes after virally-induced erythrocyte fusion have revealed that temporal redistribution and clustering of intramembrane particles (IMP) and exposure of phospholipids devoid of IMP are necessary for the cell-cell fusion[10–12]. The mobility of IMP is known to be controlled by a network of the spectrin-actin complex located at the cytoplasmic surface of the membrane[21,22]. Indeed, spectrin has been shown to participate in Sendai virus-induced cell fusion in erythrocytes. These results show that cell-to-cell fusion requires the re-organization of erythrocyte membrane proteins.

There is some evidence which indicates the alteration of the membrane architecture of erythrocytes infected with *Plasmodia.* Polyacrylamide gel electrophoresis analyses have indicated breakdown or denaturation of spectrin and other membrane proteins of erythrocytes infected with *P. chabaudi*[4,7], with *P. berghei*[3,7], and with *P. knowlesi*[5,6]. This may cause clustering of IMP in erythrocytes infected with *Plasmodium,* which has been demonstrated by McLaren et al.[23].

Thus, these changes may facilitate IMP mobility on the induction of cell fusion by Sendai virus, thereby making PE more prone to cell fusion. Whatever the explanation is, our study supports the concept that membranes of *Plasmodium*-infected erythrocytes are different from normal erythrocytes, and these differences can be detected by an increase in Sendai virus-induced cell fusion.

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Atypical base composition of foldback DNA[1]

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Summary. Salmon, calf and human foldback DNAs all exhibited hypermethylation, reduced adenine plus thymine content and an excess of adenine over thymine when compared with their respective native DNAs. The only unusual feature of the base composition of wheat germ foldback DNA was the excess of thymine over adenine.

Foldback DNA forms a distinct class of repetitive sequences by virtue of its ability to renature instantaneously at low concentrations[3]. Apart from having perfect inverted repeat sequence arrangement of the type ABC ... C'B'A'[4], foldback DNA could contain interspersed single-stranded regions[5,6]. Although foldback DNA occurs ubiquitously[6–11], its biological function remains unknown. Sequence analyses have shown that the recognition site for endonuclease R in *Haemophilus influenzae*[12] and the *lac* operon in *Escherichia coli*[13] are made up of short inverted repeats of 6 and 24 nucleotide length respectively. Heterogeneous nuclear RNA of HeLa cells possesses regions that are self-complementary and are thought to have been transcribed from inverted repeat DNA[14,15].

While it is important to know the nucleotide sequence of specific foldback regions, it is equally important to study the base composition of foldback DNA isolated as a kinetic class. It is possible that the ease of renaturation might be associated with the preponderance of certain bases apart from the inverted repeat nucleotide arrangement. This point would be best clarified by comparing the base compositions of foldback DNAs obtained from a variety of organisms. To date, only rapidly reannealing DNA fractions from rodent tissues have been so analyzed. 5-Methylcytosine (5MC) enrichment was shown in these fractions from Ehrlich ascites carcinoma[7] and mouse mastocytoma[16], and an 8 moles percent of excess of A over T was observed[7]. The broader class of rapidly reannealing DNA in Chinese hamster cells was also shown to be hypermethylated[17]. We report the analyses of the base composition of 4 eukaryotic foldback DNAs, all of which showed minor deviations from Watson-Crick base-pairing.

Materials and methods. All DNAs used were RNA-free. The preparation of foldback DNA from bovine and human DNAs employing nuclease S1-dioxane digestion and hydroxyapatite chromatography (60 °C) has been described[10], and was applied without modification. The preparations of salmon and wheat foldback DNA were similarly obtained except that 40 °C was used as the temperature of nuclease S1 digestion. At least 3 foldback and 6 native preparations of each kind of DNA were analyzed in duplicate. 2–4 mg of native or foldback DNAs were hydrolyzed (70% $HClO_4$, 100 °C, 60 min) and neutralized with KOH.

The bases were separated by descending paper chromatography[18] with the solvent system isopropanol: water: conc. HCl (170:36:44), and located under UV-light with reference to standards. Guanine was eluted with 1 M HCl, and the other bases with 0.1 M HCl, for quantitation with dual wavelength measurements[19]. In order to check for the possible contamination of 5MC fractions with C, eluates obtained from paper chromatograms were dried and reacted with 'Sil-Prep' (Applied Science Laboratories Inc., State College, Penn., USA). Gas chromatography modified from Razin and Sedat[20] was carried out on 1.5% OV-101 column ($5' \times \frac{1}{8}''$) at 147 °C with nitrogen as the carrier gas.