favours the assumption of an enhanced metabolism of ibuprofen, although the difference is not statistically significant.

Liver enlargement, as observed by Adams *et al.* [3] after many weeks treatment, might indicate an inducing property of ibuprofen, since many inducers increase liver weight [14]. This is true even for typical microsomal inhibitors like SKF 525-A [15] or phenylbutazone [16].

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Drug-induced lesions in trypanosome fine structure: a guide to modes of trypanocidal action

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Trypanocidal drugs have incompletely known modes of action derived mainly by surmise from their effects on non-trypanosomal systems [1, 2]. In the present study, representative drugs wre chosen from the main classes of trypanocide: diamidine (pentamidine, hydroxystilbamidine, Berenil); adenine nucleoside (Puromycin, Puromycin aminonucleoside, Cordycepin(3′-deoxyadenosine), Nucleocidin); aminoacridine (acriflavine); amino-phenanthridine (Ethidium); aminoquinaldine (Antrycide); naphthylamine sulphonate (suramin) and arsenical (tryparsamide, Mapharside). Most of them were assayed under standard conditions *in vitro*, using only one monomorphic strain of trypanosome (*Trypanosoma rhodesiense* (N) [3]), so that comparative inhibition of parasite motility, infectivity, respiration and glycolysis could be recorded (Table 1). As the bloodstream form of this trypanosome does not multiply *in vitro*, infectivity was used as an index of cell division; motility correlates well with respiration and glycolysis, so that simple *in vitro* tests of effects on infectivity and motility with this strain can show if the primary action of a trypanocidal drug is likely to be on macromolecular synthesis or on energy-yielding reactions.

The trypanocides were also used to treat the same trypanosome strain *in vivo*, and the ultrastructural lesions produced were analysed as a further guide to drug action. Infected mice were treated with curative intraperitoneal drug doses, and blood samples were taken 5–6 hr after treatment and processed for electron microscopy as described elsewhere [3]; in the case of Mapharside, which acts rapidly, the sample was taken 1 hr after treatment. All fixation and preparative procedures were rigidly standardized, and as none of the lesions to be described were found in the normal untreated trypanosome, these lesions have been ascribed to drug action; the lesions have also been found to be detectable and reproducible with other fixation and preparative methods (unpublished work with Dr. D. J. McLaren, to whom we are indebted for Figs. 1, 2a and 3).

In vitro, only Ethidium, Antrycide and suramin affected trypanosome infectivity (cell division) more than respiration or glycolysis (Table 1). Like the highly active acriflavine and the thiol-reactive phenylarsenoxide Mapharside, Cordycepin affected all four activities equally but was even more strongly inhibitory; its rapid cytotoxicity may be due to incorporation into and inactivation of adenine nucleotide coenzymes as occurs with the related Tubercidin [4]. The remaining drugs were not markedly selective, but nitrofurazone, an active electron-acceptor [5], inhibited respiration much more strongly than glycolysis, and pentamidine showed preferential inhibition of glycolysis. Puromycin and Puromycin aminonucleoside tended to affect infectivity more than respiration or glycolysis; like Ethidium [6] and Antrycide[7], both are powerful inhibitors of macromolecular biosynthesis[8,9]. Acriflavine and Cordycepin are also known to inhibit nucleic acid synthesis [10, 11], but strong inhibition of energetic processes is obviously an additional, if not a primary cause of rapid cytotoxicity.

Further insight into trypanocidal action was provided by study of characteristic drug-induced lesions in fine structure. Various cytotoxic agents produce injuries in the mammalian cell which can be correlated with their biochemical properties. For example, Puromycin [12] and ethionine [13] are protein synthesis inhibitors and cause changes in polyri-

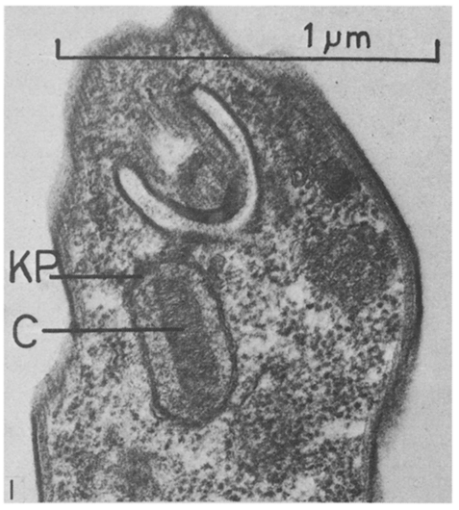


Fig. 1. Kinetoplast (KP) of normal *T. rhodesiense*, with fibrillolamellar DNA core (C).

bosomal patterns, suggestive of altered protein synthesis. Nucleolar micro- and macro-segregation (separation of granular and fibrillar elements) is almost invariably produced by inhibitors of nuclear RNA polymerase activity [14]; this is an important diagnostic lesion, as the nucleolus produces 80–90 per cent of the cell RNA.

The principal lesions found in *T. rhodesiense* were in the ribosomes, nucleus, kinetoplast (a secondary DNA-containing "nucleus-mitochondrion", Fig. 1), lysosomes and endoplasmic reticulum. The nature of these lesions permits some deductions about possible modes of trypanocidal action.

Diamidines. Although diamidines such as pentamidine did not appear to affect macromolecular synthesis selectively *in vivo*, all three diamidines produced a characteristic lesion in the kinetoplast where the looped fibrillolamellar DNA core was condensed terminally ("sliced sausage" profile) (Fig. 2a). Pentamidine also produced nucleolar segregation (Fig. 3) (indicative of inhibition of *r*RNA synthesis), extensive ribosomal alterations (aggregation, depopulation, loss of electron density, hypertrophy), and occasional cytoplasmic clefts (Fig. 4).

Adenine nucleosides. All four drugs characteristically produced electronlucent cytoplasmic clefts (Fig. 4) which were generally acicular, with one side bounded by a membrane of the endoplasmic reticulum. In addition, Cordycepin and Nucleocidin produced nucleolar segregation and ribosomal alterations; clumping and peripheral margination of nuclear chromatin were also produced by Nucleocidin.

Aminoacridines. Acriflavine, like the diamidines, produced terminal condensation of kinetoplast DNA (Fig. 2a), and also massive disruption of nuclear DNA, with loss of the nuclear membrane, scattering of chromatin into the cytoplasm and extensive nucleolar segregation. The ribosomes were altered extensively.

Aminophenanthridines. The only effect of Ethidium was to attack the kinetoplast DNA core, but the condensation was amorphous (Fig. 2b) and different from that produced by the diamidines (Fig. 2a).

Aminoquinaldines. Antrycide produced the same kind of kinetoplast DNA condensation as the diamidines (Fig. 2a). It also caused focal loss and aggregation of ribosomes.

Naphthylamine sulphonates and arsenicals. Suramin, tryparamide and Mapharside affected the ribosomes primarily and extensively, producing general and focal depopulation. The arsenicals also produced cytoplasmic clefts (Fig. 4).

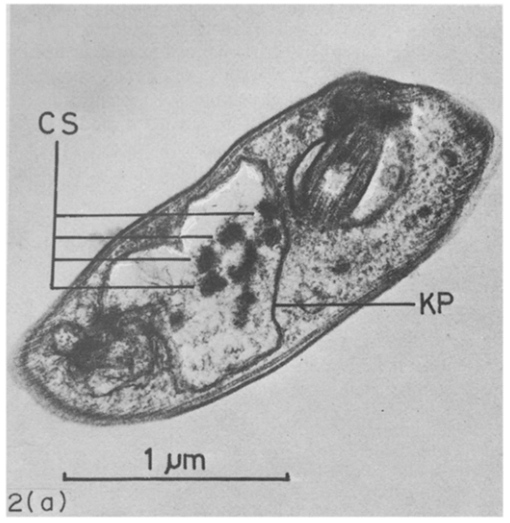
All drugs induced considerable proliferation of lysosomes, mostly vacuolated, and all drugs except ethidium caused loss of configuration of polyribosomes.

Those drugs which affect the kinetoplast are cations known to bind to DNA. Binding by Ethidium is primarily intercalative [15]; aminoacridines like acriflavine can intercalate, but may bind also by external attachment across the major groove of the helix [16]. Diamidines appear to be nonintercalative [17]; structurally they are, like Antrycide, potential cross-linking agents, so that the two forms of kinetoplast DNA core condensation (Figs. 2a, b) may reflect non-intercalative and intercalative modes of binding respectively; the prediction of non-intercalative binding of Antrycide will await confirmation by physical analysis. All these drugs, except acriflavine and pentamidine, attacked kinetoplast rather than nuclear DNA, perhaps because of the former's circularity, combined with lack of histones, dif-

Table 1. Effect of drugs on trypanosomes (*T. rhodesiense*) *in vitro*

Drug	Minimum drug concn ($\log_{10}M^{-1}$) inhibiting (90–100%)			
	Motility	Infectivity	Respiration	Glycolysis
Pentamidine	4	4	4	5
Puromycin	3	3–4	3	3
Puromycin aminonucleoside	3	3–4	3	3
Cordycepin	5	6–7	6	6–7
Acriflavine	5	5–6	6	5
Ethidium	4	5	4	4
Antrycide	3	>5	4	3
Suramin	<3	4–5	3	3
Mapharside	5	5	5	5
Nitrofurazone	3	4	4	<2

Incubations (50 min) at 37° of 10^8 trypanosomes/2 ml 50/50 inactivated horse serum/Krebs–Ringer–0·1% glucose in air, in Warburg manometer flasks with CO_2 absorption in centre well. Respiration was measured manometrically, motile forms were counted with a haemocytometer initially and after incubation, and glycolysis was determined with glucose oxidase; infectivity was assessed by injecting mice with the remaining contents of each flask, followed by daily blood examination. Drug effects were related in each case to the behaviour of control drug-free incubates.



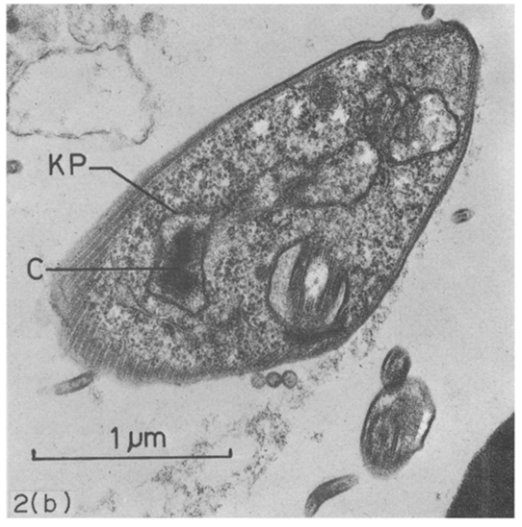


Fig. 2. (a) Kinetoplast (KP) of diamidine-treated *T. rhodesiense*. The DNA core is condensed in "sliced sausage" profiles (CS) (b) Kinetoplast (KP) of Ethidium-treated *T. rhodesiense*. The DNA core (C) shows amorphous condensation.

ferent base composition and position adjacent to the actively absorptive cytostome-like flagellar "reservoir" [18]. In addition, as in liver cells [19, 20], the mitochondrion-like kinetoplast DNA and RNA polymerases may be more drug-sensitive than the nuclear enzymes; kinetoplast DNA synthesis is known to be selectively inhibited by Berenil [21], acriflavine [22] and Ethidium [23] and possibly Antrycide [24].

Of the drugs producing nucleolar segregation, Pentamidine, Cordycepin and aminoacridines are known to inhibit *in vitro* RNA polymerases from other cells [25, 26] and Cordycepin specifically terminates *r*RNA production and inhibits synthesis of *m*RNA [27, 28], but the specificity of RNA synthesis inhibition by Nucleocidin is not known; it is a powerful protein synthesis inhibitor with little effect on RNA synthesis in liver cell preparations [29]. Nucleolar segregation was accompanied by extensive ribosomal alterations (aggregation, depopulation, loss of electron density, hypertrophy) with three of the drugs but not with nucleocidin; the latter's effects may be exerted, like Puromycin (which did not affect ribosomes), via *t*RNA, reputedly associated with the fibrillar component of the nucleolus [14]. The combination of nucleolar and ribosomal lesions suggests that the other drugs affect *r*RNA production in trypanosomes.

Puromycin and tryparsamide, which did not affect the nucleolus, produced fine, very dense microspheres scattered in the cytoplasm, similar to those described in mammalian cells treated with drugs inhibiting *r*RNA synthesis, and considered to be "structural manifestations of template inactivation" [30]. The inability of pathogenic trypanosomes to synthesize adenine [2], suggests that they will be especially vulnerable to the adenine nucleoside drugs. The trypanocidal effects of Puromycin and Nucleocidin are therefore more likely to be on nucleotide anabolism than on peptide synthesis; tryparsamide with its arsonic acid substituent may participate similarly as an organic phosphate analogue [31].

Ribosomes were primarily affected by Antrycide, suramin and arsenicals. In the case of Antrycide, this behaviour accords with its effects on the trypanosomatid flagellate *Crithidia oncopelti* [7]. Suramin is known to inhibit RNA polymerase activity, but as no nucleolar lesions and no microgranule production were found, the ribosomal alterations may be due to competition with *m*RNA binding at the ribosome, as with other polyanions [32]; trypanocidal synergism with Puromycin, to which suramin-resistant trypanosomes become hypersensitive [33], might also suggest a ribosomal locus for suramin. The effects of the arsenicals may arise from interaction with ribosomal protein thiol groups [34].

The cytoplasmic clefts produced by the nucleoside drugs, diamidines and arsenicals (Fig. 4) closely resembled those described in rat jejunal cells after absorption of long chain saturated fatty acids [35]; the cleft was considered to be an immobile, saturated, and hence osmiophobic, triglyceride deposit. *T. rhodesiense* cannot synthesize long chain fatty acids and may be vulnerable to drugs affecting their absorption or intracellular disposal. Recent autoradiographic studies [36] show that Cordycepin blocks ^{3}H-oleic acid uptake into the endoplasmic reticulum and greatly stimulates incorporation of ^{3}H-palmitic acid, about a third of which appears in the cytoplasmic clefts.

All thirteen drugs, except the rapidly-acting arsenoxide, Mapharside, induced abnormally large numbers of lysosomes, most of which were vacuolated, indicating enzyme release [37]. Various trypanocides (diamidines, Ethidium, Antrycide and suramin) are rapidly taken up by lysosomes of mammalian cells and trypanosomes [2], so that intracellular damage due to activation and release of lysosomal enzymes seems likely. Stabilization of lysosomal membranes and subsequent inhibition of mitosis [38] is possible; stilbamidine, but not suramin, has this effect [39]. The persistence of lysosomes in otherwise severely drug-damaged trypanosomes [40] supports the possibility of lysosome stabilization, but drug-lysosome interaction in trypanosomes requires much further experimental investigation.

Not all the structural lesions can be linked directly with a lethal action on the trypanosomes. Drug-induced condensation of kinetoplast DNA will obviously prevent transmission of those African trypanosomes which undergo cyclical development in the tsetse vector, where the necessary kine-

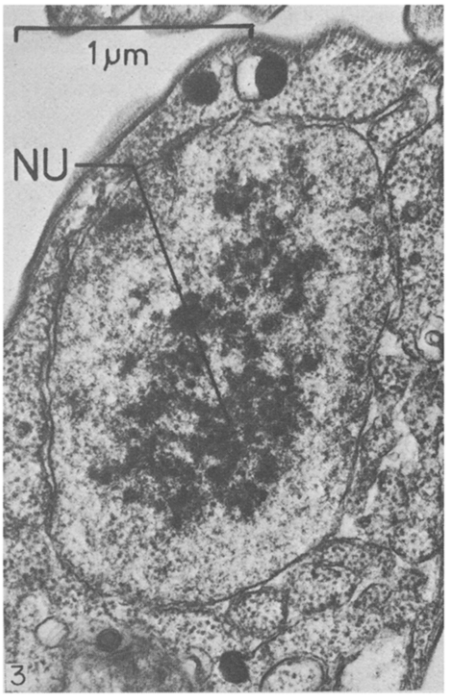


Fig. 3. Nucleus of diamidine-treated *T. rhodesiense* with dispersed nucleolus showing segregation into fibrillar (dark) and granular (light) masses.

toplast-mitochondrion proliferation [41] is dependent on an intact kinetoplast DNA core, but so-called dyskinetoplastic forms, with a highly condensed kinetoplast core, can still multiply effectively in a mammalian host, although they are not capable of cyclical development. Drugs which attack the kinetoplast, like acriflavine, Ethidium and Antrycide must therefore kill trypanosomes by additional mechanisms,

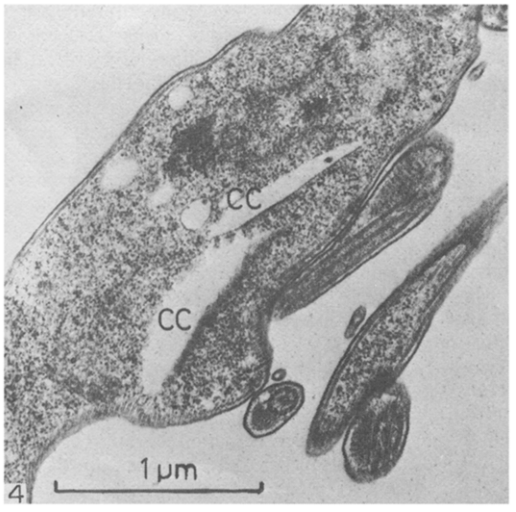


Fig. 4. Cytoplasmic clefts (CC) in Cordycepin-treated *T. rhodesiense*.

some of which are indicated by effects on respiration and glycolysis as well as on cell organelle structure.

The implications of the drug-induced lesions in trypanosome fine structure, together with a preliminary comparison of inhibitory effects of the drugs on trypanosomes *in vitro*, appear in general to confirm some surmised modes of drug action [2] and to render a few others less plausible; in some cases, unexpected lesions such as cytoplasmic clefts, indicate new lines of enquiry.

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Inhibition of rabbit mitochondrial monoamine oxidase by iprindole*

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The action of tricyclic antidepressant drugs has been attributed to inhibition of neuronal reuptake of the biogenic amines, norepinephrine (NE) and/or 5-hydroxytryptamine (5-HT), in brain [1–3]. This pharmacological property is common to the majority of the known antidepressant drugs in clinical use today. However, it has recently been shown that the antidepressant agent, iprindole [5-(3-dimethylaminopropyl)-6,7,8,9,10,11-hexahydro-5-H-cyclooct [*b*] indole–HCl; WY-3263], is much less effective than other tricyclic antidepressant drugs in preventing the reuptake of catechol or indole amines in rat and mouse brain and heart tissue [4–7]. Also, unlike the action of other tricyclic antidepressants, iprindole failed to alter the concentration of 5-HT in human platelets [8]. Thus, the mechanism of the clinical mode of action of this tricyclic drug is not consistent with the hypothesis described above.

It has been shown in several laboratories, including our own, that tricyclic antidepressant drugs inhibit mitochondrial monoamine oxidase (MAO) [9–11]. We recently reported that the antidepressant, imipramine, reversibly inhibited both type A and B forms of rabbit mitochondrial MAO. The type B form of the oxidase was further shown to be more susceptible to inhibition by this drug than was the type A form. Since inhibition of MAO by tricyclic antidepressant drugs may contribute to the clinical action of these substances, it was of interest to examine the effect of iprindole on the activity of both forms of the oxidase.

Male albino rabbits weighing approximately 2 kg were used in all experiments. Preparation of brain mitochondria and the assay used for MAO activity have been described previously [11]. In brief, reaction mixtures containing 1·8 μM ^{14}C-β-phenylethylamine or ^{14}C-5-hydroxytryptamine and varying amounts of inhibitor were incubated with rabbit brain mitochondrial MAO at 37° for varying lengths of time. The ^{14}C-deaminated products formed were separated from the amine starting material by cation-exchange (Bio Rex-70) chromatography and the radioactivity of effluents containing the deaminated products was measured in a liquid scintillation spectrometer (Packard TriCarb model 3320). All experiments were repeated at least twice.

5-Hydroxytryptamine-2-^{14}C creatinine sulfate (sp. act., 58 mCi/m-mole) was purchased from Amersham Searle Co., Arlington Heights, Ill. and β-phenylethylamine-2-^{14}C–HCl (sp. act., 7 mCi/m-mole) was purchased from New England Nuclear Corp., Boston, Mass. Iprindole was a gift from Dr. Michael R. Maise of Wyeth Laboratories, Philadelphia, Pa.

The effect of iprindole on the deamination of phenylethylamine (PEA), a specific type B MAO substrate [12], and 5-

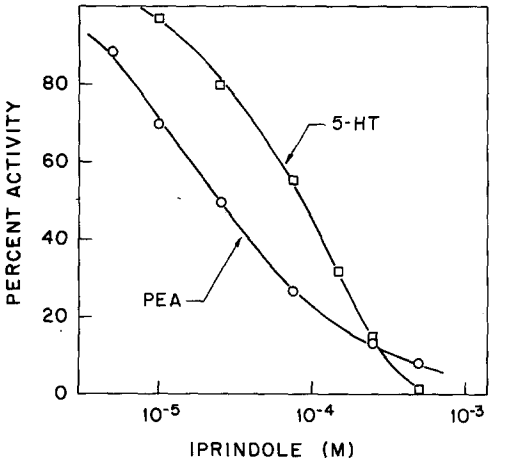

Fig. 1. Reaction mixtures containing 3·6 nmoles ^{14}C-PEA or ^{14}C-5-HT, 0·45 mg protein and varying concentrations of iprindole in a total of 2 ml of 0·05 M potassium phosphate buffer, pH 7·4, were incubated at 37° for 5 and 60 min respectively. The amounts of phenylacetic acid formed in the absence of drug were 1·10 and 0·32 nmoles respectively.

* This investigation was supported by Public Health Service Grant 13315 from the National Heart and Lung Institute.