but is known to occur as a temporary wound response in potato slices (*8*). In the case of heating aroid inflorescences, at least a partial involvement of lipid oxidation has been suggested in *Sauromatum* (*13*), which, unlike *P. selloum*, contains glyoxysomes (*14*). The absence of glyoxysomes or peroxisomes in *P. selloum* sterile florets suggests that lipid was being respired by mitochondrial β-oxidation, which occurs in animal tissues, rather than by glyoxysomal β-oxidation, which is common in plant tissues (*15*). The prevalence of mitochondria in the tissues of *P. selloum* is consistent with this suggestion. It has been proposed that the alternative pathway (the so-called cyanide-resistant pathway) in mitochondria is responsible for thermogenicity in aroids and other plants (*16*).

The lipid degradation that occurs during heat generation in *P. selloum* appears to be the most dramatic example of direct lipid oxidation yet reported for plant tissues. Furthermore, the use of lipids by *P. selloum* has interesting similarities to that found in animal tissues with high energy requirements, such as heart and wing muscles (*17*).

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References and Notes

1. In *P. selloum*, up to a few thousand florets are closely packed along an elongate stalk; the stalk plus attached florets is called a spadix. Three types of florets are arranged along the spadix: those producing pollen (fertile male) are located along the top, those producing heat (sterile male) are along the middle, and those producing seeds (fertile female) are along the bottom. Surrounding the spadix is a protective leaflike sheath called the spathe. Together the spathe and spadix constitute the inflorescence.
2. K. A. Nagy, D. K. Odell, R. S. Seymour, *Science* **178**, 1195 (1972).
3. R. S. Seymour, G. A. Bartholomew, M. C. Barnhart, *Planta*, in press.
4. The respiratory quotient is the ratio of the amount of CO_2 released to the amount of O_2 consumed during complete oxidation of a substrate. Complete oxidation of a carbohydrate such as starch results in equal amounts of CO_2 released for O_2 consumed, a quotient of 1.0. For lipids more O_2 is consumed than CO_2 released, and the quotient can often be as low as 0.7, depending on the lipid being oxidized.
5. R. Park and S. Epstein, *Geochim. Cosmochim. Acta* **21**, 110 (1960); *Plant Physiol.* **36**, 133 (1961); B. N. Smith, *BioScience* **22**, 226 (1972); _____ and C. R. Benedict, *Plant Cell Physiol.* **15**, 949 (1974).
6. $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ values, where

$$\delta^{13}C \text{ (per mil)} = \left[\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1\right] \times 1000$$

The standard is the PDB carbonate.
7. J. S. Hsu and B. N. Smith, *Plant Cell Physiol.* **13**, 689 (1972).
8. B. S. Jacobson, B. N. Smith, S. Epstein, G. G. Laties, *J. Gen. Physiol.* **55**, 1 (1970).
9. B. S. Jacobson, B. N. Smith, A. V. Jacobson, *Biochem. Biophys. Res. Commun.* **47**, 398 (1972).
10. We were unable to isolate enough starch to analyze from the sterile florets and therefore purified starch from the stalk of the spadix along the region where the sterile florets were attached. Because both the stalk and the florets are achlorophyllous tissues requiring import of all materials for growth through the vascular system, it is reasonable to assume that the $\delta^{13}C$ values for the starch accumulated in the stalk would be similar to those of starch in the adjacent sterile florets.
11. H. Beevers, in *The Biochemistry of Plants*, P. K. Stumpf, Ed. (Academic Press, New York, 1980), vol. 4, pp. 117–130.
12. The ranges and means of the respiratory rate (in micromoles of CO_2 respired per gram, wet weight, of sterile florets per minute) were 0.60 to 1.09 and 0.83 ± 0.16 for preheating specimens (N = 7), 1.27 to 7.05 and 3.67 ± 1.83 for heating specimens (N = 17), and 0.03 to 1.56 and 0.40 ± 0.53 for postheating specimens (N = 9).
13. R. H. Wilson and B. N. Smith, *Z. Pflanzenphysiol.* **65**, 124 (1971).
14. C. Berger and E. Schnepf, *Protoplasma* **69**, 237 (1970).
15. T. Galliard, in *The Biochemistry of Plants*, P. K. Stumpf, Ed. (Academic Press, New York, 1980), vol. 4, pp. 85–116.
16. B. J. D. Meeuse, *Annu. Rev. Plant Physiol.* **26**, 117 (1975).
17. R. L. Hazelwood, in *Avian Biology*, B. S. Farner, J. R. King, K. C. Parkes, Eds. (Academic Press, New York, 1972), vol. 2, pp. 471–526.
18. M. J. DeNiro and S. Epstein, *Geochim. Cosmochim. Acta* **42**, 495 (1978).
19. M. Kates and F. M. Eberhardt, *Can. J. Botany* **35**, 895 (1957).
20. V. P. Skipski, A. F. Smolowe, R. C. Sullivan, M. Barclay, *Biochim. Biophys. Acta* **106**, 386 (1965).
21. G. W. Pucher, C. S. Leavenworth, H. B. Vickery, *Anal. Chem.* **20**, 850 (1948).
22. R. K. Stump and J. W. Frazer, *Nucl. Sci. Abstr.* **28**, 746 (1973).
23. D. W. Northfelt, M. J. DeNiro, S. Epstein, *Geochim. Cosmochim. Acta* **45**, 1895 (1981).
24. H. Lück, in *Methoden der Enzymatischen Analyse*, H. U. Bergmeyer, Ed. (Verlag Chemie, Weinheim, 1962), pp. 885–894.
25. We thank G. Bartholomew, E. Gonzalez, and G. Laties for discussions and H. Ajie and D. Winter for technical assistance. Supported by a grant from the UCLA Academic Senate Committee on Research and by NSF grant ATM 79-24591.
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Primary Murine Bone Marrow Cultures Support Continuous Growth of Infectious Human Trypanosomes

Abstract. *The human parasite* Trypanosoma brucei gambiense *grew continuously at 37°C in primary cultures of murine bone marrow. Cultured parasites remained virulent for mice. Rapid parasite growth coincided with the appearance of adherent adipocyte-epitheloid cell aggregates that also promoted hematopoiesis. This culture system should permit studies of host cell control of trypanosome proliferation, pathogenic effects of trypanosomes on blood cell development, and the relative trypanocidal and marrow suppressive activities of drugs.*

The *Trypanosoma brucei* subgroup of African trypanosomes includes important pathogens of humans and domestic animals (*1*). Although these trypanosomes are usually described as blood parasites, most of them develop in the extravascular tissue space (*2*). Parasites isolated from tissue sites differ antigenically (*3*) and in reproductive activity (*4, 5*) from peripheral blood parasites. Reinvasion of the blood by tissue parasites is thought to be an important factor in relapse following drug treatment (*6*). Factors that influence the tissue distribution and physiological activity of trypanosomes in different sites are unknown. However, certain established cell lines potentiate the growth of *T. brucei* in vitro at 37°C (*7*). Interactions between trypanosomes and mammalian cells in these culture systems appear to be specific since few cell lines support parasite growth and not all parasite strains grow with the same cell line (*7*). Similar specific interactions between trypanosomes and host tissue cells could influence parasite multiplication in vivo. Bone marrow is one tissue that harbors a high proportion of dividing trypano-

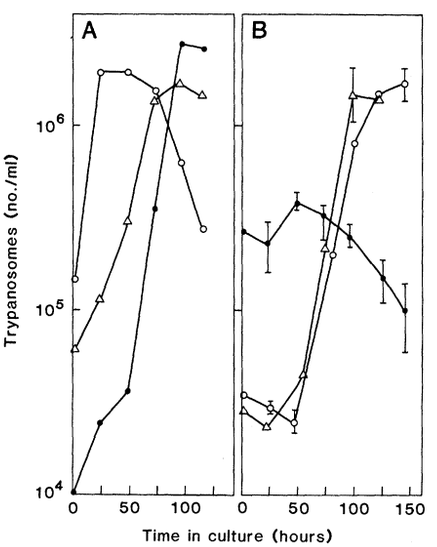

Fig. 1. Growth of *T. b. gambiense* in primary murine bone marrow cultures. (A) Marrow from BALB/cCr mice was cultured for 3 weeks and then inoculated with trypanosomes at different initial cell densities. (B) W/W^V cultures of different ages: (●), 2 weeks; (○), 3 weeks; (△), 4 weeks. The flasks were gently shaken at the times indicated, and 0.2 ml of supernatant was removed for hemacytometer counts. In (A) the values are for a single flask at each cell input. In (B) the mean and range for three flasks is shown at each culture age. All ranges are not shown to avoid obscuring mean values; variation at all time points was comparable to data shown.

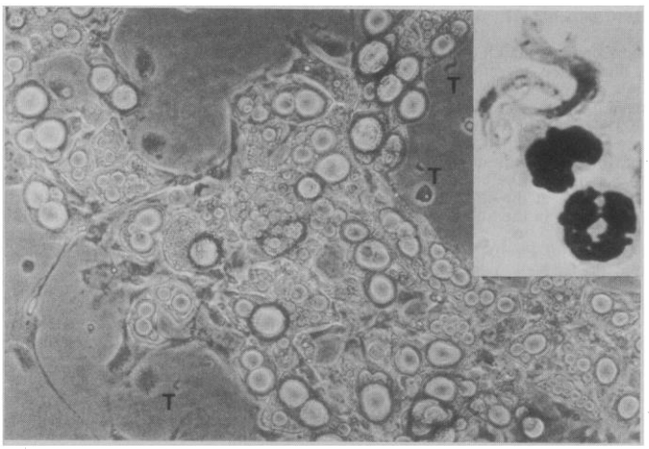

Fig. 2. Adipocyte colonies with supporting epitheloid and fibroblastic elements in 10-week-old BALB/cCr marrow culture. Trypanosomes (*T*) are visible near the adherent cells. Phase contrast. (Inset) Slender bloodstream trypomastigotes and maturing leukocytes from the supernatant of a 3-week-old BALB/cCr marrow culture. Giemsa-stained cytofuge preparation.

somes during remissions of parasitemia in *T. brucei* infections of mice (*4*). In this report I demonstrate that primary cultures of murine bone marrow support rapid growth of infective mammalian forms of the human pathogen *T. b. gambiense* in vitro.

Established methods for initiating bone marrow cultures in the absence of trypanosomes were modified to support the growth of parasites (*8*). Hydrocortisone was used to stimulate the development of adipocytes in the adherent cell layer (*9*). RPMI 1640 tissue culture medium was used because trypanosomes did not grow in Fisher's medium. Primary marrow cultures were not recharged with fresh marrow after adherent stromal cell layers were formed (*8, 9*). The development of bone marrow cultures over 3 weeks was similar to what has been reported previously (*8–10*). Specifically, islands of interacting epitheloid cells, monocytes, and adipocytes, which are known to be necessary for hematopoietic stem cell proliferation in culture, became numerous in the adherent cell layer during the third week (*9, 10*). Hematopoietic activity, as evidenced by production of nonadherent blasts, mature granulocytes, and monocytes was intense for 3 to 4 weeks and then subsided.

Initially, *T. gambiense* was grown in vitro by establishing bone marrow cultures from lightly infected mice (*11*). Trypanosomes from infected marrow persisted in such cultures for more than 2 weeks, but parasite populations remained below 5×10^4 per milliliter until about the third week. Subsequently, trypanosomes multiplied to populations approaching 2×10^6 per milliliter in the same flasks.

When parasites were inoculated into 3- to 9-week-old bone marrow cultures established from uninfected mice, vigorous growth began rapidly. In such cultures, the trypanosomes multiplied exponentially with a doubling time of about 6 hours. This doubling time is similar to that seen in vivo. The parasites multiplied until approximately 2×10^6 parasites were present per milliliter of culture supernatant. This maximum cell yield is comparable to other culture systems with established cell lines (*7*). Parasite counts decreased exponentially after this density was reached (Fig. 1A). The parasites could be maintained for over 2 months in a single marrow culture by weekly feeding if the density was adjusted to about 10^4 per milliliter at the time of feeding. Trypanosomes maintained in such cultures remained fully infective for mice; six out of six mice were successfully infected with ten trypanosomes from a line that had been maintained in culture for 10 weeks. The prepatency period (4 days) and time to death (6 days) were comparable to results obtained when mice were infected with ten blood-passaged trypanosomes. Thus, the virulence of this strain was maintained during growth in culture. Analysis of radioiodinated trypanosomes by electrophoresis on sodium dodecyl sulfate–polyacrylamide gels revealed no differences in the surface proteins of trypanosomes harvested from mice infected with cultured or blood-passaged parasites (*12*).

In established cultures, the trypanosomes grew as typical slender bloodstream trypomastigotes. Most of the trypanosomes were free in the culture supernatant (Fig. 2); a few were associated with the feeder layer, as reported in other culture systems (*7*). More trypanosomes remained in the feeder layer in bone marrow cultures that were less than 3 weeks old or when cultures were established in 2-cm^2 tissue culture wells instead of in flasks. When parasites and marrow were cultured together for extended periods, many of the fibroblastic and epitheloid cell colonies disappeared from culture. The remaining cells included many adipocytes organized in clusters or long strands with epitheloid cells and fibroblasts. Almost all the trypanosomes were in the supernatant of such cultures. Cultures that did not contain adipocyte clusters did not support trypanosome growth, even if large numbers of fibroblastic cells were present. Attempts to culture trypanosomes without bone marrow cells, with nonadherent cells, or with conditioned medium from long-term marrow cultures (*13*) were unsuccessful. These results suggest that the adherent cells that develop during the first 3 weeks of cultivation of bone marrow potentiate trypanosome growth and that adipocyte-containing clusters either are the critical cells or serve as a reliable marker for the development of the critical cells.

Results consistent with this hypothesis were also obtained from studies with bone marrow cultures derived from genetically anemic W/W^V mice (*8*). Bone marrow from these mice lacks hematopoietic stem cells but produces adherent cells that will support the development of competent stem cells from other strains in vivo and in vitro (*14*). Thus if adherent cells in marrow that support hematopoiesis also support trypanosome growth, one would expect W/W^V marrow to be able to promote the growth of *T. gambiense* in vitro. In addition, competition for nutrients between trypanosomes and developing blood cells would be diminished in W/W^V marrow cultures. Figure 1B shows that 2-week-old W/W^V marrow cultures supported trypanosome growth poorly; trypanosome numbers decreased over a week. On subsequent feeding of these cultures, however, vigorous parasite growth occurred. Infection of 3- and 4-week-old W/W^V cultures resulted in parasite densities similar to those obtained in comparable BALB/cCr marrow cultures (Fig. 1B). The lag phase in the W/W^V cultures was somewhat longer than in BALB/cCr cultures; this may reflect the lower initial bone marrow cell input in the W/W^V cultures. Again, the ability of the W/W^V marrow cultures to support trypanosome growth coincided with the development of adipocyte-epitheloid clusters. Little hematopoiesis occurred in these cultures, as expected. Attempts to grow trypanosomes with conditioned medium from W/W^V cultures were unsuccessful. Six of six mice were lethally infected with ten trypanosomes from a line maintained 6 weeks in W/W^V marrow cultures.

Lines of *T. gambiense* have been continuously cultivated in vitro by subinoculating parasites into 3- to 5-week-old

murine bone marrow cultures at 4- to 6-week intervals. Growth in subculture remains vigorous for 1 to 3 months. The line currently being carried has been serially passaged through five marrow cultures in 7 months. Both BALB/cCr and W/W^V marrow has been used successfully to prepare subcultures.

Cultivation of pathogenic trypanosomes with primary cultures of normal host cells offers a means to explore a variety of physiological interactions between host tissues and these extracellular parasites. My results suggest that bone marrow adipocyte-epitheloid complexes that potentiate hematopoiesis may also potentiate trypanosome growth. Three-week-old bone marrow cultures are known to support active hematopoiesis for several months when recharged with fresh marrow as a source of stem cells (*8, 9*). Thus this culture system may also prove useful in elucidating postulated mitogenic or toxic influences of trypanosomes on blood cell precursors (*15*). In addition, it should permit the simultaneous screening of the trypanocidal and marrow-suppressive effects of drugs or other agents in vitro. In this regard, it is significant that human bone marrow can be grown by methods similar to those described here (*16*).

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References and Notes

1. C. A. Hoare, *The Trypanosomes of Mammals* (Blackwell, Oxford, 1972).
2. G. J. Losos and B. O. Ikede, *Vet. Pathol.* **9** (Suppl.), 8 (1972).
3. J. R. Seed and H. G. Effron, *Parasitology* **66**, 269 (1973).
4. A. E. Balber, *Exp. Parasitol.* **35**, 209 (1974).
5. G. S. Z. Ssenyonga and K. M. G. Adam, *Parasitology* **70**, 255 (1975).
6. A. B. Clarkson, Jr., and F. H. Brohn, *Science* **194**, 204 (1976).
7. H. Hirumi, J. J. Doyle, K. Hirumi, *ibid.* **196**, 992 (1977); H. Hirumi, *Bull WHO* **55**, 405 (1977); G. C. Hill, S. P. Shimer, B. Caughey, L. S. Sauer, *Science* **202**, 763 (1978); *Acta Trop.* **35**, 201 (1978); R. Brun, L. Jenni, M. S. Schonenberger, K. F. Schell, *J. Protozol.* **28**, 470 (1982).
8. T. M. Dexter and N. G. Testa, *Methods Cell Biol.* **14**, 387 (1976). The marrow donors were female BALB/cCr (Duke University) or WBB6F1 (W/W^V; Jackson Laboratories) mice aged 8 to 15 weeks. Femoral and tibial marrow was flushed into RPMI-1640 tissue culture medium supplemented with 25 percent horse serum (Gibco), 10 m*M* Hepes, penicillin (100 U/ml), streptomycin (100 μg/ml), Fungizone (25 μg/ml), and 2×10^{-7} *M* sodium hydrocortisone hemisuccinate. The cells were dispersed by two passages through a 10-ml pipette and dispensed into 25-cm^2 Corning 125 flasks. The initial cell input varied between 1 and 2×10^7 cells per flask in 10 ml depending on yield. Cell yields from W/W^V mice were lower than from BALB/cCr, and cultures from the former were initiated with 6×10^6 cells. Cultures were maintained at 37°C in air containing 5 percent CO_2. Loosely adherent cells were removed, and cultures were fed at least weekly by removing and replacing half the culture medium. No differences in trypanosome growth were noted with two different batches of horse serum.
9. J. S. Greenberger, *Nature (London)* **275**, 752 (1978).
10. T. D. Allen and T. D. Dexter, *Differentiation* **6**, 191 (1976).
11. The monomorphic Wellcome TS (TxTaT-1) strain of *T. b. gambiense* was obtained from R. Edwards, University of North Carolina. Bone marrow cultures were prepared from BALB/cCr mice 12 hours after the mice were infected with 10^7 parasites. Alternatively, heparinized cardiac blood from infected BALB/cCr mice was diluted in phosphate-buffered glucose saline, and trypanosomes were separated from most of the blood cells by differential centrifugation (*4*). The parasites were washed twice in culture medium and inoculated into established bone marrow cultures from uninfected mice.
12. A. E. Balber and J. E. Sturtevant, in preparation.
13. Nonadherent cells were centrifuged (800*g*, 10 minutes), washed in culture medium, and resuspended in flasks as described for marrow cultures. Conditioned medium was harvested from 1- to 6-week marrow cultures at the time of weekly feeding. Cells were removed by centrifugation. The medium was either diluted in fresh medium without further treatment or concentrated by pressure dialysis on an Amicon PM10 membrane and added in various proportions to fresh medium, after being sterilized by filtration.
14. J. M. Dexter and M. A. S. Moore, *Nature (London)* **269**, 412 (1977).
15. C. E. Clayton, M. E. Selkirk, C. A. Corsini, B. M. Olilvie, B. A. Askonas, *Infect. Immun.* **28**, 824 (1980).
16. J. S. Greenberger, *In Vitro* **15**, 823 (1979); M. A. S. Moore, H. E. Broxmeyer, A. P. C. Sheridlin, P. A. Meyers, N. Jacobson, R. J. Winchester, *Blood* **55**, 683 (1980); W. E. Hocking and D. W. Golde, *Blood* **56**, 118 (1980); S. Gartner and H. S. Kaplan, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4756 (1980); I. R. G. Toogood *et al.*, *Leuk. Res.* **4**, 449 (1980).
17. Supported by grant CA 14049 from the National Cancer Institute. I thank D. B. Amos for support and encouragement.

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Serum Ferritin as a Predictor of Host Response to Hepatitis B Virus Infection

Abstract. *With hemodialysis patients, a high serum ferritin before there was serological evidence of hepatitis B virus infection increased the likelihood that the infection would be persistent. This finding suggested that hepatitis B virus is likely to infect and actively replicate in liver cells with the propensity for increased ferritin synthesis. The virus itself could stimulate the synthesis of ferritin in a cyclic positive feedback mechanism that increases intracellular ferritin concentration and, eventually, intracellular iron. Transformed liver cells have low iron content, do not replicate hepatitis B virus, and require iron for growth. Infected, nonmalignant liver cells could supply iron to the transformed cells and nourish their expansion.*

Variation in the response of humans to infection by hepatitis B virus (HBV) is one of the most perplexing aspects of the biology of this virus. Females are more likely than males to make antibody to the viral surface antigen (HBsAg), whereas males are more likely to become carriers and to have HBsAg persisting in their blood. The prevalence of antibody to HBsAg increases with age, whereas surface antigen frequency increases initially and then declines after the teenage years in most populations.

To identify factors that might contribute to the carrier state, we examined males with Down's syndrome because they are highly susceptible to HBV infection (*1*). We measured serum iron, in addition to other variables, because iron is often increased in patients with hepatitis. We found that serum iron and transferrin saturation values were higher in the patients who were carriers than in those who were not carriers. These findings were confirmed in a study of male and female patients undergoing hemodialysis treatment (*2*).

Those studies established that the association of increased serum iron with the HBV carrier state was independent of the increased serum iron resulting from liver cell breakdown as measured by alanylaminotransferase (ALT; formerly referred to as SGPT). However, it was not possible to ascertain whether the increased serum iron was a consequence of HBV infection or, alternatively, whether the carrier state occurred more frequently in individuals with increased serum iron.

In a retrospective cohort study of patients treated with hemodialysis, changes in iron level were measured during a period in which HBV infection was known to have occurred (*3*). Three conditions had to be met for a patient to be included in the study: (i) the patient had to have at least one serum sample that was positive for HBsAg; (ii) the serum sample obtained 2 months before HBsAg was first detected had to be negative for both HBsAg and its antibody; and (iii) a serum sample had to be obtained 12 months after HBsAg was first detected (*4*). Fifty-four patients who met these criteria during the period 1974 to 1981 were included in the analysis. The key finding was that the amount of iron in the serum before the initial finding of HBsAg was no greater in individuals destined to be carriers than in those who proved to be transiently infected.

In blood, iron is transported by transferrin and is stored primarily in the liver and spleen in the form of ferritin. Although most of the ferritin in the body is intracellular, it is found in low concentrations in the serum. In normal individ-