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MAR 79 A C ZAHALSKY, R L WEINBERG DAMD17-74-C-4140

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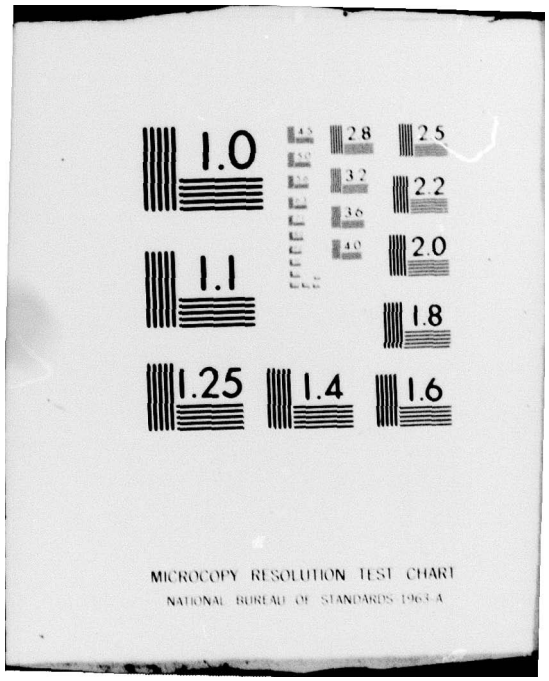
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DRUG EFFECTIVENESS AND TRYPANOSOME BURDEN IN MICE*

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ABSTRACT

The response of trypanosome infected mice to Antrycide (quinapyramine), Hydroxystilbamidine and Isometamidium was found to depend on the parasite burden at the time of drug administration. With a monomorphic infection some variables that were found to influence the study of drug action in vivo include: (i) parasitemia level at the time of drug administration, (ii) drug dose at the selected parasitemia level and (iii) time span to the onset of clearing when the conditions in (i) and (ii) have been chosen.

It has been reported that the effective clearing of some African bloodstream trypanosomes is related to the level of parasitemia attained at the time a drug is given (Walker and Opiyo, 1973). Apart from other criteria of activity, trypanocidal compounds have been described as 'early' or 'late' acting depending upon the time of onset of clearing.

Studies on the mode of action of trypanocidal agents in vivo often require that sufficient numbers of organisms be harvested from the host at some critical time after drug administration, i.e. an adequate quantity of drug-exposed organisms may be needed to extract labeled macromolecules. To properly time the introduction of labeled precursor it was necessary to have information on the duration and level of parasitemia attained after injection of varying drug dosages.

In the present study we examine the relationship of initial trypanosome burden to effective drug dose, the course of parasitemia following drug administration, and the onset and duration of the period of clearing. Utilizing these criteria comparisons are drawn between effects obtained with a diamidine and a phenanthridine class compound. The monomorphic, rodent-adapted strain of Trypanosome brucei used in these studies produces a fulminating parasitemia in untreated mice resulting in death of the animal, usually within 4-5 days. The results obtained are discussed with reference to their value in predicting the time to chemotherapeutic cure (in the mouse) and the likely advantage of introducing high specific activity labeled precursor during drug exposure but before a parasitemia peak is reached.

METHODS

Parasitemia was routinely maintained by intraperitoneal (ip.) passage of a small volume (0.2 ml) of diluted whole blood into näive 22-25g male NLW mice (National Laboratory Animal Co., O'Fallon, Mo.). Experimental animals were first grouped according to size and then injected ip. with a known no. of organisms contained in a TRIS-glucose-EDTA buffer (pH 7.4) volume of 0.2 ml. The rise in parasitemia was initially followed by examination of wet smears and then by hemocytometry of tail blood samples when a level of ~5×10^6 organisms/ml blood was attained. Animals exhibiting near replicate parasitemias, as determined solely by hemocytometry, were grouped and injected ip. with drug(s) as indicated in Figures 1-3. Drug solutions were always made up fresh in deionized water. After drug injection animals were monitored every 4-5 hr for numbers of trypanosomes/ml blood by hemocytometry of tail blood samples. A TRIS-glucose-EDTA buffer, pH 7.4, was routinely used as diluent for the red cell counting pipettes. All animals were provided with food and water *ad libidum* during the course of these experiments.

RESULTS AND DISCUSSION

In all graphs each time point represents the mean of hemocytometer counts performed on tail blood samples of 3-6 mice having similar parasitemia and having been identically injected with drug at the dosages indicated. As a point of reference for our findings with Antrycide, Hydroxystilbamidine and Isometamidium, Table 1 gives summary data on these compounds cited by Hawking (1963).

Antrycide toxicity to the host appears to be dependent on the parasite burden, i.e. 10 mg/kg ip. is tolerated at a parasitemia level of 9×10^6 - 10^7 organisms/ml blood whereas in uninfected mice or in animals exhibiting a low infection (1-2 trypanosomes per HD field) this same drug dose represents an LD_{50}. Likewise, the minimum curative dose is dependent on the parasite burden, i.e. 10 mg/kg ip. did not clear or cure when administered at an infection of 9×10^6 - 1.8×10^7 organism/ml (Figure 1) whereas 8 mg/kg cured a low infection of 1-2 organisms/HD field after a latent period of 15-26 hr.

A latent period of 8-16 hr was noted when hydroxystilbamidine was administered to animals exhibiting parasitemia's in the range 0.8 - 1.9×10^8 organisms/ml (Figure 2). As shown, the lag period appears to be dependent on drug dose whereas clearance shows dependence on both drug dose and initial parasite burden, e.g. at 10 mg/kg and a parasitemia of 8×10^8 organisms/ml clearing occurs at about 50 hr compared to 70 hr when 7 mg/kg are given at a starting infection of 2×10^8 organisms/ml. Although hydroxystilbamidine was effective in clearing at relatively high initial parasite burdens, ~2×10^8 organisms/ml, at the minimum effective dose

(1.7 mg/kg) and effective dose (3.4 mg/kg) parasitemia re-emerged and the animals died, usually within 72 hr after detection. Curative doses noted were 7 mg/kg at a parasitemia level of 1.8×10^8 organisms/ml and 3 mg/kg at an initial infection of 2.5×10^7 organisms/ml. During the clearing period the morphology of the trypanosomes was altered - the parasites appeared swollen and a decrease in motility was evident.

The decline in parasitemia noted with Isometamidium was preceded by a latent period of 36-46 hr (Figure 3). At 40, 10 or 3 mg/kg and at a parasitemia of $1\text{-}2.5\times10^7$ organisms/ml all animals were cured and remained clear for several months. At 1 mg/kg and 3×10^6 organisms/ml 4 of 6 animals were cured whereas at a starting parasitemia of 2.5×10^7 organisms/ml only half the animals were cured.

Accuracy in the hemocytometry of tail blood samples requires a minimum parasitemia level of $\sim5\times10^6$ organisms/ml blood. Drugs were therefore injected when animals showed parasitemias in the range $8\times10^6\text{-}4\times10^7$ organisms/ml blood. With Isometamidium (Fig. 3) the relationship of drug dose to the time of attainment of a peak in parasitemia, and the trypanosome numbers/ml at this peak were: 1mg/kg, 39-42hr, $8\times10^8\text{-}2.4\times10^9$; 3mg/kg, 34-38hr, 1.5×10^9; 10mg/kg, 26-28hr, 10^8 and 40 mg/kg, 22hr, 6×10^7. In previous studies with Berenil (ibid) a post-drug parasitemia of $\sim2\times10^9$ cells/ml was required for analysis of DEAE-purified organisms. Since the generation time of this rodent adapted laboratory strain is 5-5.5hr, 3mg/kg Isometamidium at a starting parasitemia of $\sim4\times10^7$ organisms/ml provides sufficient time for the drug to act (up to 7 generations) yet results in the yield of an adequate amount of cell material. In the case of Hydroxystilbamidine (Fig. 2) 1.7 mg/kg resulted in a parasitemia

of 1.1-1.2×10^9 organisms/ml ~24 hr after drug administration. Although the onset of clearing with this compound was dose dependent, a cure was not achieved at 3.4 mg/kg, i.e. a reoccurrence of the parasitemia followed remission. Only at the highest doses (7 & 10 mg/kg) was cure achieved. The least effective agent in this type of infection was Antrycide. At the highest dose used, 10 mg/kg, corresponding to an LD_{50} in mice (Hawkings, 1963), no effect on parasitemia was seen when the initial infection was ~10^7 organisms/ml blood.

Predicting the start and duration of drug clearing of a monomorphic infection at a known initial parasitemia level also provides information on the time to attain a peak in parasitemia in the presence of drug. This interval in a fulminating parasitemia confers the distinction between early and late acting drugs. Preferably during this time span a radiolabeled precursor may be introduced to monitor drug effect(s) on macromolecular syntheses. Considering the decay in serum radioactivity following ip. injection, and the competition by host cells for precursor during this period, the introduction of label should preferably be timed to occur within 2 hr after drug administration. Drug-treated organisms exposed to isotopes and harvested within 18-20 hr after treatment contain sufficient radioactivity in their nucleic acids and protein to permit chemical analyses (Zahalsky and Zahalsky).

With an established strain, application of the criteria noted in these studies may permit the design of 'trypanosome nomograms' for investigators desiring to know ... "how much drug for how many organisms and with what yield."

LITERATURE CITED

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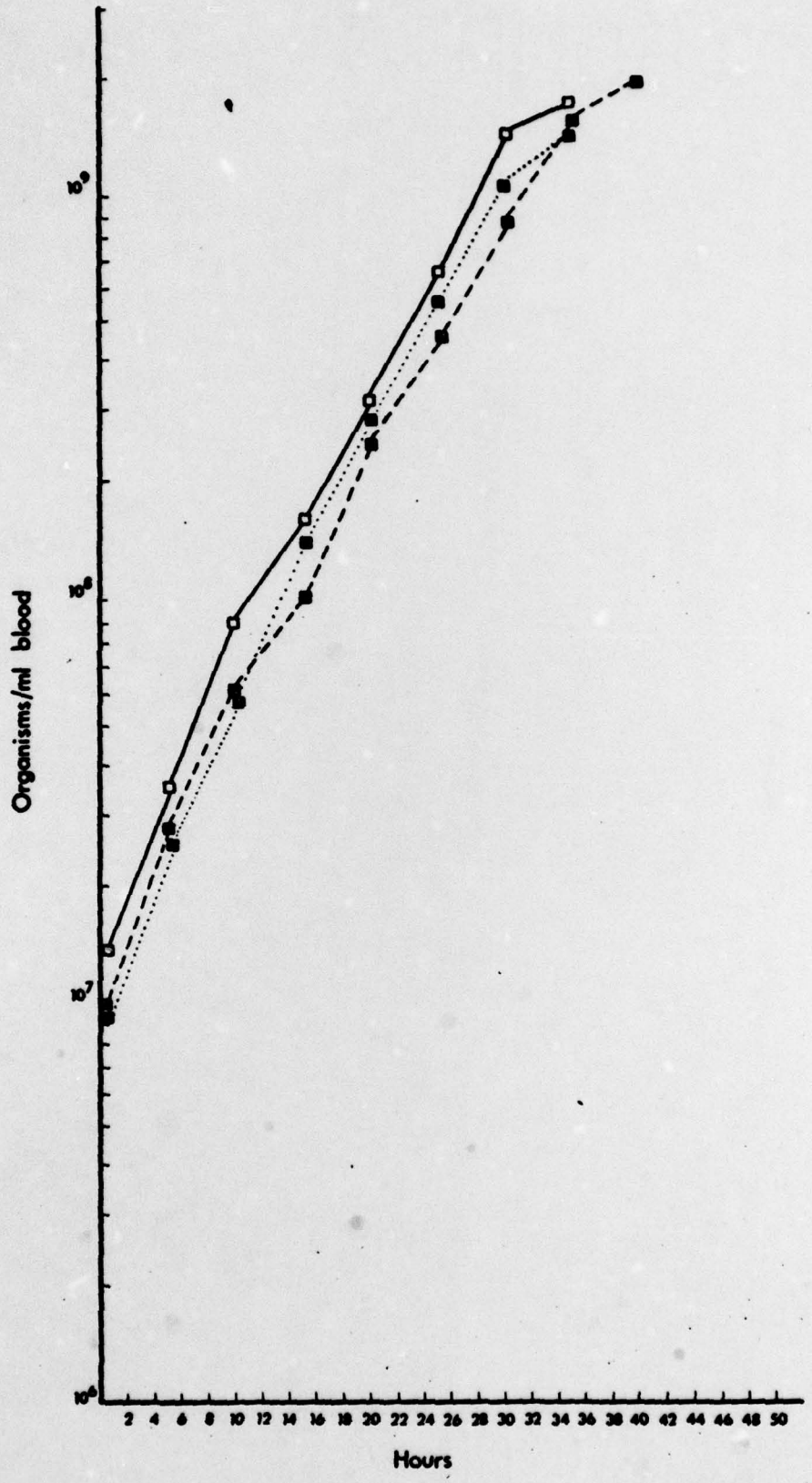

Fig. 1 Effect of Antrycide on T.brucei Parasitemia

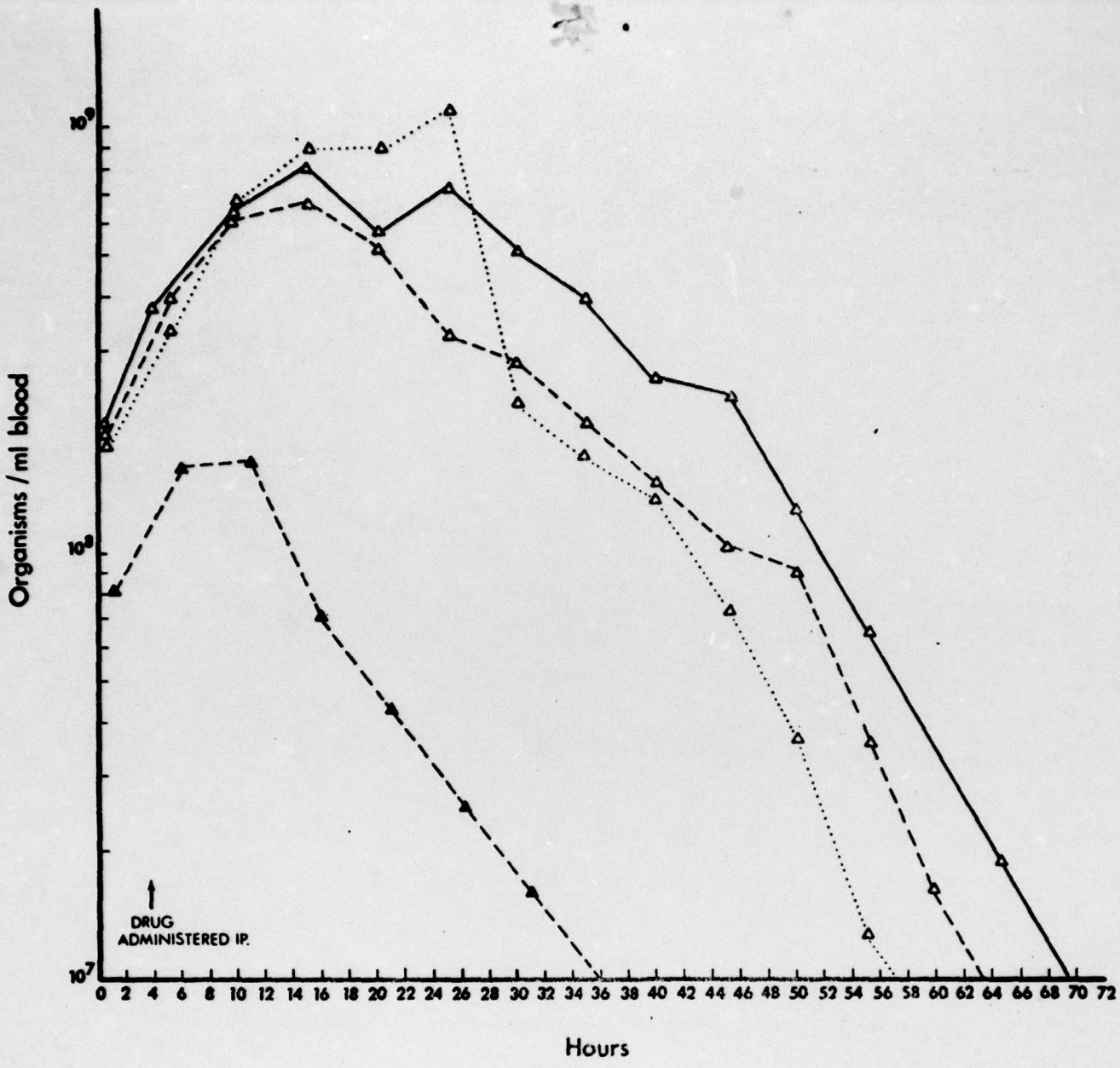

Fig. 2 Effect of Hydroxystilbamidine on T. brucei Parasitemia

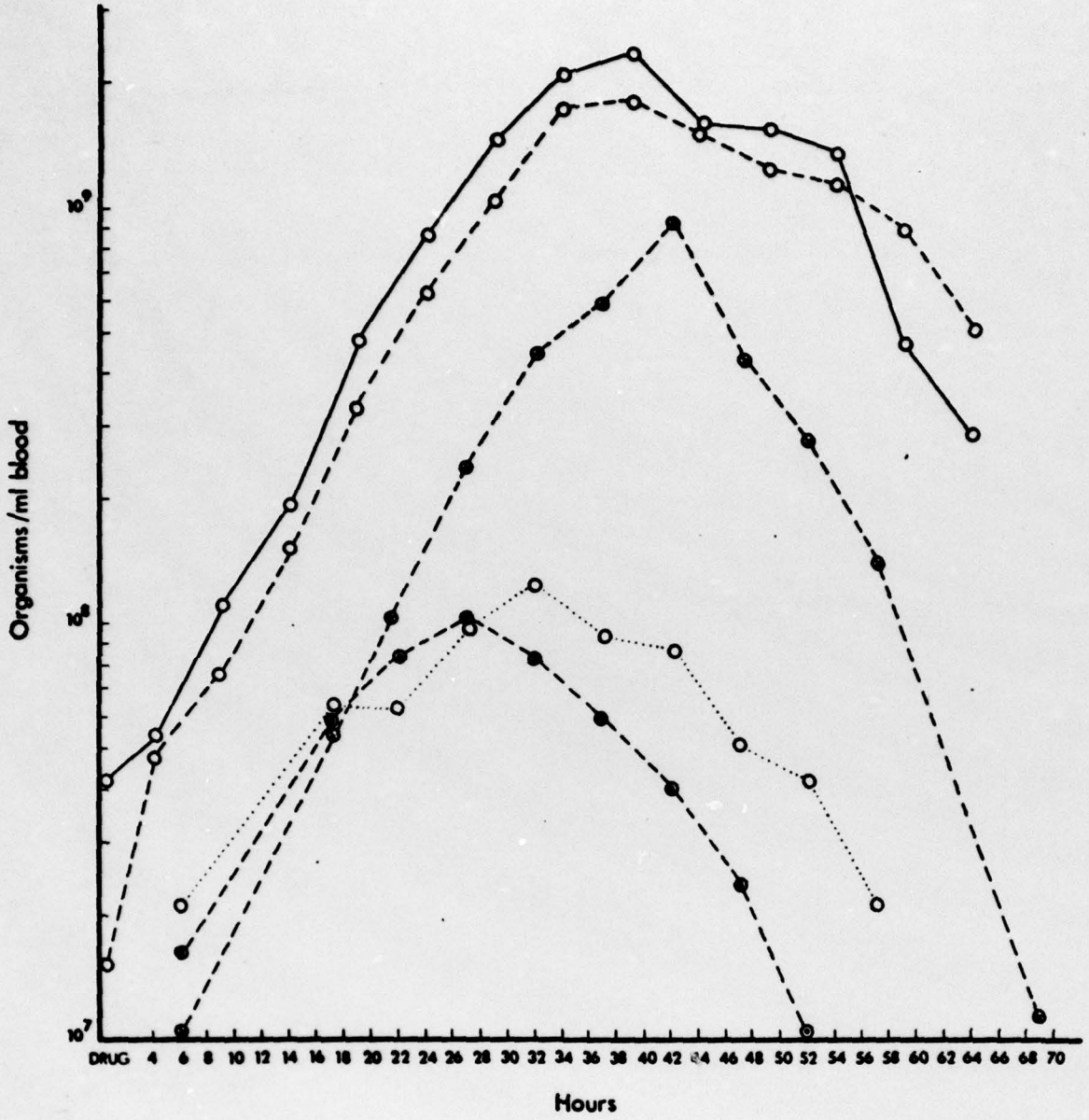

Fig. 3 Effect of Isometamidium on T. brucei Parasitemia

Table I. Reported Effectiveness of Some Trypanocidal Compounds

(All values shown in mg/kg; administration by intraperitoneal route unless otherwise indicated)

Drug	Organism	LD_{50}	MED	MCD	Comments
Antrycide	T. brucei (Liverpool strain)	15-20	2.5 subcutan	2.5 subcutan	Parasitemia decreases after a latent period of 24-48 hr.
Hydroxystilbamidine	T. rhodesiense	50	0.25	2.5	MED for T. congolense = 12.5
Isometomidium	T. congolense	50	.005	.01	Parasitemia decreases after a latent period of 24-48 hr. Phenanthridinium compounds generally more active against T. congolense and T. vivax than on T. gambiense group.

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