References and Notes

1. R. T. Menzies, *Appl. Phys. Lett.* **22**, 592 (1973).
2. E. D. Hinkley and P. L. Kelley, *Science* **171**, 635 (1971); E. D. Hinkley, *Opto-Electronics* **4**, 69 (1972).
3. R. T. Menzies, *Appl. Opt.* **10**, 1532 (1971); *Opto-Electronics* **4**, 179 (1972).
4. R. H. Dicke, *Rev. Sci. Instrum.* **17**, 268 (1946).
5. J. H. McElroy, *Appl. Opt.* **11**, 1619 (1972).
6. P. Brockman and R. K. Seals, Jr., paper L-8814, and R. K. Seals, Jr., paper L-8866, presented at the Sixth Fluid and Plasma Dynamics Conference of the American Institute of Aeronautics and Astronautics, Palm Springs, Calif., July 1973.
7. D. L. Fried, *Proc. IEEE (Inst. Electr. Electron. Eng.)* **55**, 57 (1967).

8. Supported by NASA contract NAS 7-100.

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Brain Capillary Blockage Produced by a Virulent Strain of Rodent Malaria

Abstract. *A sudden enhancement in virulence of a mild* Plasmodium berghei yoelii 17 x *strain resulted in fulminating and fatal infections in CF1 and A/J mice. The virulent strain has maintained its characteristics after ten cyclical transmissions through* Anopheles stephensi. *The visible expression of virulence of the mutated strain is its ability to cross the blood-brain barrier and cause intravascular sequestration of injected erythrocytes and blockage of brain capillaries. We, therefore, believe that the virulent line of* Plasmodium berghei yoelii 17 x *could serve as a useful laboratory model for the study of "cerebral malaria."*

Obstruction of brain capillaries by parasitized erythrocytes occurs in falciparum (malignant tertian) malaria of man and can lead to death. Consequently, "cerebral malaria" has been studied both clinically and histologically since the early part of this century (*1*). Recently, attempts have been made to discover the causes of intravascular sequestration of infected erythrocytes in primate malaria models, such as, *Plasmodium knowlesi* and *P. coatneyi* in rhesus monkeys (*Macaca mulatta*) and *P. falciparum* in the night monkey (*Aotus trivigatus*) (*2*).

The findings of these investigations have clarified some of the mechanisms of intravascular sequestration and the changes in parasitized erythrocytes which enhance their ability to adhere and subsequently block the lumen of capillaries. These studies have also revealed the early and late sites of vascular schizogony in other organs. The pathological process involved, according to Maegraith (*3*), is stasis and loss of fluids and proteins which lead to cytotoxic anoxemia.

Rudzinska and Trager reported changes in the fine structure of infected erythrocytes (*4*), and Miller reported a decrease in the deformability of these cells (*5*). The different sites and organs of deep vascular schizogony early in the infection were demonstrated for *P. knowlesi, P. coatneyi,* and *P. falciparum* in their experimental primate hosts (*6*). However, there is no cerebral involvement in any of these infections in spite of the very high parasitemias. The histopathological picture of "cerebral malaria" in fatal cases of *P. falciparum* infections in man has not been observed in any other mammalian plasmodial infection.

We have discovered a suitable laboratory model for the study of brain involvement and cerebral capillary blockage in malaria infection. *Plasmodium berghei yoelii 17 x* normally causes an infection which is low in parasitemia and mild in its course. A strain of this plasmodium underwent an enhancement in virulence after its removal from our deep freeze. This virulent line caused fulminating and fatal infections in CF1 and A/J mice within 6 to 7 days after intraperitoneal injection of 10^6 parasitized erythrocytes. Parasitemia rose rapidly and reached 71 to 85 percent in the terminal phase of the infection. The virulent line of *P. b. yoelii* maintained its virulence during ten cyclical transmissions through *Anopheles stephensi* and 28 blood transfers. Its enzyme patterns were the same as that of the mild parent strain (*7*). Mice which recover from the mild *P. b. yoelii 17 x* were immune to the virulent *P. b. yoelii 17 x* line (*8*).

This enhanced virulence appears to involve the brains of the infected mice. Fine petechial hemorrhages were seen on the surface and in sections of the brain. Blockage of brain capillaries by infected erythrocytes were observed in brain smears stained in Giemsa (brain squash preparations), and 10 to 20 percent of the capillaries which we counted were affected. Both the fine capillaries which permit the passage of a single red cell and wider capillaries (Fig. 1, A, B, and C) were involved. The various stages of schizogonic development could be seen in the adhering, infected cells. "Ballooning" and aneurism formations in blocked capillaries were often observed (Fig. 1D). The ballooned areas were packed with infected erythrocytes and pigment. The cause and mechanism of the ballooning within the capillaries have not yet been determined. Twenty-two of 24 CF1 mice infected with the virulent line of *P. b. yoelii 17 x* showed this involvement of the brain capillaries. In a more recent experiment, eight CF1 mice and four

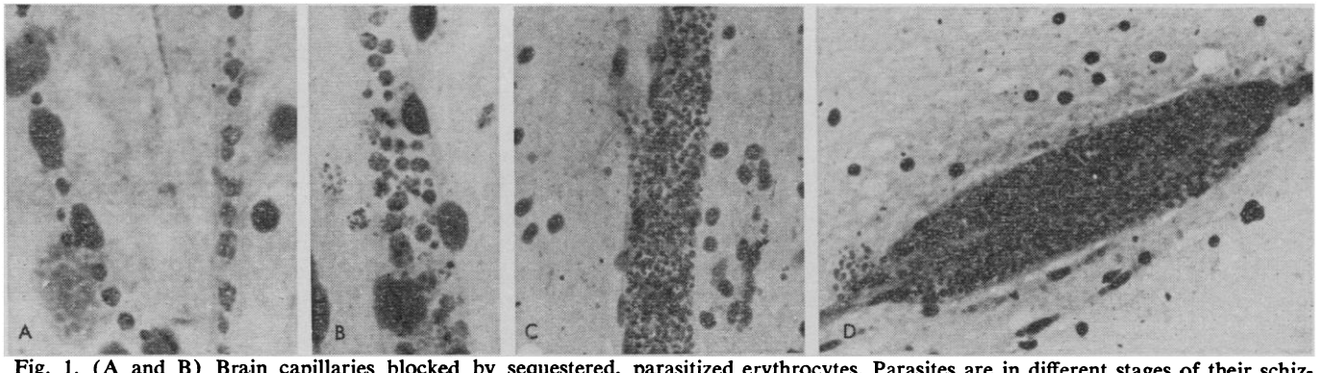

Fig. 1. (A and B) Brain capillaries blocked by sequestered, parasitized erythrocytes. Parasites are in different stages of their schizogonic development (brain smear stained in Giesma; × 960). (C) Large brain capillary blocked by sequestered, parasitized erythrocytes (Giemsa; × 420). (D) "Ballooning" in a brain capillary packed with parasitized erythrocytes (Giemsa; × 420).

A/J mice were injected with 5×10^6 parasitized erythrocytes per mouse. All 12 mice died 5.5 days after inoculation. Blood smears taken 6 hours before death showed a peripheral parasitemia of 56 to 86 percent. Brain smears stained in Giemsa showed infected cell sequestration and heavy blockage of fine and large brain capillaries in all of these mice.

Past studies with *P. berghei berghei* NK65 and KS11 strains (*9*), in which a systematic search for brain involvement was carried out, failed to reveal any cerebral vascular blockage. In fatal infections of *P. berghei berghei* in hamsters, young albino rats, tree rats, or mice, phagocytosis of hemozoin pigment has been seen in some endothelial cells of brain capillaries. However, the engulfment of malaria pigment in brain capillaries of these animals was minor compared with the hemozoin phagocytosis in the liver, spleen, or lungs. Moreover, no sequestration of infected erythrocytes in brain capillaries has ever been observed in spite of parasitemias of 70 to 85 percent. *Plasmodium vinckei vinckei*, which we have kept for many years in our laboratory, has always produced a fulminating, fatal injection within 6 to 8 days, and terminal parasitemias have ranged from 85 to 100 percent (*10*). In spite of high parasitemias in the lumen of vessels of internal organs and massive hemozoin engulfing, the brains of these mice, when examined in stained smears, crush preparations, or histological sections, were almost free from parasites. Only rarely have infected erythrocytes been seen within a cerebral capillary, and the blood-brain barrier in *P. vinckei vinckei* infected mice has appeared to remain intact until death.

From the results with the virulent *P. b. yoelii 17 x* line, and from our past studies with other rodent malaria parasites, we conclude that the virulence of the *P. b. yoelii 17 x* line is due, at least partially, to its ability to cross the blood-brain barrier, develop intraerythrocytic schizogony within the lumen of cerebral vessels, induce intravascular sequestration of infected erythrocytes, and block small and large capillaries of the brain. We feel this strain is a useful model for the study of physiological and pathological aspects of "cerebral malaria."

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References and Notes

1. E. Marchiafava and A. Bignami, *Sulle Febri Estivo-Autunnali* (Loescher, Roma, 1892), pp. 104–157.
2. Q. M. Geiman and M. Y. Maeghen, *Nature (Lond.)* **215**, 437 (1967).
3. B. G. Maegraith, *Pathological Processes in Malaria and Blackwater Fever* (Blackwell, Oxford, 1948), pp. 260–289; *Bull. N.Y. Acad. Med.* **45**, 1060 (1969).
4. M. A. Rudzinska and W. J. Trager, *J. Protozool.* **15**, 73 (1968); S. S. Luse and L. H. Miller, *Am. J. Trop. Med. Hyg.* **20**, 655 (1971).
5. L. H. Miller, S. Chien, S. Usami, *Am. J. Trop. Med. Hyg.* **21**, 133 (1972).
6. R. S. Desowitz, L. H. Miller, R. D. Buchanan, B. Permanich, *Trans. R. Soc. Trop. Med. Hyg.* **63**, 198 (1969); L. H. Miller, *Am. J. Trop. Med. Hyg.* **18**, 860 (1969); L. H. Miller, H. N. Fremont, S. A. Luse, *ibid.* **20**, 816 (1971).
7. M. Yoeli, B. Hargreaves, R. Carter, D. Walliker, *Ann. Trop. Med. Parasitol.*, in press.
8. M. Yoeli, B. Hargreaves, D. Walliker, *ibid.*, in press.
9. M. Yoeli, unpublished.
10. ———, R. Nussenzweig, S. Upmanis, H. Most, *Nature (Lond.)* **211**, 50 (1966).
11. The work has been supported by a grant from the World Health Organization. We thank Y. Ortiz and C. Young for their help.

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Thiocarbamate Sulfoxides: Potent, Selective, and Biodegradable Herbicides

Abstract. *Sulfoxidation of thiocarbamates yields a new class of chemicals having increased herbicidal activity along with greater tolerance of corn and soybeans in greenhouse tests. However, their thermal stability is not favorable. These sulfoxides are intermediates in the mammalian metabolism of thiocarbamates, being formed by liver microsomal oxidases and cleaved in a system consisting of glutathione and a soluble enzyme from liver.*

The production of agricultural crops is facilitated by the use of effective and selective herbicidal chemicals such as thiocarbamates (Table 1) (*1*). Thiocarbamates undergo rapid biodegradation (*2*), and hence persisting residues are not a problem. However, the mechanism of their biodegradation has not been defined biochemically. We find that thiocarbamate sulfoxides, which form in high yields on treatment of thiocarbamate herbicides with equimolar *m*-chloroperoxybenzoic acid (MCPBA), are not only potent and biodegradable herbicides, but are intermediates in the mammalian metabolism of the thiocarbamates themselves.

Seven commercial thiocarbamate herbicides and many related compounds were treated with equimolar quantities of MCPBA in chloroform or methylene chloride, yielding, in each case, the corresponding thiocarbamate sulfoxide (Table 1). The monooxygenated thiocarbamates are characterized as the sulfoxides by appropriate infrared, nuclear magnetic resonance, and mass spectra and by further reaction with equimolar quantities of MCPBA to give the corresponding sulfones $[RS(O)_2C(O)NR_1R_2]$.

Laboratory tests establish that sulfoxides of EPTC (*S*-ethyl di-*N*,*N*,-propylthiolcarbamate) and pebulate (0.3 to 0.5 part per million) in aqueous solution strongly inhibit the root growth of germinating oat seedlings, whereas concentrations seven to eight times greater are required for the same effect with EPTC and pebulate. Greenhouse and field tests in which the soil and herbicide were mixed before the seeds were planted verify that, in general, the thiocarbamate sulfoxides are more potent herbicides than the corresponding thiocarbamates, particularly when tested on broadleaf weeds and, to a lesser extent, on grass weeds. Table 1 illustrates the increase in potency for control of broadleaf weeds and the reduction in corn injury on sulfoxidation of the thiocarbamates, as judged by greenhouse trials. The crop tolerance is improved by sulfoxidation not only in the case of corn, but also with vernolate on soybeans, although this is not the case with cycloate and pebulate on sugar beets. The selectivity is striking in greenhouse tests on corn and weeds where 0.5 kg/ha (1 kg/ha ~ 1 pound/acre) of the sulfoxides of butylate, EPTC, and vernolate control crabgrass (*Digitaria sanguinalis*), foxtail (*Setaria viridis*), watergrass (*Echinochloa crus-galli*), and wild oat (*Avena fatua*), yet this crop is unharmed even at 27 kg/ha of these sulfoxides. In paddy rice culture, there is little or no advantage in using the sulfoxides of benthiocarb and molinate as compared with the parent thiocarbamates. The sulfoxides have certain advantages over the corresponding thiocarbamates: increased potency in control of some weeds when moist soils are treated; increased safety factor for corn and soybeans, and reduced volatility so that the requirement for immediate incorporation in soil is less critical. Features which may limit their commercial potential are hydro-