Table 1. Mutation rates per unit irradiance. Mean values of the mutation rate per unit irradiance, Y, and standard errors of Y, are given for cultures grown aerobically (+) or anaerobically (−). The absolute standard errors for the 100-watt lamp are somewhat larger than those shown, since they include an error of about 20 percent in the determination of the average value of the irradiance because of the rather nonuniform spatial distribution of radiation from this lamp. Y = (mutant/bacterium per day)/(watt/mm^2).

Oxygen	Filter	Y ± S.E.
	4-watt lamp	
+	None	0.45 ± 0.03
+	No. 5680	.51 ± .02
−	None	.26 ± .02
	100-watt lamp	
+	None	.37 ± .03
+	No. 5680	.57 ± .10

ond. Mutation rates were increased by about one order of magnitude for exposures up to 1 hour, but there was significant killing after this time; about half of the cells were inactivated after an exposure of 8 hours.

To investigate the nature of mutagenesis with near-visible light, continuous cultures were grown anaerobically (5 percent CO_2, 95 percent N_2) and irradiated as before. For these, the average mutation rate was 1.55×10^{-6} mutant per bacterium per day, about 60 percent of the aerobic rate (rates from 1.9 to 13 divisions per day). This result shows that induction of mutation with near-visible light involves at least two mechanisms, one of which requires oxygen and therefore may be a photodynamic effect. The results are similar to those of Cabrera-Juarez, who found oxygen-dependent and independent inactivation in transforming DNA (*2*).

Near-visible light is very inefficient compared to ultraviolet light at 260 mμ. Under the same growth conditions, a much smaller irradiance of ultraviolet light, 0.051 erg/mm^2 per second, induced mutation to T5-resistance in a closely related strain at almost half the aerobic rate mentioned above, 1.27×10^{-6} mutant per bacterium per day (*6*). Since the well-known 260-mμ absorption peak of DNA extends to 310 mμ, this great sensitivity to ultraviolet light suggested the possibility that our light sources emitted some ultraviolet light below 320 mμ, and that this component was responsible for the induction of mutation. For this reason, exposures were made through a filter (7.5 by 7.5 cm, Corning No. 5860) that transmitted less than 1 percent of the incident irradiation at wavelengths less than 310 mμ (Fig. 1). With this filter, the irradiance was reduced to 18.6 percent of the unfiltered rate for the 4-watt lamps, and to 21 percent for the 100-watt lamp. However, the presence of the filter did not decrease the value of the mutation rate per unit irradiance (Table 1). Thus, there could have been no material fraction of mutations induced by light of those wavelengths that were severely attenuated by the filter. For this reason, with the "continuous" spectrum most of the mutants were produced by wavelengths between 330 and 380 mμ. The results with the "line" spectrum are even clearer: almost all of the mutants must have been induced with light near 365 mμ.

The photochemical reactions initiated in mutagenesis with near-visible light are quite unlikely to be identical with those initiated in mutagenesis by ultraviolet light. Absorption by the 260-mμ peak falls precipitously at wavelengths greater than 300 mμ; extrapolated values of the molar absorption coefficients are reduced from the value at 260 mμ by factors of about 10^4 at 320 mμ and 10^6 at 365 mμ. This precipitous decline is inconsistent with the mutation rates observed both with and without the filter. That is, this absorption mechanism should have led to few or no mutants with the "line" spectrum, whereas with the 100-watt lamp the observed specific mutation rate per unit irradiance was more than half of the value for the 4-watt lamps. Thus mutations induced with near-visible light do not show the wavelength dependence expected for mutations induced with ultraviolet, and other photochemical reactions must be involved.

H. E. KUBITSCHEK
Argonne National Laboratory, Argonne, Illinois 60439

References and Notes

1. A. Hollaender and C. W. Emmons, *Cold Spring Harbor Symp. Quant. Biol.* **11**, 78 (1946).
2. E. Cabrera-Juarez, *J. Bacteriol.* **87**, 771 (1964).
3. A. Hollaender, *ibid.* **46**, 531 (1943).
4. H. E. Kubitschek and H. E. Bendigkeit, *Mutation Res.* **1**, 113 (1964).
5. R. B. Webb and M. M. Malina, personal communication.
6. H. E. Kubitschek and H. E. Bendigkeit, *Mutation Res.* **1**, 209 (1964).
7. Supported by the AEC.

9 February 1967

Eperythrozoon coccoides: Influence on Course of Infection of Plasmodium chabaudi in Mouse

Abstract. *Mice infected with* Plasmodium chabaudi *obtained from two sources were found to be contaminated with* Eperythrozoon coccoides. *At each transfer of blood parasitized with plasmodia, eperythrozoa were also passed. In the presence of these organisms, the malarial infection assumed a low-level, chronic course infrequently resulting in death of the mice. When the eperythrozoa were eliminated through treatment with oxophenarsine hydrochloride, the malarial infection took an acute course always ending in death.*

Several studies (*1*) indicate that organisms such as *Eperythrozoon coccoides* influence the course of *Plasmodium berghei* infections. These reports emphasize the need to examine for the presence of such contaminating organisms whenever unexpected host responses occur. Such responses were observed in our laboratory in mice infected with *Plasmodium chabaudi* Landau and were found to be caused by an intercurrent infection with eperythrozoa.

We followed the malarial infection in groups of CF_1 male mice (approximately 20 g) by counting, usually every other day, parasitized cells in Giemsa-stained thin blood-smears and by counting, with a hemacytometer, red blood cells diluted by the unopette method (*2*). In all cases the mice were inoculated intravenously with 1×10^6 cells parasitized with plasmodia, unless specified otherwise. We prepared the inocula by appropriately diluting heart blood from a single animal when the malarial infection was near its peak.

The low-level, chronic course of infection with *Plasmodium chabaudi* (*3*), reported by others (*4, 5*) and seen by us (Fig. 1), was characterized by extreme anemia and extensive reticulocytosis. These responses exceeded those expected as a result of the malarial parasitemia. Severe anemia was evident on the 4th day after inoculation, when

only 1.5 percent of the cells were parasitized (Fig. 1). By this time the counts of red blood cells had dropped from 9×10^6 to 3×10^6 per microliter. Reticulocytic response to this anemia was already obvious on day 7, at which time 18 percent of the red blood cells were immature. By day 13, reticulocytes accounted for 68 percent of the cells. The percentage of parasitized cells never exceeded 15, and all the mice survived the infection. The slope of the line for the increase in the number of parasites was lowered between days 3 and 4 to the extent that the percentage of parasitized cells remained almost stationary during that time. By the 5th day, the initial rate of parasite increase had resumed. Maximum parasitemia occurred on day 11. After that time, the parasitemia fluctuated, but by day 17 it had decreased to less than 5 percent. It remained below this level for a 5-week period. With the decrease in parasitemia, the counts of red blood-cells increased, and the reticulocytosis diminished.

Eperythrozoa, accidentally introduced as contaminants of the infected blood used for inoculation, cause an infection that is patent for a shorter period of time than the malarial infection is. The eperythrozoa reach maximum numbers on the 3rd day after inoculation and have almost disappeared again by day 7. The inordinate drop in red blood-cell counts and the interference with increase in malarial parasites seen on day 4 are both associated with the presence of the maximum number of eperythrozoa.

When the eperythrozoa were eliminated by treatment with oxophenarsine hydrochloride (*6*), neither of these phenomena occurred. The course of the malarial infection was acute, consistently resulting in death of all animals on day 7, the day of maximum parasitemias of 75 to 90 percent (Fig. 2). No animal among the more than 200 so inoculated survived the infection in our laboratory. The counts of red blood cells remained in the normal range until after 30 percent of the cells were parasitized, and then they fell to less than 1×10^6 per microliter on day 7. Reticulocytosis reached a level of only 10 percent on day 7, and no early inhibition of parasite increase was observed.

That the eperythrozoa were responsible for altering the pattern of malarial infection and the host responses was further demonstrated (Fig. 3). The two infections were experimentally combined in a manner different from ordinary blood passage. Mice infected with pure malarial parasites (free of eperythrozoa) on day 0 were later inoculated with eperythrozoa on day 3. A complicating factor of this experiment was that a pure strain of eperythrozoa (free of malarial parasites) was not available. Therefore, when the mice were inoculated on day 3 with eperythrozoa, they also received a relatively small, superinfecting dose of malarial parasites. The mice received 1×10^6 parasitized cells free of eperythrozoa on day 0 and 1×10^5 parasitized cells plus eperythrozoa on day 3 (group 1). Groups 2, 3, and 4 served as controls. Groups 2 and 3 were inoculated with pure malarial parasites on day 0 ($1 \times 10_6$).

On day 3, group 3 was superinfected with pure malarial parasites (1×10^5), and group 4 was inoculated with malarial parasites (1×10^5) plus eperythrozoa. The infection in groups 2 and 3 increased to a peak on day 7, and all animals died on that day. In group 1 the introduction of eperythrozoa on day 3 altered the course of the malarial infection. The slope of the line for increase in the number of malarial parasites was lowered between days 5 and 6. Significantly, this was the time when the maximum numbers of eperythrozoa were present. The malarial parasitemia on day 6 was only 30 percent, and no animals died on the 7th day as a result. A second peak of 37 percent occurred on the 12th day. It coincided with the time of the maximum malarial parasitemia for group 4, which had received only the smaller (1×10^5) dosage of malarial parasites plus eperythrozoa. Three mice in group 1 died on day 12, but by day 25 five animals were still alive. This represents only a 37 percent mortality rate as compared to 100 percent for groups 2 and 3, where no eperythrozoa were involved.

Obviously, the eperythrozoa contributed to the reduction in pathogenicity of the malarial infection. The inhibition of increase in malarial parasites noted 3 to 4 days after eperythrozoan inoculation (Figs. 1 and 3, group 1) might indicate competition between the

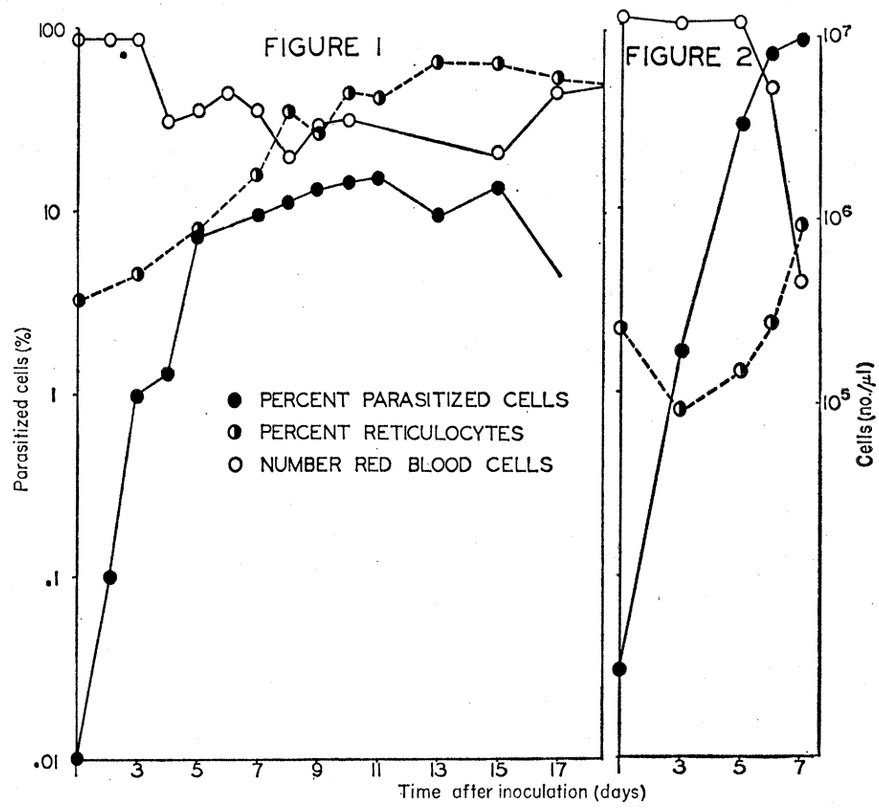

Fig. 1. Course of infection of *Plasmodium chabaudi* in a group of five mice inoculated intravenously with 1×10^6 cells parasitized with plasmodia from heart blood of a donor mouse contaminated with eperythrozoa. Fig. 2. Course of infection of *Plasmodium chabaudi* in a group of five mice inoculated intravenously with 1×10^6 cells parasitized with plasmodia from heart blood of a donor mouse free of eperythrozoa.

two organisms for substrate or even production of toxic products by the eperythrozoa. This direct effect of the eperythrozoa is reinforced by an indirect one associated with the early anemia and subsequent reticulocytosis. There is evidence indicating that, if reticulocytosis is high at the time when malarial parasitemia is high, the malarial infection is less severe, and fewer mice succumb. The number of reticulocytes is high on day 7 and increases as the parasitemia slowly approaches the peak (Fig. 1). This is associated with low mortality. Although not shown, the time when the number of reticulocytes of group 4, in the experiment represented in Fig. 3, was high did not coincide with the time of high parasitemia. Reticulocytosis was greatest on day 10, but it had already decreased appreciably by day 12, the day of greatest parasitemia. The malarial parasitemia and the mortality rate of this group were greater than in any other instance of the dual infection. This correlation between lack of severity of the malarial infection and reticulocytosis has been repeatedly observed. In a separate experiment, even when reticulocytosis was stimulated with phenylhydrazine, the phenomenon was seen. Eighty percent of a group of animals treated with phenylhydrazine survived the otherwise invariably fatal infection *(7)*.

The *Plasmodium chabaudi* infection in mice heretofore described as a low-level, chronic one, causing death only occasionally, was found to take this course because of the presence of eperythrozoa. When the contaminating organisms were eliminated, the malarial infection was found to resemble that of *Plasmodium vinckei*. The description of the infection of *P. vinckei (5)* could easily describe that for *P. chabaudi* in mice free of eperthyrozoa in that it causes fulminating infection invariably fatal within 5 to 7 days after intraperitoneal inoculation of a million parasitized red blood cells. Maximum parasitemias range between 51 and 96 percent. The fact that neither *P. vinckei* nor *P. chabaudi* preferentially invade reticulocytes also adds to their resemblance. In addition, the disc-electrophoretic patterns of a single sample of *P. chabaudi* extract reportedly closely resembles that of *P. vinckei (8)*. These observations might call for a reevaluation of methods for differentiating one species from another.

KAREN J. OTT
LESLIE A. STAUBER
Bureau of Biological Research,
Rutgers University,
New Brunswick, New Jersey

FIGURE 3

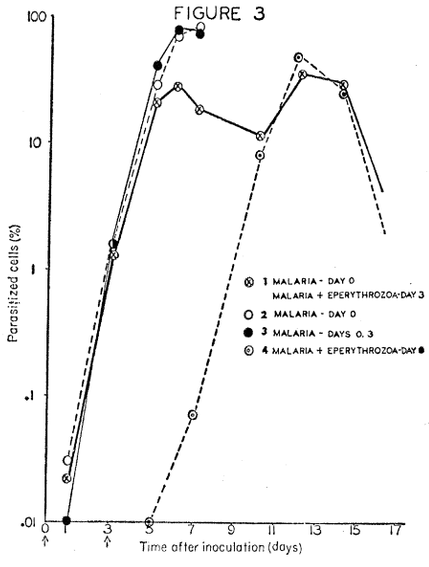

Fig. 3. Course of infection of *Plasmodium chabaudi* in four groups of eight mice: (1) Infected on day 0 with 1×10^6 cells parasitized with plasmodia from a donor mouse free of eperythrozoa and superinfected on day 3 with 1×10^5 parasitized cells plus large numbers of eperythrozoa from a contaminated donor mouse; (2) infected on day 0 with 1×10^6 parasitized cells from an eperythrozoan-free donor mouse; (3) infected on day 0 with 1×10^6 and superinfected on day 3 with 1×10^5 parasitized cells from eperythrozoan-free mice; and (4) infected only on day 3 with the inoculum of large numbers of eperythrozoa and small numbers (1×10^5) of malaria-parasitized cells identical with the superinfecting dose given to group 1. All inoculations were intravenous. The arrows indicate the days of infection and superinfection.

References and Notes

1. D. Y. M. Hsu and Q. M. Geiman, *Amer. J. Trop. Med. Hyg.* **1**, 747 (1952); J. P. Thurston, *Parasitology* **45**, 141 (1955); W. Peters, *Exp. Parasitol.* **16**, 158 (19(→ P. E. Thompson and A. Bayles, *J. Parasitol.* **52**, 674 (1966).
2. H. W. Gerarde, *Med. Bull.* **20**, 358 (1960).
3. Two strains of *P. chabaudi* are maintained in our laboratory; both are contaminated with eperythrozoa. Dr. Meir Yoeli of New York University School of Medicine provided the strain used in our work, and the other was obtained from Dr. Irene Landau through the courtesy of Dr. Elvio H. Sadun's laboratory at Walter Reed Army Institute of Research.
4. I. Landau, *Compt. Rend.* **260**, 3758 (1965).
5. M. Yoeli, R. Nussenzweig, R. S. Upmanis, H. Most, *Nature* **211**, 49 (1966).
6. The compound, obtained from Dr. Paul E. Thompson of Parke, Davis and Company, was dissolved and diluted in sterile distilled water and administered in 0.2-ml doses intraperitoneally at a level of 10 mg/kg of body weight daily for 3 to 5 days.
7. K. J. Ott (unpublished results).
8. D. Spira, J. Hamburger, A. Zuckerman, *J. Protozool.* **13** (Suppl.) 34 (1966).
9. This paper is contribution No. **149** from the Army Research Program on Malaria (grant DA 49-193-MD 2747). We thank Mrs. Nelda Davis and Frederick J. Levy for technical assistance.

16 January 1967

2,4,5-Trichlorophenoxyacetic Acid: Effect on Ethylene Production by Fruits and Leaves of Fig Tree

Abstract. *Production of ethylene by fruits and leaves of the fig tree was stimulated by the application of 2,4,5-trichlorophenoxyacetic acid (20 parts per million) to levels that apparently were responsible for hastened maturation of fruit, epinasty, and senescence of leaves.*

Several phenoxy growth regulators stimulate growth and hasten the maturation of fig fruits as much as 60 days *(1)*. Increase in growth is a characteristic response of plant tissues to auxins *(2)*, but the stimulation of maturation in fig and other fruits by these regulators has not been explained. Evidence presented here indicates that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) induces production of ethylene (C_2H_4) by fig fruits and thus stimulates their maturation. Exogenously applied C_2H_4, at concentrations of 0.1 part per million (ppm) or more, stimulates ripening in many fruits, and it has been suggested that this compound is a ripening hormone *(3)*. Auxins stimulate production of C_2H_4 in plant tissues *(4)*, and while maturation must not be equated with ripening, 2,4,5-T may stimulate maturation indirectly through induced production of C_2H_4.

Large branches on each of three Calimyrna fig trees were sprayed on 21 June 1966 with an aqueous solution of 20 ppm of 2,4,5-T. Controls were comparable branches on untreated trees. Samples of 20 fruits and 50 leaves were taken from each tree 24 hours after spraying and periodically thereafter. All fruits from either treated or untreated trees were mixed and then divided equally into three samples. Daily measurements of the production of CO_2 and C_2H_4 were made in triplicate. Leaf samples were handled in the same way. Respiratory rates at 20°C were measured on each continuously aerated lot by the method of Claypool and Keefer *(5)*, and the rates of C_2H_4 production simultaneously by the method of Maxie *et al.* *(6)*.

Figure 1 shows the effect of 2,4,5-T on the rates of production of CO_2 and C_2H_4 by fig fruits sampled 24 hours after treatment. Treated fruits had a higher initial respiratory rate and showed a climacteric-like rise and fall, reaching a peak on the 7th day. Untreated fruits showed a progressive decline in respiratory rate until the 10th