Acknowledgements—This investigation was supported in part by grant MH-02435 from the National Institute of Mental Health, USPHS, and in part by a grant from Initiative 171, State of Washington. We wish to thank Mr. Don M. Kennedy for his technical assistance.

Department of Pharmacology,
School of Medicine,
University of Washington,
Seattle, Wash., U.S.A.

A. HORITA
M. A. CARINO

REFERENCES

1. P. GULLINO, M. WINITA, S. M. BIRNBAUM, J. CORNFIELD, M. C. OTEY and J. P. GREENSTEIN, *Archs Biochem. Biophys.* **64**, 319 (1956).
2. S. M. HESS and W. DOEPFNER, *Archs int. Pharmacodyn. Thér.* **134**, 89 (1961).
3. H. GREEN, S. M. GREENBERG, R. W. ERICKSON, J. L. SAWYER and T. ELLISON, *J. Pharmac. exp. Ther.* **136**, 174 (1962).
4. L. J. WEBER and A. HORITA, *Biochem. Pharmac.* **14**, 1141 (1965).
5. J. V. HODGE, J. A. OATES and A. SJOERDSMA, *Clin. Pharmac. Ther.* **5**, 149 (1964).
6. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **117**, 82 (1956).

Biochemical Pharmacology, Vol. 19, pp. 1524–1526. Pergamon Press. 1970. Printed in Great Britain

Specific inhibition by ethidium bromide of the incorporation of ^{3}H thymidine into the kinetoplastic DNA of *Trypanosoma cruzi*

(*Received* 2 *September* 1969; *accepted* 13 *November* 1969)

WE NOW know that the kinetoplast of Trypanosomatids is a part of the mitochondrial apparatus in which a great deal of DNA is accumulated. In *Trypanosoma cruzi* the kinetoplastic DNA represents more than 20 per cent of the total DNA. The kinetoplastic DNA has a higher AT content than the nuclear DNA; this property is used for its fractionation. Ethidium bromide (EB) is a trypanocidal drug which binds specifically to the kinetoplastic DNA when used at low concentration (0·5–5 μg/ml of culture medium). Electron microscope studies of the ultrastructure of trypanosomes growing in a medium supplemented with 0·5 μg EB/ml reveal alterations of the kinetoplastic DNA with a progressive loss of this DNA after a few divisions of the trypanosomes.[1] A high percentage of dyskinetoplastic trypanosomes were obtained when cells were treated with ethidium bromide. Nuclear DNA was not affected by EB at this concentration. Dyskinetoplastic trypanosomes are not viable and they can only survive for 4 weeks after weekly transplantation in a new medium without EB.[2] In this paper we report studies on the incorporation of ^{3}H thymidine into the kinetoplastic and nuclear DNA. We noted a specific inhibition of the incorporation of the radioactive compound by EB, in the kinetoplastic DNA of trypanosomes.

The methods used were autoradiography of trypanosomes and liquid scintillation counting of the DNA. Trypanosomes (*Trypanosoma cruzi* strain Institut Pasteur) were cultured as described in previous works.[3, 4] After 4 days culture, during the exponential phase of trypanosomes' growth, EB in sterile water solution was added to the culture medium to obtain a final concentration of 0·5 μg/ml. After 2 hr culture, Me^{3}H thymidine (specific activity 9·7 c/mM) was added to the medium to a concentration of 0·5 μc/ml. After 2 hr the pulsing medium was removed, the trypanosomes washed twice

with cold SSC. (0·15 M NaCl, 0·015 M sodium citrate). Pulse labeling experiments without preliminary addition of EB were undertaken in a similar manner.

Smears of trypanosomes were autoradiographed by the stripping film method described by Pelc[5] using Ilford K2 emulsion. After an exposure time of one week the slides coated with emulsion were developed, fixed then colored by Giemsa's stain.

In normal medium, nuclei and kinetoplasts were labeled by Me ^{3}H thymidine while in medium supplemented with EB there was an inhibition of the incorporation of the radioactive compound into the kinetoplastic DNA (Table 1).

TABLE 1. AVERAGE VALUES OF SILVER GRAINS COUNTED ON NUCLEUS AND KINETOPLAST OF TRYPANOSOMES GROWING IN MEDIUM WITHOUT EB AND IN PRESENCE OF EB (0·5 μg/ml)

	Nucleus	Kinetoplast
Number of silver grains (medium without EB)	6·2 ± 0·5	3·2 ± 0·4
Number of silver grains (medium + EB)	6·0 ± 0·4	0·7 ± 0·2

In our culture conditions the cell cycle of trypanosomes was about 24 hr. As trypanosomes cultures were not synchronized there is a portion of cell population without labeling (about 35 per cent). In normal medium the kinetoplast was always labeled before the nucleus.

Nuclear and kinetoplastic DNA fractionation was described in previous work.[6] Kinetoplastic and nuclear DNA fractions were collected and used for radioactive counting. DNA sample were transferred on Whatman glass fiber GF/A filters. The dried filters were immersed in 10 ml toluene–PPO–POPOP and counted in a Beckman liquid scintillation counter. Kinetoplastic DNA was also submitted to a fractionation of its molecular forms by density gradient centrifugation in EB as described in a previous work.[4] Two bands, labeled a and b, were obtained (Fig. 1). Band a corresponds to open DNA circles and b to covalently closed DNA circles.[4] These two bands were submitted to radioactive scintillation counting. The amount of DNA used for counting was determined by the spectrofluorometric method of Le Pecq.[7] Results of radioactive counting are summarized in Table 2.

TABLE 2. SPECIFIC ACTIVITY (dpm/μg) OF DNA FOUND IN NUCLEAR DNA, TOTAL KINETOPLASTIC DNA AND BANDS a AND b OBTAINED FROM KINETOPLASTIC DNA AS REFERRED IN THE LEGEND OF FIG. 1

	Specific activity* (dpm/μg of DNA) (normal medium)	Specific activity* (dpm/μg of DNA) (medium + EB)
Kinetoplastic DNA	52,620	11,060
Band a	55,280	8230
Band b	44,410	13,120
Nuclear DNA	50,330	60,670

* Mean value of three experiments.

It can be seen from these results that EB produces a specific inhibition of the incorporation of ^{3}H thymidine into the kinetoplastic DNA whereas the nuclear DNA normally incorporates this compound. Recently it has been shown that EB exerts a selective inhibitory effect on purified rat liver mitochondrial DNA polymerase, which was 37 times more sensitive than the nuclear polymerase to EB.[8] The process of this selective inhibition is not well understood.

When the trypanosomes are cultured in presence of EB (1·5 μg/ml) for several days a new physiological state which affects specifically kinetoplastic DNA is induced. Abnormal circular DNA molecules (circular dimers, trimers, tetramers and pentamers) are induced with a high frequency (more than 30% of kinetoplastic DNA).[9] The mechanism of this drug action is quite puzzling and its study is in progress.

Department of Molecular Pharmacology,
Gustave Roussy Institute,
94-*Villejuif*, *France*

GUY RIOU

REFERENCES

1. E. DELAIN and G. RIOU, *Compt. Rend.* **268**, 1327 (1969).
2. G. RIOU, E. DELAIN and R. PAUTRIZEL, *Ann. Inst. Pasteur Paris*, in press.
3. G. RIOU, *Compt. Rend.* **265**, 2004 (1967).
4. G. RIOU and E. DELAIN, *Proc. natn. Acad. Sci. U.S.A.* **62**, 210 (1969).
5. S. R. PELC, *Int. J. Appl. Rad. Iso.* **1**, 172 (1956).
6. G. RIOU and C. PAOLETTI, *J. molec. Biol.* **28**, 377 (1967).
7. J. B. LE PECQ and C. PAOLETTI, *Analyt. Biochem.* **17**, 100 (1966).
8. R. R. MEYER and M. V. SIMPSON, *Biochem. biophys. Res. Commun.* **34**, 238 (1969).
9. G. RIOU and E. DELAIN, *Proc. natn. Acad. Sci. U.S.A.* **64**, 618 (1969).

Biochemical Pharmacology, Vol. 19, pp. 1526–1528. Pergamon Press. 1970. Printed in Great Britain

The effect of some carbonyl compounds on rat liver glutathione levels

(*Received* 9 *October* 1969; *accepted* 12 *November* 1969)

SOME foreign compounds that are metabolized to mercapturic acids after administration to animals depress liver GSH levels,[1–4] and an initial stage in mercapturic acid biosynthesis is the enzyme catalysed conjugation of these compounds with GSH.[1, 5] The reactions of GSH with many $\alpha\beta$-unsaturated carbonyl compounds are catalysed by glutathione S-alkenetransferases that are present in liver preparations from several animal species,[6, 7] suggesting that these compounds would be partly metabolized to mercapturic acids. Further evidence is provided by results reported in this paper which show that certain $\alpha\beta$-unsaturated carbonyl compounds lower liver non-protein thiol levels (mainly GSH[8]) after administration to rats.

Experimental

The carbonyl compounds, which were all liquids, were administered by intraperitoneal injection to female rats (Chester Beatty strain) weighing between 200 g and 380 g. For most of these compounds, published toxicity data refer to oral administration only, and for intraperitoneal injection a quarter or less of the oral LD_{50} was given. Where the doses were small, the compound was dissolved in arachis oil or in 0·1 M-orthophosphate buffer, pH 7·4. Control rats were dosed with arachis oil or with buffer. Of the treated rats, two were killed after 30 min and four after 2 hr; the controls (usually three) after 2 hr. The livers were immediately removed, homogenized in 5 vol. of 0·1 M-orthophosphate buffer, pH 7·4, an equal volume of 4% (w/v) sulphosalicylic acid was added, and the mixture centrifuged at approx. 2000 *g* for 30 min. All operations were carried out below 10°. The supernatant was assayed for GSH by the 5,5′-dithiobis-(2-nitrobenzoic acid) method.[9] Recoveries of GSH added to the whole homogenate ranged between 87–101 per cent (mean 96 per cent). Mean control GSH (forty-four rats) was 155 mg/100 g liver with values ranging between 96–220 mg/100 g liver, but the majority were within 20 per cent of the mean value. Johnson[2] reported 151 mg/100 g liver by a similar assay method, and Woodward[10] obtained 172 mg/100 g liver by the glyoxalase method. In cases where GSH levels fell to about 90 per cent of the control value, the result is not considered significant.