In contrast, in suckling rats no definite histological changes were observed. This dependence of liver damage from age, along with the striking central necrosis of the adult animals, i.e. involving the cells where the drug metabolism system is located[5], might suggest a metabolic activation of the compound to a proximate toxic metabolite which in turn could act on specific cellular sites.
Chronic poisoning: After 4 months of TBA feeding, with about half of the experimental animals dead, the liver morphology of all survivors was severely distorted by large masses of proliferating bile ducts, with a variable amount of collagen and mononuclear inflammatory cells among them. Whereas some ducts appeared dilated and lined by a single layer of flattened cells, others showed a columnar epithelium lining a narrow lumen containing cells, cell debris and homogeneous PAS positive material (figure, b). Bile duct proliferation was always pronounced, in some cases involving nearly entire liver lobes.
Hepatocytes, severely reduced in number and trapped by proliferating biliary tissue, were arranged in nodules of varying size and shape. They showed a large variety of pathological features, ranging from fat deposition to glycogen storage to deep cytoplasmic basophilia. Nuclei were enlarged with typical prominent nucleoli. Some hyperplastic nodules were also observed[6].
After 4 months of recovery, during which another 10% of rats died, the liver showed a distorted pattern with collagen septa surrounding hepatocyte pseudolobules. Very large areas of cholangiofibrosis were still present, strongly suggesting adenomatous changes in places.
As a conclusion, TBA administration in small amounts induces marked changes in the rat liver. Some of them (central necrosis, liver cirrhosis, bile duct proliferation and cholangiofibrosis) cannot be considered as specific of this compound, because they are present during treatment with a large variety of liver poisons. Nevertheless, their morphological features, time of appearance and extent closely parallel those induced by TAA. Taking also into account the nuclear and especially nucleolar enlargement, a strict similarity between the effects of TBA and TAA is evident, thus indicating that the chemical group common to these molecules (i.e. the $-CSNH_2$ moiety) is the active one, directly during or after metabolic transformations.

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Some observations on the effect of Ro 7-1051 on Trypanosoma cruzi, particularly in cell culture[1]

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Summary. The drug Ro 7-1051 showed a deleterious effect on the intracellular and extracellular forms of Trypanosoma cruzi represented by nuclear pyknosis, fragmentation and lysis of parasites and by its reduced susceptibility to infection.

Reports have been made in the literature on the actions of drugs on Trypanosoma cruzi 'in vivo' and 'in vitro'. Silva et al.[4] have shown that the aminonucleoside of Stylomicin produces gross alterations to the intracellular form, and to some extent to the extracellular form, of the parasite in cell culture. Fernandes et al.[5] studied the activity of Mitomycin® C, Actinomycin D and analogues of pyrimidine on growth, protein synthesis, nucleic acid synthesis and on the activity of Trypanosoma cruzi, in culture.
Therapeutic studies previously carried out include: Browning et al.[6] and Goodwin et al.[7] with phenanthridine derivatives; Pizzi[8], with primaquine; Goble[9], with 6-methoxy-8-amino-quinolines; Packchamian[10], with several antibiotics; Bock et al.[11], with Bay-2502, nitrofurfurilidine derivatives, and Richle[12], with Ro 7-1051.
With reference to the chemotherapy of human Chagas' disease, studies previously carried out include: Lugones et al.[13] and Tourres[14], both using Bay-2502, and Ferreira[15], with nitrofurazone, levofuraltadone (NF-602) and Bay-2502.
The work presented here studied the effect of Ro 7-1051 (N-benzyl-2-nitro-1-imidazoleacetamide) on Trypanosoma cruzi, in cell culture.
Materials and methods. The experiments were divided into groups after the following treatments:
a) HeLa cells, cultured in flasks, were infected with Trypanosoma cruzi, Y strain, metacyclic or blood forms, using floating coverslip slides; 0.8 ml of nutrient medium (Eagle minimal medium with 2% inactivated calf serum) containing 10 µg, 50 µg or 100 µg/ml of Ro 7-1051 was added and the flasks were incubated at 37 °C for 1, 2, 3 or 4 days.
b) Metacyclic forms of Trypanosoma cruzi, Y strain, were incubated at 37 °C for 6, 12 and 24 h in a nutrient medium containing 100 µg/ml of Ro 7-1051. The parasites were

1 Acknowledgments. The authors are deeply thankful to Roche Products for supplying the drug and for valuable information. The authors are also grateful to Prof. Nelson Villa for criticism and to Miss Edna Bertin for technical assistance.
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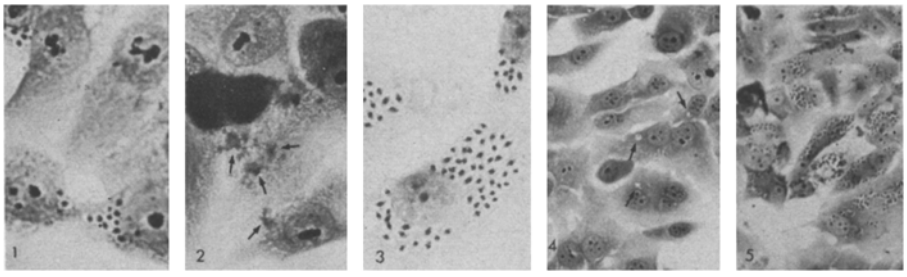

Fig. 1. HeLa cell culture infected with Trypanosoma cruzi, Y strain, incubated with Ro 7-1051, at a concentration of 100 µg/ml, for 24 h, at 37 °C. Note the rounding of the amastigote forms. Giemsa stain. × 500. Fig. 2. HeLa cell culture infected with Trypanosoma cruzi, Y strain. 48 h incubation at 37 °C with Ro 7-1051, at a concentration of 100 µg/ml. Note the karyorrhexis and lysis of the parasites within the host cell (arrows). Giemsa stain. × 500. Fig. 3. Control culture of HeLa cells infected with Trypanosoma cruzi, Y strain. Note the numerous, highly infected cells. Giemsa stain. × 500. Fig. 4. HeLa cell culture infected with Trypanosoma cruzi, Y strain (metacyclic form, incubated with Ro 7-1051 at a concentration of 100 µg/ml for 24 h at 37 °C). Incubation at 37 °C for 4 days. Note the scarcity and degeneration of parasites (arrows). Giemsa stain. × 190. Fig. 5. Control culture of HeLa cells infected with Trypanosoma cruzi, Y strain. Incubated at 37 °C for 4 days. Note the large number of infected cells, each cell containing numerous parasites. Giemsa stain. × 190.

then centrifuged at 2,500 rpm for 15–20 min and the pellets both resuspended and washed in Hanks-Wallace solution and recentrifuged. The pellets were added to HeLa cells, cultured in flasks containing floating coverslip slides, 1.5 ml of nutrient medium without the drug was added to each flask and the flasks incubated for 1, 2, 3 or 4 days.

After incubation, the slides were washed with balanced saline, fixed with Bouin's solution for 15 min and stained with Giemsa stain. Control cultures, without the drug, were run concurrently and at least 10 slide preparations of each experimental group were set up.

Results. Within 24 h of incubation (figure 1), the preparations from the first group showed an early tendency to rounding of the amastigote forms, and after 48 h incubation (figure 2), showed pyknosis, karyorrhexis and lysis of the parasites. Intensity of effect varied according to incubation time and dosage of Ro 7-1051.

Slide cultures previously treated with a dose of 100 µg/ml, and incubated for 4 days, showed very few parasites, those present being highly degenerate.

The control cultures showed plenty of highly infected cells with no signs of degeneration of cells or parasites (figure 3).

The infected cells from the second group preparations (figure 4) contained fewer parasites compared to the controls (figure 5) and most of these parasites showed degenerative signs. The intensity of degeneration varied according to the contact time between the parasite and the drug.

Discussion. Richle[12] demonstrated, in infected mice, that Ro 7-1051 acts on Trypanosoma cruzi by promoting complete destruction of the pseudocyst form. Similarly, our results show that this 2-nitroimidazole derivative caused deleterious effects on the intracellular forms of the parasite, even at a dose as low as 10 µg/ml. The degenerative signs were similar to those described by Brener[16] in Trypanosoma cruzi infected chick embryo tissue cultures, in which the amastigote forms showed nuclear pyknosis and fragmentation and eventual lysis, when submitted to the action of nitrofuran compounds, and phenanthridine derivatives.

In addition, the results of the pre-incubation of the metacyclic forms with the drug, suggests an action on the extracellular forms of the parasite, by reducing the ability of the parasite to infect the cell.

Conclusion. The drug Ro 7-1051 showed deleterious effects on the intracellular forms of Trypanosoma cruzi, represented by nuclear pyknosis, fragmentation and lysis of the parasites, at doses as low as 10 µg/ml. At higher doses, up to 100 µg/ml, the drug appeared to have no harmful effect on the cells, only on the parasites. Results also suggest an action of the drug on the extracellular forms of the parasite, as demonstrated by the reduced ability of the parasite to infect the cell.

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Desmosomal abnormalities in the liver of methotrexate-treated psoriatics

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Summary. Electron microscopy of the liver of methotrexate-treated psoriatic patients revealed junctional abnormalities consisting of detachment of desmosomal plaques between hepatocytes. Mitochondria anchoring desmosomal microfilaments were frequently noted.

While studying the fine structural changes in the liver of a psoriatic patient treated with the folic acid antagonist methotrexate (MTX), a marked abnormality of desmosomes was noted. The lesion consisted of the separation of junctional surfaces and accumulation of microfilaments at the cytoplasmic aspect of desmosomal plaques. Often, adjacent mitochondria appeared to anchor the microfilaments of desmosomes, being in direct contact