cubation. Figure 2 shows the effects of various T3 concentrations on the rate of glucose metabolism in myocardial cells after 40 to 60 hours of incubation. The maximum increase in the rate of glucose utilization was approximately twice the rate in cultures containing hypothyroid calf serum and a half-maximal biologic effect was observed at an estimated free T3 concentration of $2.4 \times 10^{-11} M$.

Nuclear binding of thyroid hormone in cardiac tissue in vivo has been reported (*13*), but such binding has not been related to specific myocardial responses. We compared the affinity constant of T3 for isolated nuclei in vitro obtained from newborn and adult rat hearts, using methods previously described (*6, 14*). Figure 3 shows that K_d was $2.5 \times 10^{-10} M$ for nuclei from newborn rat heart and $0.9 \times 10^{-10} M$ for nuclei from adult rat heart. At these concentrations, with the T3 occupying 50 percent of the nuclear binding sites, the results were virtually identical to those previously reported for isolated rat liver and GH_1 cell nuclei (*6*). With isolated nuclei in vitro, the K_d values were approximately five times the values obtained with intact cells (*6*). Although the exact mechanism of the effect of T3 on glucose utilization is not known, the similarities in the K_d values for T3 binding to isolated nuclei from newborn rat heart and from GH_1 cells in vitro, and the similar biologic responses in the two systems support the concept that the effect of T3 on cultured myocardial cells is regulated at the nuclear level (*3, 12*). This concept is also supported by the kinetic studies illustrated in Fig. 1 in which the effect of T3 is consistent with a time-dependent induced change in the system rather than an immediate response to thyroid hormone.

JIR S. TSAI
AMY CHEN

Endocrine Division, Department of Medicine, New York University School of Medicine, New York 10016

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Trypanosomiasis: An Approach to Chemotherapy by the Inhibition of Carbohydrate Catabolism

Abstract. *When the infected mammalian host of* Trypanosoma brucei brucei *is injected with a solution of the iron chelator salicyl hydroxamic acid and glycerol, the aerobic and anaerobic glucose catabolism of the parasite is blocked and the parasite is rapidly destroyed.*

The protozoans *Trypanosoma brucei rhodesiense* and *T. brucei gambiense* are the causative agents of African human sleeping sickness, and the closely related *T. brucei brucei* is one of several tyrpanosomes that cause a similar disease in cattle. All three parasites are transmitted by the tsetse fly vector to a mammalian host (*1*). No new effective drugs against these diseases have been developed in recent years, and resistance of the parasites to most of the drugs now in use has been reported (*2*). We are able to block both aerobic and anaerobic pathways of adenosine triphosphate (ATP) production in these parasites by the simultaneous administration of salicyl hydroxamic acid (SHAM) and glycerol to the host. Because the production of energy in the form of ATP is entirely dependent on carbohydrate metabolism (*3*), the parasites are immediately immobilized and almost entirely cleared from the blood within 5 minutes.

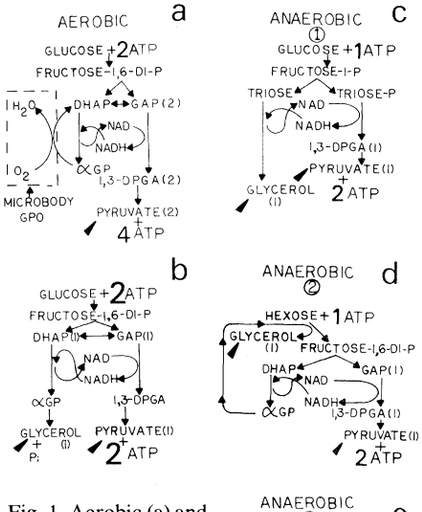

Fig. 1. Aerobic (a) and possible anaerobic (c through e) metabolic pathways in *T. b. brucei*. The pathway illustrated in (b) would generate no net ATP. Metabolic end products are marked by arrowheads. Abbreviations: ATP, adenosine triphosphate; fructose-1,6-di-P, fructose-1,6-diphosphate; DHAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; NAD, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; αGP, L-α-glycerophosphate; 1,3-DPGA, 1,3-diphosphoglyceric acid; GPO, L-α-glycerophosphate oxidase; fructose-1,6-di-P, fructose-1,6-diphosphate; Pi, inorganic phosphate; fructose-1-P, fructose-1-phosphate; GA, glyceraldehyde.

Carbohydrate metabolism of the trypanosome in the insect vector is complete—that is, it includes glycolysis, a tricarboxylic acid (TCA) cycle, and reoxidation of coenzymes by cytochromes in the kinetoplast-mitochondrion (*4*). In contrast, only glycolysis occurs in the long slender (LS) form, the principal stage of the parasite in the bloodstream of the mammal. The other stage in mammals, the short stumpy (SS) form, develops in the bloodstream from the LS form. The SS forms are believed to be preadapted to the insect vector and have a partial TCA cycle but no functional cytochromes (*3*). It is the LS forms that divide in the mammal and probably account for the pathology of the disease. Evidence indicates that the SS forms are at an end point in the mammal and do not reproduce unless taken up by the vector (*5*). Parasitemias in mammals infected with these organisms differ in their ratios of LS forms to SS forms, the ratios varying with the strain of the trypanosome and the stage of infection, even in cloned populations of parasites. To diminish this variation and to concentrate on the LS form, we used a clone of *T. brucei brucei* that was isolated from EATRO (East African Trypanosomiasis Research Organization) strain 110 and made monomorphic (LS form only) by 30 rapid pas-

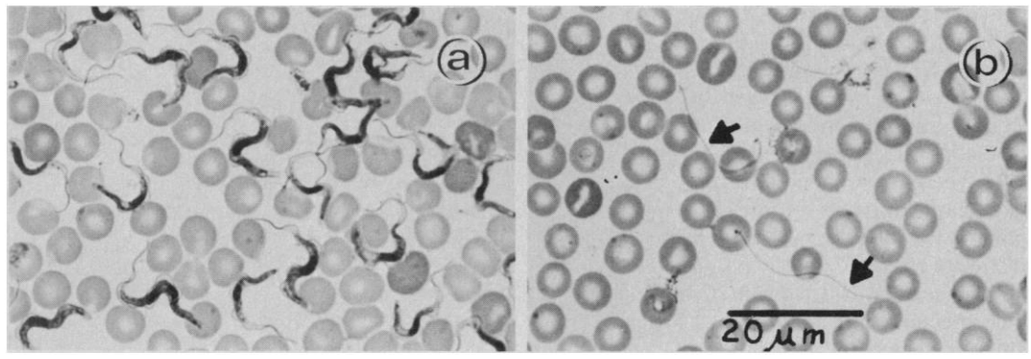

Fig. 2. Giemsa-stained blood film of a rat infected with *T. b. brucei*. (a) Before treatment. (b) Three minutes after treatment. Lysed trypanosome debris is evident and two flagella with attached kinetoplasts are marked with arrows.

sages in rats. In experiments with *T. brucei rhodesiense*, we used EATRO strain 1895 which produces both LS and SS forms.

No lactate is produced by the LS forms of either *T. b. brucei* or *T. b. rhodesiense*, and pyruvate is the chief end product of glucose catabolism. The highest reported yield of pyruvate generated from glucose by *T. b. brucei* is 92 percent (*6*). Although this is better than the previous high value of 83 percent, which was obtained from apparently rapidly deteriorating cells (*7*), we find that under the proper conditions the true conversion of glucose to pyruvate is 100 percent. By using a more suitable phosphate-saline buffer and better means of separating the protozoans from host blood cells (*8*), we are able to maintain the parasites in good morphological condition in vitro for at least 12 hours, and glucose consumption is linear over a 4-hour period at 25°C. Using Sigma glucostat reagent for glucose and the change in absorbance at 340 nm in the presence of excess oxidized nicotinamide adenine dinucleotide (NAD+) with lactate dehydrogenase added to measure pyruvate, we found that a suspension of the LS forms of *T. b. brucei* (2.5×10^8 cells per milliliter) consumed 4.06 ± 0.3 μmole (± S.E.) of glucose and produced 8.15 ± 0.20 μmole of pyruvate per hour. These values were constant over a 3-hour period. Aeration was maintained by constantly bubbling air through the cell suspension. We conclude that in the LS forms, pyruvate is the only end product of glycolysis in the presence of adequate molecular oxygen.

Reoxidation of reduced nicotinamide adenine dinucleotide (NADH) produced by glycolysis is required for continuous energy production. However, this is not accomplished by either lactate dehydrogenase or cytochromes in the organisms but by a glycerophosphate oxidase system (*9*) which is localized in the microbody organelles (*10*) (Fig. 1a). The pathway shown in Fig. 1a requires the presence of molecular oxygen; however, both the LS and SS forms of these parasites can survive anaerobically for several hours. The end products of cell metabolism under anaerobic conditions are primarily pyruvate and glycerol in equimolar amounts (*6, 7*). To account for both the stoichiometry of these end products and the reoxidation of NADH formed under anaerobic conditions, it has been proposed (*6, 11*) that cells regenerate NAD+ from NADH by reducing dihydroxyacetone phosphate to L-α-glycerol phosphate (αGP), and subsequently degrade the αGP, which is not reoxidized anaerobically, to glycerol and orthophosphate (Fig. 1b). It has been pointed out most clearly by Opperdoes *et al.* (*6*) that because this pathway will generate zero net ATP it cannot possibly account for anaerobic glycolysis in these organisms.

We have reached somewhat the same conclusions reported by Opperdoes *et al.* (*6*). Hexose monophosphate aldolase activity could reduce ATP input and yield both a triose and a triose phosphate. The triose phosphate could yield two molecules of ATP per mole with the triose reduction to glycerol mediated by way of NADH (Fig. 1c). Aldolase activity with fructose-1-phosphate is present; however, we cannot detect triose reductase activity. It is also possible that αGP is used to transphosphorylate a hexose, thus reducing the ATP input requirement and yielding net positive ATP production (Fig. 1d). A third possibility—one not previously considered—is that a monophosphate aldolase produces a triose and a triose phosphate, the triose phosphate being reduced by NADH to αGP and the αGP used to transphosphorylate the triose. This would generate glycerol and a second triose phosphate which could yield two molecules of ATP (Fig. 1e).

Notwithstanding uncertainties concerning the pathway of anaerobic glycolysis in *T. b. brucei*, if aerobic and anaerobic glycolysis formed the only basis of energy production in these cells, simultaneous inhibition of these activities should result in cell death. All of the anaerobic schemes discussed here include glycerol as an end product of fermentation in a reaction that would involve a small negative change in Gibbs free energy (ΔG or ΔF) or a slightly positive ΔG, depending on the microenvironment. If this were the case, glycerol should block the alternate pathway by mass action. Glycerophosphate oxidase function is blocked by the lack of molecular oxygen and also by several hydroxamic acids, including SHAM (*6, 12*). We have found that 1 m*M* SHAM in the presence of 2.5 m*M* glycerol causes immediate loss of motility of *T. b. brucei* in vitro, and within 3 minutes extreme alteration and damage to the cells is visible by phase contrast microscopy.

The effect of SHAM and glycerol on the parasites in vivo was tested by using rats heavily infected with *T. b. brucei* (1.2×10^9 parasites per milliliter of blood). After injecting intravenously (5 ml per kilogram of body weight) a solution of SHAM (125 m*M*) and glycerol (600 m*M*) (96 and 276 mg/kg, respectively), all the parasites were immobilized within 1 minute, and no intact motile parasites and few ghosts were observed after 3 minutes. All of the uninfected control animals treated with six times the amount of SHAM and glycerol given the experimental animals survived.

A Giesma-stained blood smear of a rat infected with *T. b. brucei* (1.5×10^9 cells per milliliter) is shown in Fig. 2a. Blood taken from the same rat 3 minutes after we injected into the tail vein both SHAM and glycerol (182 mg/kg and 552 mg/kg, respectively) is shown in Fig. 2b. Only a few whole parasites are visible and these are clearly abnormal. Many flagella, some apparently with the kinetoplast portion of the kinetoplast-mitochondrion still attached, and what appeared to be parasite nuclei, were seen in the blood

from treated animals. No parasites or parasite debris were seen 24 hours after treatment.

Mice that were infected with *T. b. rhodesiense* and that showed both the LS forms and SS forms of the parasite in the blood were also injected with SHAM and glycerol. One hour after treatment only a monomorphic SS form was present in the blood, and after 24 hours no parasites were observed.

Six days after the treatment of rats and mice infected with *T. b. brucei*, the parasites reappeared in the blood and the animals died within a few days. Without treatment, the animals would have died within a few hours. Mice infected with *T. b. rhodesiense* also showed a recurrence of parasitemia after treatment. We have found no regimen of treatment that prevents the recurrence of parasitemia, although the response of the parasite population to successive treatments remains the same and the parasites do not appear to become resistant to the treatment. Two possible explanations for the recurrence are (i) the entire population of parasites contains a few resistant cells with a partial TCA cycle, and (ii) the effective trypanocidal levels of SHAM and glycerol are not reached in some tissues and parasites in those tissues survive. The second hypothesis seems more probable, because temporal separation of SHAM and glycerol administration by 5 minutes abolishes their therapeutic value, suggesting that they are cleared very rapidly from the blood. By finding a substitute for glycerol or a different method of administration, it might be possible to maintain therapeutic levels of the drugs long enough for all infected tissues to be reached. Similarly, other hydroxamic acids or iron chelators might be more effective in blocking the activity of glycerophosphate oxidase.

The results presented here promise a rational approach to trypanosome chemotherapy based on knowledge of the peculiar carbohydrate catabolic pathways of these parasites.

ALLEN B. CLARKSON, JR.
FREDERICK H. BROHN

Laboratory of Parasitology,
Rockefeller University,
New York 10021

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Depletion of Brain Catecholamines: Failure of Ocular Dominance Shift After Monocular Occlusion in Kittens

Abstract. *Monocularly deprived kittens were compared with littermates that had had their eyelids sutured for the same time but that had, in addition, been treated with 6-hydroxydopamine to deplete their forebrains of catecholamines. The visual cortices of all the catecholamine-depleted kittens showed high proportions of binocular neurons, in contrast to the control group, most of whose visual cortical neurons were driven exclusively by the nondeprived eye. Catecholamines may play an important role in the maintenance of cortical plasticity during the critical period.*

A valuable paradigm for the effects of experience on brain function is the change in binocularity of kitten visual cortical neurons which occurs after eye occlusion. Monocular visual experience results in increased numbers of neurons which respond exclusively to the previously open eye, with a loss of the normal, binocularly activated neurons. These changes in ocular dominance are virtually permanent (*1*), occur rapidly (*2*), and are confined to a well-defined postnatal period of susceptibility or "critical period" (*3*). A key question concerns the factors which determine the beginning and end of this period of neural plasticity.

In this report we present a preliminary test of the hypothesis that the catecholamine neurohormones are required for the maintenance of visual cortical plasticity during the critical period. Our research was stimulated both by the hypotheses of Kety (*4*) and Crow and coworkers (*5*) that the brainstem monoamine pathways are involved in the forebrain's plasticity, and by recent work linking the monoamines with brainstem pathways which have powerful effects on the visual pathway (*6*), and which appear to mature during the critical period (*7*). To examine the role of catecholamines we used 6-hydroxydopamine (6-OHDA), a specific neurotoxin that is taken up from the cerebrospinal fluid by axon terminals which contain norepinephrine or dopamine and results in their destruction (*8*). Electrophysiological recording supports the hypothesis to the extent that the usual changes in binocularity of cortical neurons do not follow monocular occlusion in kittens whose cortices have been depleted of catecholamines by 6-OHDA.

Four pairs and one trio of littermates were obtained from our quarantined cat colony, a partially inbred line of tabby cats. Each pair included a control and an experimental kitten, and the trio (JT4, JT2, and TJ12; see Table 1) included two different kinds of controls as well as an experimental animal. Each of the 11 kittens had a fine stainless steel cannula with trocar implanted in the right lateral ventricle under ketamine anesthesia (30 to 40 mg/kg) during the fourth to seventh week after birth. The right eyelid of all kittens was sutured under Fluothane anesthesia (for rapid recovery) during the same period, some time after ventricular cannulation (Table 1). Each procedure was timed to coincide as closely as possible for both members of an experimental-control pair (Table 1).

Using the permanently implanted cannula as a guide, we injected into the ventricle a dose of 6-OHDA plus vehicle (16 μg of 6-OHDA per microliter of 0.05 to 0.1 percent ascorbic acid in Ringer solution), or the vehicle solution alone (*9*). Our choice of dose was guided by previous studies on neonatal rats and adult cats (*10*) and by close observation of the behavioral effects following injection (*11*). Because we do not have reliable data on the lower limits of catecholamine levels achieved by our treatment (*12*), and because some evidence indicates that the nerve terminals recover rapidly after cessation of treatment (*13*), we gave repeated large injections for more than 1 week. For example, 200 μg of 6-OHDA