thepin may depress 5-HT-inhibitory but not excitatory responses. The correlation between the reduction of MRN-evoked inhibition and inhibition by exogenous 5-HT of the same SN neurones by methiothepin is compatible with a suggested MRN-SN serotoninergic pathway[4]. However, the additional interactions of methiothepin with DA observed in this and other studies[8] suggests the necessity for further pharmacological experiments to confirm the identity of 5-HT as the neurotransmitter in the MRN-SN pathway.

Liver changes following thiobenzamide poisoning

G. Malvaldi

Istituto di Patologia Generale, Università di Pisa, via Roma 55, I–56100 Pisa (Italy), 15 March 1977

Summary. Single thiobenzamide administration to rats induces liver necrosis. Chronic poisoning is followed by biliary cirrhosis. Areas of cholangiofibrosis are still evident after 4 months of recovery.

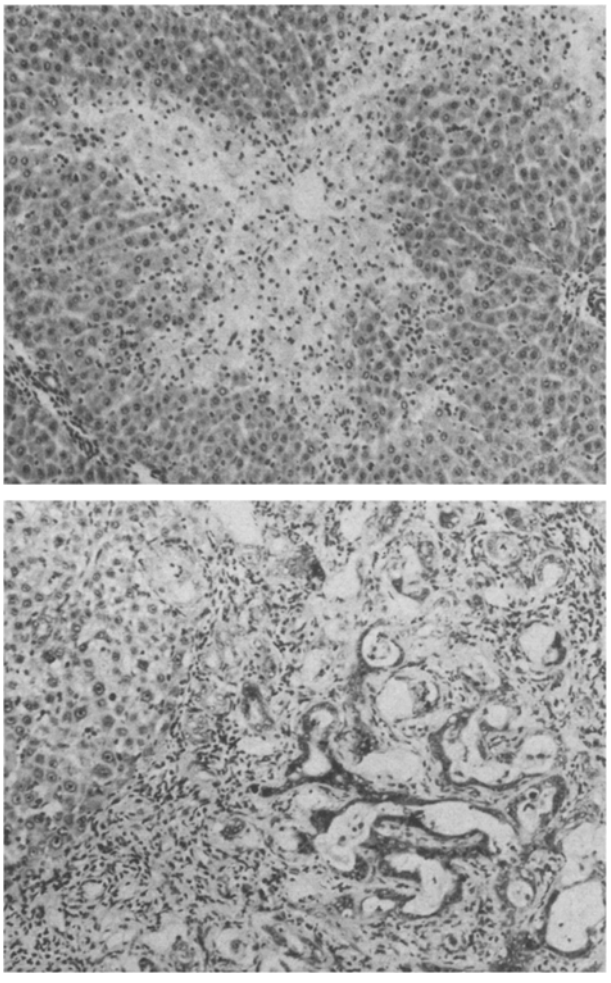
Thiobenzamide (TBA) (C_6H_5–$CSNH_2$) is an yellow, water-insoluble compound widely used as an intermediate for synthesis. It is also endowed with antibacterial activity against mycobacteria[1]. Even if there is little reason to add another compound to the ever-increasing list of those studied as able to cause liver damage, a toxicological study was undertaken for TBA in view of its structural similarity with the well-known toxic and carcinogen compound thioacetamide (TAA) (CH_3–$CSNH_2$). In fact some aspects of the mechanism of action of TAA are still debatable, in particular as regards the importance of the entire molecule or of an active group, the =S-moiety being strongly suspected[2].

The results reported here, showing that TBA causes a liver damage very similar to that due to the TAA, indicate that the lipid soluble TBA could be a useful tool in this field.

Methods. Male Sprague-Dawley rats bred in our colony were used. Acute intoxication was performed in suckling (7 days old) and adult rats starved for 12–16 h by i.p. administration of TBA suspended in 0.1% rat serum albumin at dose level of 30 mg/100 g; blocks of liver from 2 lobes were taken after 6, 12, 24, 48 and 120 h.

Chronic poisoning was obtained by feeding rats with the stock diet containing 0.050% TBA for a period of 4 months. The livers were examined after every month of intoxication, as well as after 4 additional months of normal diet. The liver tissue was fixed in Carnoy fluid and 10% buffered formalin. Frozen sections were stained with oil red for fat. Paraffin sections were stained with haematoxylin and eosin, PAS method with and without diastase for glycogen and Van Gieson method for connective tissue. Control rats (normal and TAA treated at equimolecular amounts) were used, with results identical to those reported by Gupta[3,4].

Results and discussion. Only the relevant changes observed in the liver will be reported and briefly discussed. Acute poisoning: 6 h after the TBA dose, centrolobular hepatocytes showed loss of cytoplasmic basophilia and glycogen and the mediolobular ones were filled with small fat droplets. The lobular distribution of liver damage was particularly evident after 24 h, when a central necrosis involved about one half of the lobule (figure, a), with a sharp boundary between the damaged hepatocytes and the periferal ones which retained their normal glycogen content, but had sligthly enlarged nuclei. The finding could be due to the incipient regeneration which was evident as a mitotic burst after 48 h, when the necrotic parts were filled with mononuclear inflammatory cells. 5 days later, the liver picture was indistinguishable from that of control animals.



a Centrolobular necrosis. *b* Area of cholangiofibrosis; hepatocytes on the left upper corner show prominent nucleoli.

1 K. Waisser, M. Celadnik, J. Sova and K. Palat, Čslká Farm. *24*, 423 (1975).
2 J. A. Castro, N. D'Acosta, E. C. de Ferreyra, C. R. de Castro, M. I. Diaz Gomez and O. H. de Fenos, Toxic. appl. Pharmac. *30*, 79 (1974).
3 D. N. Gupta, J. Path. Bact. *72*, 183 (1956).
4 D. N. Gupta, J. Path. Bact. *72*, 415 (1956).

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.

5 J. C. Wanson, P. Drochmans, C. May, W. Penasse and A. Popowski, J. cell. Biol. *66*, 23 (1975).
6 E. Farber, Meth. Cancer Res. *7*, 345 (1973).

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

S. Yoneda, E. Kimura[2] and Beatriz Ribas Castellani[3]

Department of Histology and Embryology, Institute of Biomedical Sciences, University of São Paulo, São Paulo (Brazil), 14 December 1976

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.
2 Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo (Brazil).
3 Post-Graduate of Microbiology, Institute of Chemistry, University of São Paulo, São Paulo (Brazil).
4 L. H. P. Silva, S. Yoneda and J. F. Fernandes, Exp. Parasit. *8*, 486 (1959).
5 J. F. Fernandes, M. Halsman and O. Castellani, Exp. Parasit. *18*, 203 (1966).
6 C. H. Brownings, K. M. Calver and M. W. Leckie, Nature *157*, 263 (1946).
7 L. G. Goodwin, M. D. Goss, J. A. Lock and L. P. Walls, Br. J. Pharmac. Chemother. *5*, 277 (1950).
8 T. Pizzi, Proc. Soc. exp. Biol. Med. *78*, 643 (1951).
9 F. C. Goble, Antibiotics Chemother. *11*, 265 (1952).
10 A. Packchamian, Am. J. trop. Med. Hyg. *2*, 243 (1953).
11 M. Bock, R. Gönnert and A. Haberkorn, Bol. chil. Parasit. *24*, 13 (1969).
12 R. Richle, Progve Med. *101*, 282 (1973).
13 H. S. Lugones, F. Peralta, D. C. Feijóo and A. E. A. Marteleur, Bol. chil. Parasit. *24*, 10 (1969).
14 C. L. B. Tourres, Bol. chil. Parasit. *24*, 24 (1969).
15 H. O. Ferreira, Bol. chil. Parasit. *24*, 101 (1969).