D. Hoffman, *Adv. Cancer Res.* **8**, 249 (1964).
7. L. W. Wattenberg, J. L. Leong, P. J. Strand, *Cancer Res.* **22**, 1120 (1962).
8. A. H. Conney, E. C. Miller, J. A. Miller, *J. Biol. Chem.* **228**, 753 (1957); A. H. Conney, M. Sansur, F. Soroko, R. Koster, J. J. Burns, *J. Pharmacol. Exp. Ther.* **151**, 133 (1966).
9. R. M. Welch, Y. E. Harrison, A. H. Conney, P. J. Poppers, M. Finster, *Science* **160**, 541 (1968); R. M. Welch, Y. E. Harrison, B. W. Gommi, P. J. Poppers, M. Finster, A. H. Conney, *Clin. Pharmacol. Ther.* **10**, 100 (1969); D. W. Nebert, J. Winkler, H. V. Gelboin, *Cancer Res.* **29**, 1763 (1969); M. R. Juchau, *Toxicol. Appl. Pharmacol.* **18**, 565 (1971); R. M. Welch, A. Loh, A. H. Conney, *Life Sci.* **10**, 215 (1971).
10. W. Lijinsky and P. Shubik, *Science* **145**, 53 (1964).
11. Y. E. Harrison and W. L. West, *Biochem. Pharmacol.* **20**, 2105 (1971).
12. L. W. Wattenberg, *Cancer* **20**, 99 (1971); in *Environment and Cancer*, R. D. Cumley, Ed. (Williams & Wilkins, Baltimore, 1972), p. 241.
13. E. S. Vesell, G. T. Passananti, F. E. Greene, J. G. Page, *Ann. N.Y. Acad. Sci.* **179**, 752 (1971); B. Alexanderson, D. A. P. Evans, F. Sjöqvist, *Br. Med. J.* **4**, 764 (1969).
14. A. H. Conney, *Pharmacol. Rev.* **19**, 317 (1967); ———, R. Welch, R. Kuntzman, P. J. Poppers, M. Finster, J. A. Wolff, A. D. Munro-Faure, A. W. Peck, A. Bye, R. Chang, M. Jacobson, A. Poland, *Ann. N.Y. Acad. Sci.* **179**, 155 (1971).
15. S. H. Curry, A. D'Mello, G. P. Mould, *Br. J. Pharmacol.* **42**, 403 (1971).
16. L. Rivera-Calimlim, J. P. Morgan, C. A. Dujovne, J. R. Bianchine, L. Lasagna, *Biochem. Pharmacol.* **20**, 3051 (1971); D. N. Wade, P. T. Mearrick, J. L. Morris, *Nature (Lond.)* **242**, 463 (1973).
17. R. Tissot, G. Bartholini, A. Pletscher, *Arch. Neurol.* **20**, 187 (1969); F. K. Goodwin, H. K. H. Brodie, D. L. Murphy, W. E. Bunney, Jr., *Lancet* **1970-I**, 908 (1970); D. L. Dunner, H. K. H. Brodie, F. K. Goodwin, *Clin. Pharmacol. Ther.* **12**, 212 (1971).
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Ventral Medial Hypothalamus: Involvement in Hypoglycemic Convulsions

Abstract. *After the ventral medial hypothalamus of mice was lesioned with gold thioglucose, the dose of insulin required to produce convulsions in 50 percent of the animals was doubled compared to that in nonlesionad controls. No dose of insulin, up to 50 milliunits per gram, produced convulsions in more than 60 percent of the lesioned mice, even though blood glucose levels fell to approximately 24 milligram percent.*

The ventral medial hypothalamus (VMH) is thought to be a site of glucose receptors in the brain (*1*). Gold thioglucose (GTG) injected intraperitoneally into mice results in lesions of the VMH (*2*) and consequent hyperphagia leading to obesity. Other gold thio compounds such as gold sodium thiomalate (GTM) do not produce such lesions (*3*). The extent of hypothalamic lesions produced by a given dose of GTG appears to be directly related to the rate of glucose utilization at the time of injection (*4*). Mice that have been made diabetic before GTG injection do not develop hypothalamic lesions (*5*); however, intravenous (*6*) or intrahypothalamic (*7*) injections of insulin have been found to restore the sensitivity of the VMH to lesion by GTG. The mechanism by which the intrahypothalamic injection of insulin restores GTG sensitivity to diabetic mice is not fully understood. It suggests a local effect of insulin in the brain, a concept which is at odds with the generally accepted idea that glucose utilization by brain is not influenced by insulin (*8*).

Severe hypoglycemia following insulin administration causes convulsions. Since it has been postulated that the VMH may sense glucose levels (*9*), we investigated a possible role of the VMH in initiating the behavioral response (convulsion) to hypoglycemia.

Young adult female CBA/J mice (Jackson Laboratories) weighing 18 to 22 g were divided randomly into three groups and housed in groups of ten. The mice were fasted for 24 hours before being given a single intraperitoneal injection of either GTG (0.4 mg per gram of body weight), GTM (0.4 mg/g), or saline (*10*). The fast was continued for an additional 24 hours following injection and then the mice were allowed free access to food. No mice died during the 30 days following injection and 76 percent of the mice injected with GTG attained a weight 2 standard deviations greater than the mean weight of the control groups during the 73-day period following injection. A typical lesion produced by this dose of GTG under the conditions described is shown in Fig. 1 (*11*).

Weights were recorded periodically, and 73 days after treatment a series of insulin injections was begun. At that time the average fasting weight was 21 percent greater for GTG-treated mice than for control groups treated with either GTM or saline ($P < .01$; Student's t-test). The mice were fasted for 18 hours before each late morning insulin injection (*12*). After each injection the mice were observed for 70 minutes in their home cages with no food available. A convulsion was scored

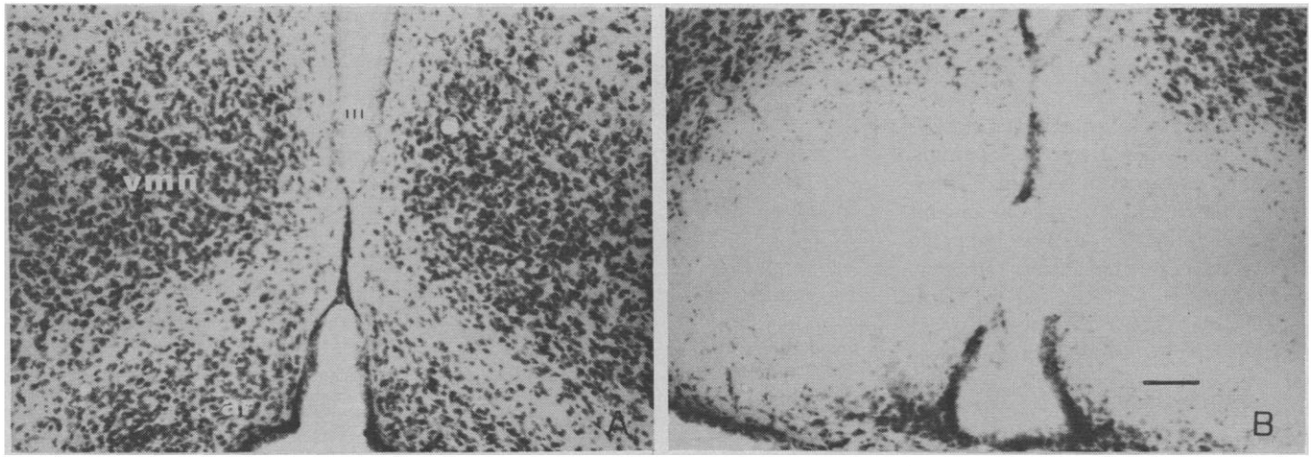

Fig. 1. Transverse sections 40 μm thick through the hypothalamus of CBA mice: (A) control and (B) GTG treated. The structures identified are: *vmn*, ventromedial nucleus; *ar*, arcuate nucleus; and *III*, third ventricle. Scale bar, 100 μm; magnification, $\times 78$.

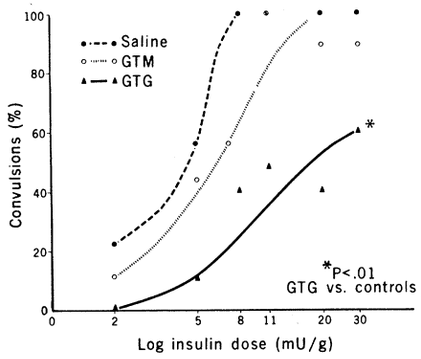

Fig. 2. Log dose–response curves for saline controls ($N = 8$); GTM controls ($N = 7$); and GTG-treated group ($N = 25$). See text for details.

when a mouse displayed tonic or clonic spasms or nose-to-tail axial torsion spasms. Each mouse that convulsed was immediately injected intraperitoneally with 25 mg of glucose dissolved in water. This quickly reversed the hypoglycemic convulsion in both the lesioned and nonlesioned groups. At the end of an observation period, all mice were given free access to food and water and allowed 5 days to recover before the next insulin injection.

Compared to the nonlesioned controls, the GTG-lesioned group showed a lower incidence of convulsions at all insulin dose levels used ($P < .01$) (Fig. 2) (*13*). There was no significant difference between the GTM and saline groups. The doses of insulin required to produce convulsions in 50 percent of the mice (CD_{50}) were 4.7 and 5.8 milliunits (mU) per gram for the saline and GTM groups, respectively, and 11 mU/g for the GTG group. The doses of insulin required to produce convulsions in 90 percent of the mice were 7 and 9 mU/g for the saline and GTM groups, respectively; however, no dose of insulin up to 50 mU/g elicited convulsions in more than 60 percent of the GTG group.

In order to determine whether mice subjected to repeated exposures to increasing doses of insulin on successive test days had developed a resistance to insulin, the saline and GTM control groups were challenged with a second dose of 11 mU/g at the end of the experiment. All of the saline and all but one of the GTM group convulsed. We concluded that insulin resistance had not developed. This conclusion is supported by measurements of the blood glucose content, which was determined after an 18-hour fast and again 60 minutes after injection of insulin (14 mU/g) or during convulsions, whichever occurred first. In all animals blood glucose fell from a fasting level of approximately 65 mg percent (3.6 m*M*) to 23 mg percent (1.2 m*M*). There were no significant differences in blood glucose content among the groups (Table 1) (*14*).

In order to determine whether the apparent protection conferred by the GTG lesion was specific for hypoglycemic convulsions or was a more general protection against convulsions, the three groups of mice were treated with pentylenetetrazol (35 mg/kg, administered intraperitoneally). There were no differences among the groups; 88 percent of the lesioned and 93 percent of the nonlesioned groups convulsed. Although these studies are limited in scope, the protection conferred by the hypothalamic lesion appears to be specific for hypoglycemic convulsions.

It is not possible at this time to give a simple explanation for the apparent protection against insulin-induced hypoglycemic convulsions seen in mice with VMH lesions. However, certain possibilities exist which are consistent with the observations of others.

Wooley and Timiras (*15*) have shown that the threshold for electroshock convulsions varies with the time of estrus in rats. No attempt was made to gauge the time of estrus of the mice in the study reported here. However the magnitude of the differences in the CD_{50} and CD_{90} between the lesioned and nonlesioned groups argues against the possibility that time of estrus significantly affected these results.

Decreases in high-energy phosphate (adenosine triphosphate and phosphocreatine) have been demonstrated in brains of mice undergoing electrogenic (*16*) and audiogenic (*17*) seizures. Paradoxically, no significant decreases in high-energy reserves were observed in cerebral cortex of mice (*18*) or whole brain of rat (*19*) during insulin-induced hypoglycemic seizures. Tarr *et al.* (*19*) suggested that a decrease of high-energy phosphates might occur only in small but critical areas of the brain and that these changes might be obscured when assayed as part of a larger area. The VMH may be such an area and may play a role in initiating hypoglycemic convulsions.

Another possibility is suggested by the studies of the response of fasted animals to insulin-induced hypoglycemia. In one study (*20*), mice fasted for 48 hours displayed significant protection against insulin-induced hypoglycemic convulsions when compared to nonfasted controls. The increased resistance to convulsions appeared to be specific for hypoglycemic convulsions since fasting did not confer protection against convulsions induced by pentylenetetrazol or strychnine. Thus the brain may be able to utilize other fuels, such as ketoacids, more efficiently after a period of fasting. It is possible that lesions in the VMH induce changes similar to those caused by fasting in the metabolic requirements of certain areas of the brain or of the whole brain, thus making lesioned mice resistant to hypoglycemic convulsion.

The results suggest that an intact hypothalamus is necessary for the full expression of insulin-induced hypoglycemic convulsions.

Table 1. Blood glucose content (*14*) of the GTG, GTM, and saline groups after fasting and after injection of insulin (14 mU/g). Values are means ± standard errors of the means.

Group	Blood glucose (mg percent) determined after	
	Fasting	Insulin
GTG	67.6 ± 1.3	24.0 ± 0.8
GTM	64.2 ± 4.2	21.8 ± 1.2
Saline	63.1 ± 1.5	23.0 ± 1.0

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References and Notes

1. J. Mayer, *Physiol. Rev.* **33**, 472 (1953).
2. N. B. Marshall, R. J. Barrnett, J. Mayer, *Proc. Soc. Exp. Biol. Med.* **90**, 240 (1955).
3. J. Mayer and N. B. Marshall, *Nature (Lond.)* **178**, 1399 (1956).
4. P. M. Edelman, I. L. Schwartz, E. P. Cronkite, G. Brecher, L. Livingston, *J. Exp. Med.* **121**, 403 (1965); H. J. Likuski, A. F. Debons, R. J. Cloutier, *Am. J. Physiol.* **212**, 669 (1967).
5. A. F. Debons, I. Krimsky, H. J. Likuski, A. From, R. J. Cloutier, *Am. J. Physiol.* **214**, 652 (1968).
6. A. F. Debons, I. Krimsky, A. From, R. J. Cloutier, *ibid.* **217**, 1114 (1969).
7. A. F. Debons, I. Krimsky, A. From, *ibid.* **219**, 938 (1970).
8. F. Baker, J. F. Fazekas, H. E. Himwich, *J. Biol. Chem.* **125**, 545 (1938); A. Beloff-Chain, R. Cantangars, E. B. Chain, I. Masi, F. Pocchiari, *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **144**, 22 (1955); C. R. Park, L. H. Johnson, J. H. Wright, Jr., H. Bastel, *Am. J. Physiol.* **191**, 13 (1957).
9. J. Mayer, *Ann. N.Y. Acad. Sci.* **63**, 15 (1955).
10. All injections were given in saline in a volume equal to 0.01 cm^3 per gram of body weight. The GTG was a gift of M. Steinberg of the Schering Corporation, Bloomfield, N.J.
11. The sections shown in Fig. 1 were taken from brains of mice fixed 24 hours after injection of GTG (0.4 mg/g) or saline since it has been shown that the lesion becomes increasingly difficult to visualize with time [G. Brecher, G. L. Laqueur, E. P. Cronkite, P. M. Edelman, I. L. Schwartz, *J. Exp. Med.* **121**, 395 (1965)]. The mice were anesthetized with so-

dium pentobarbital and perfused through a heart puncture with saline and then with 10 percent buffered formalin. Frozen sections of the hypothalamus were cut at 40 μm and stained with cresyl violet.
12. All insulin injections were given intraperitoneally in saline in a volume equal to 0.012 cm^3 per gram of body weight. The insulin used was U-40 beef and pork insulin (Lilly).
13. The statistical methods employed were two-way analysis of variance and Tukey's test.
14. Blood glucose content was determined on tail vein samples by using the fluorometric procedure of O. H. Lowry, J. V. Passonneau, F. X. Hasselberger, and D. W. Schultz [*J. Biol. Chem.* **239**, 18 (1964)].
15. D. E. Wooley and P. S. Timiras, *Am. J. Physiol.* **202**, 379 (1962).
16. J. A. Ferrendelli and D. B. McDougal, Jr., *J. Neurochem.* **18**, 1197 (1971).
17. ———, *ibid.*, p. 1207.
18. L. J. King, O. H. Lowry, J. V. Passonneau, V. Veason, *ibid.* **14**, 559 (1967).
19. M. Tarr, D. Brada, F. E. Samson, Jr., *Am. J. Physiol.* **203**, 690 (1962).
20. G. C. Tamasi and E. J. Drenick, *Endocrinology* **92**, 1277 (1973).
21. Supported in part by NIH training grant GM 01417.
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Invasion of Erythrocytes by Malaria Merozoites

Abstract. *An electro-optical system was developed to record microscope images with high resolution at low light intensities. The system was used to study the invasion of erythrocytes by malaria merozoites. Invasion consists of attachment of the anterior end of the parasite to the erythrocyte, deformation of the erythrocyte, and entry of the parasite by erythrocyte membrane invagination.*

The events that occur during the erythrocytic stage of a malaria infection have long been known. For example, the rupture of an infected erythrocyte results in the liberation of individual parasites (merozoites) which infect other erythrocytes. This is a particularly crucial sequence of events, for if the penetration of erythrocytes by merozoites could be interrupted, the erythrocytic cycle of the parasite would be broken and infection terminated.

Observations of the invasion of erythrocytes by merozoites have been reported (*1*). However, the problems of maintaining host cells and parasites under optimum physiologic conditions and observing and recording their interactions under optimum optical conditions have limited the extent of these studies. In this report we present a method for overcoming these problems and describe events that occur during the rupture of schizont-infected erythrocytes and the subsequent invasion of other erythrocytes by merozoites. A hypothesis is presented to explain some of the phenomena observed.

Erythrocytes containing mature schizonts of *Plasmodium knowlesi* were transferred from infected monkeys to culture medium, mixed with uninfected erythrocytes (*2*), and inoculated into Dvorak-Stotler controlled-environment culture chambers (*3*). After the erythrocytes had settled onto the lower cover glass, the chambers were continuously perfused with fresh culture medium and maintained at a temperature of 35° ± 0.2°C (*4*).

Microscopy was performed with an inverted microscope (Leitz Diavert) equipped with Smith differential interference optics. The image from the microscope was projected onto the faceplate of an RCA type 8673 image orthicon installed in a high-gain video camera suitable for the requisite operation at low light intensities. The resulting video signal was processed by an image enhancer (*5*) and recorded on 1-inch video tape for storage. The video signal from selected tape segments was processed by a time base corrector (*6*) and subsequently used to produce a 16-mm motion picture negative by electron beam recording (*7*).

The rupture of schizont-infected erythrocytes is always preceded by the coalescence of malaria pigment into a single unit within a residual body (Fig. 1a). This is followed by vesiculation of the erythrocyte membrane (Fig. 1b), distortion of the erythrocyte due to movement of intracellular merozoites (Fig. 1c), and finally swelling (Fig. 1d) and rupture of the erythrocyte (Fig. 1e) and release of the merozoites with explosive suddenness (Fig. 1f). Erythrocyte swelling begins about 9 seconds before rupture and results in a net increase in volume of approximately 20 percent. Rupture of the infected erythrocyte occurs within 1 minute after the first appearance of erythrocyte membrane vesiculation. Occasionally, merozoites remain attached to the residual body. Aside from Brownian motion, individual merozoites which settle onto the lower cover glass of the chamber display pivotal motion, bending, or generalized contraction with a variable point of contact to the glass; these movements are random relative to surrounding erythrocytes.

Penetration of an erythrocyte occurs only if the anterior end of the merozite, containing the paired organelles, contacts the erythrocyte (see cover, upper left photograph; 0 second). The initial attachment between the anterior end of the merozoite and an erythrocyte results in a rapid and marked deformation of the erythrocyte for a period of 5 to 10 seconds (cover, left to right, photographs 2 to 6; 0.4, 1.3, 1.5, 5.2, and 6.6 seconds). Deformation

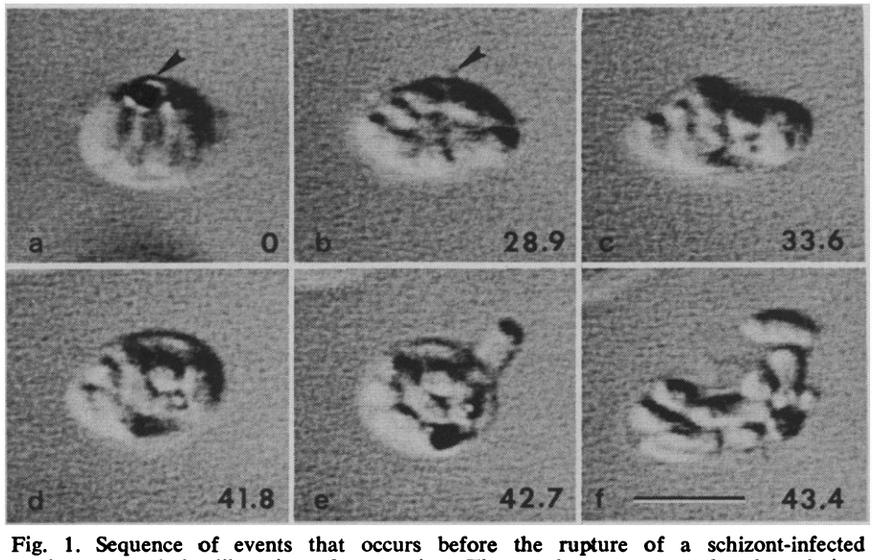

Fig. 1. Sequence of events that occurs before the rupture of a schizont-infected erythrocyte and the liberation of merozoites. The numbers represent the elapsed time in seconds. Scale marker, 5 μm.