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ASPECTS OF PYRIMIDINE BIOSYNTHESIS OF *TRYPANOSOMA CRUZI*

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SUMMARY

The Authors have studied the pyrimidine metabolism of intra- and extracellular forms of *Trypanosoma cruzi*. In every evolutive form, grown both in culture medium and cell culture, the parasite was shown to exhibit a large incorporation of uridine and an almost negligible uptake of orotic acid. These data strongly suggest that in *T. cruzi*, the pyrimidic compounds are built up preferentially through a "salvation" pathway.

INTRODUCTION

The metabolism of *Trypanosoma cruzi* has been investigated under several aspects.

LITTLE & OLESON [10] and CITRI & GROSSOVICZ [2] observed, respectively, that orotic and cytidilic acids do estimulate the growth of the parasite.

REY & FERNANDES [13] have shown that, in diphasic medium, *Trypanosoma cruzi* synthesizes its pyrimidic ring preferentially by "salvation" pathway and, for the synthesis of nucleic acids and acid-soluble nucleotides, its uptake of orotic acid for the pyrimidine fraction is low, whereas the incorporation of uracil for the biosynthesis of pyrimidine nucleotides is high. This particular behaviour is comparable to the parasite's purine metabolism, in which the extracellular forms utilize the "salvation" metabolic pathway (FERNANDES & CASTELLANI [4]).

In tissue culture, YONEDA [18] observed that the intracellular amastigote and metacyclic trypomastigote forms exhibit respectively, "de novo" and "salvation" metabolic pathways in their purine synthesis.

In the present research, we have tried to settle the metabolic behaviour of the intra- and extracellular forms of *T. cruzi* with regard to pyrimidine synthesis.

MATERIAL AND METHODS

Two different sets of preparations were used: a) Culture forms of Y strain *T. cruzi* (*) (SILVA & NUSSENSZWEIG [14]), were centrifuged at 3,000 r.p.m. for 15 minutes. The sediment, after washing with HANKS-WALLACE [5] solution was submitted to a 12 to 24 hour incubation at 37°C in Eagle's minimal medium with 2% inactivated fetal calf serum added of either tritiated orotic acid (**) or tritiated uridine (***), both in final concentration of 0.5 μc/ml. Further treatment of this sediment is described under (c). b) Cell culture forms of *T. cruzi*

(*) *T. cruzi* culture, kindly supplied us by Prof. José Ferreira Fernandes from Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brasil.

(**) 5-H^3-Orotic acid, specific activity 14.1 c/Mmol Schwarz Bioresearch Inc. Orangeburg, New York, kindly supplied by Dr. Maria Mitzi Brentani and Prof. Ricardo Renzo Brentani, from Laboratório de Oncologia Experimental, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil.

(***) H^3-Uridine, specific activity, 25.6 c/mM, kindly supplied by Prof. Antonio Sesso, from Departamento de Histologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

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were obtained through previous infection of HeLa [8] or Sirc [9] cell monolayer cultures. These were then incubated for 12 to 24 hours at 37°C with tritiated orotic acid or tritiated uridine, both added to the Eagle's minimal nutrient medium in the final concentration of 0.5 μc/ml. Hereafter the monolayers were submitted to dissociation and rupture of the substrate cells, through 0.1% trypsin treatment for 30 minutes at 37°C. The resultant suspension of cell residues plus parasites was then treated as specified under (c). c) Both sediments, from (a) and (b) were centrifuged and twice washed with Hanks-Wallace solution [5] before beeing treated as stated under (d). d) Smears of (a) and (b), fixed with Bouin solution for ten minutes were submitted to autographic processing after Messier & Leblond [11] and Kopriva & Leblond [7] with K^5 nuclear emulsion (Ilford, England) the exposition time varying from 45 to 78 days, and stained with Giemsa.

The minimal number of smears for each isotope labelling was of ten. Control smears, made up of unlabelled substrata, were also prepared.

RESULTS

Group (a) — The culture form parasites, among which epimastigote forms (crithidiae) prevailed, were highly positive when treated by H^3-uridine, exhibiting a large amount of silver granules (Fig. 1), and weakly positive, with scarce and irregular number of granules, when treated by H^3-orotic acid (Fig. 2).

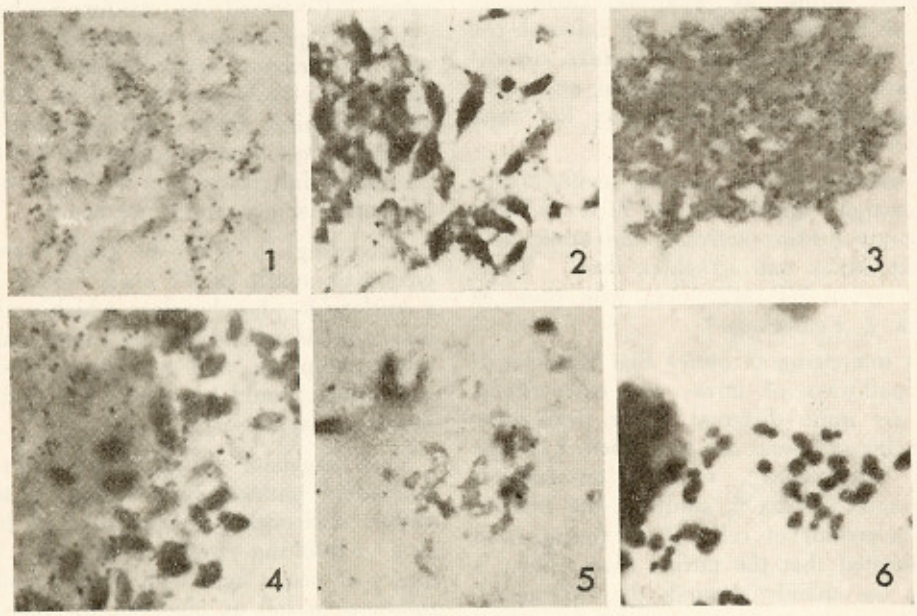

Fig. 1 — Epimastigote forms (crithidiae) of *T. cruzi* grown in culture medium. H^3-uridine uptake; abundant and regular distribution of silver granules. Giemsa stain, 1,160 X.

Fig. 2 — Epimastigote (crithidiae) forms of *T. cruzi* grown in culture medium. H^3-orotic acid uptake; scarce and irregular distribution of silver granules. Giemsa stain, 1,160 X.

Fig. 3 — Amastigote (leishmania) and epimastigote (crithidiae) forms of *T. cruzi* grown in cell culture. H^3-uridine uptake; abundant and regular distribution of silver granules. Giemsa stain, 1,160 X.

Fig. 4 — and 5 — Amastigote (leishmania) and epimastigote (crithidiae) forms of *T. cruzi* grown in cell culture. H^3-orotic acid uptake; scarce and irregular distribution of silver granules. Giemsa stain, 1,160 X.

Fig. 6 — Amastigote (leishmania) forms of *T. cruzi* grown in cell culture; control preparation; no silver granules present. Giemsa stain, 1,160 X.

Group (b) — This group behaved very much like group (a). The intracellular amastigote (leishmania) and epimastigote forms as well as the extracellular trypomastigotes (trypanosome) showed large numbers of silver granules with H^3-uridine (Fig. 3), and scarce and irregular number of granules in the H^3-orotic acid group (Figs. 4, 5).

Control preparations were consistently negative (Fig. 6).

DISCUSSION

REY & FERNANDES [13] have shown the low uptake of orotic acid in the synthesis of acid soluble nucleotides and nucleic acid pyrimidines by *T. cruzi* cultivated in diphasic medium. KIMURA & FERNANDES [6], on the other hand, observed that every epimastigote, blood trypomastigote and metacyclic trypomastigote forms, as well as amastigotes obtained by cell disruption of infected cell culture or hemocultures, do uptake uridine vigorously, while not incorporating any orotic acid, or incorporating it at very low rates when the pH is lowered.

As shown by our results, epimastigote, trypomastigote and amastigote *T. cruzi* forms incorporate uridine actively, and orotic acid in much lower and irregular rates. These results are in full agreement with those of KIMURA & FERNANDES [6].

It is interesting to notice that the biosynthetic pathways of intra- and extracellular forms are quite different where pyrimidine and purine syntheses are concerned. FERNANDES & CASTELLANI [4], working on cultural forms, and YONEDA [18], studying the biochemical behaviour of cell culture forms, have demonstrated that the purine biosynthesis is carried out mainly through the "salvation" pathway in the trypomastigote stage of the parasite, and, according to YONEDA [18], through the "de novo" pathway, predominantly, in the intracellular amastigote stage grown in cell culture.

However, our present results have proved that, in *T. cruzi*, the biosynthetic pathways involved in the synthesis of the pyrimidine ring are directed otherwise: in the various morphological stages of the parasite, an intense incorporation of uridine, against a weak and irregular incorporation of orotic acid was recorded. This would rather point to a "salvation" pathway operating for the synthesis of the pyrimidic ring.

ARVIDSON et al.[1], working with rats, and WRIGHT et al.[15, 16], studying *Lactobacillus bulgaricus 09* have verified a heavy utilization of orotic acid in the organisms studied, along with the finding by WRIGHT et al. [16] that *Lactobacillus arabinosus*, on the other hand, utilizes uracil. Furthermore, CRAWFORD et al. [3] have observed, in the last organism cited, a pathway in which uridine-5'-phosphate and 5'-phosphoribosyl pyrophosphate are utilized. These same Authors have also studied a bacteria able to take up both, uracil and orotic acid, an evidence for the actual co-operation of both pathways in the synthesis of pyrimidine.

MOORE & BOYLEN [12] have investigated a mutant of *Escherichia coli* which incorporates 2-C^{14}-uracil to uridine-5'-phosphate, cytidine-5'-phosphate and probably to thymidine deoxyribonucleotide. In this case, uracil would be a likely precursor of all ribonucleic acid pyrimidic bases and, probably, of its deoxyribonucleic acid, where the initial steps preceding interconversion to nucleoside or nucleotide stages are concerned.

According to REY & FERNANDES [13] the enzymatic outfit of *T. cruzi* would include a nucleoside phosphorylase which splits uridine and deoxyuridine into uracil plus pentose-1-phosphate, and a uridine phosphokynase which would operate at the pyrimidine nucleotide synthesis.

This flagellate could, thus, resort to a "salvation" pathway for building up its pyrimidine nucleotides, in opposition to the bacteria exhibiting preferential and intense intake of orotic acid with a minimum utilization of uracil as observed by WRIGHT & MILLER [17].

RESUMO

Aspectos da biossíntese de pirimidinas no Trypanosoma cruzi

Os Autores estudaram o metabolismo de pirimidinas nas formas intra e extracelulares do *Trypanosoma cruzi*.

Observaram que em cada uma das suas formas evolutivas em meio de cultura e em

cultivo de células, os parasitas apresentaram elevada incorporação de uridina e mínima, quase nula, utilização do ácido orótico, mostrando preferência pela via biosintética de "salvação" na formação de seus compostos pirimídicos.

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REFERENCES

1. ARVIDSON, H.; ELIASSON, N.A.; HAMMARSTEIN, E.; REICHARD, P. & von UBISH, H. — Orotic acid as a precursor of pyrimidines in the rat. *J. Biol. Chem.* **179**: 169-173, 1949.

2. CITRI, N. & GROSSOWICZ, N. — A liquid medium for the cultivation of *Trypanosoma cruzi*. *Nature* **173**:1110-1111, 1954.

3. CRAWFORD, I.; KORNBERG, A. & SIMMS, E.S. — Conversion of uracil and orotate to uridine 5'-phosphate by enzymes in Lactobacilli. *J. Biol. Chem.* **226**:1093-1101, 1957.

4. FERNANDES, J.F. & CASTELLANI, O. — Nucleotide and Polynucleotide Synthesis in *Trypanosoma cruzi*. I. Precursor of Purine Compounds. *Exp. Parasit.* **7**:224-235, 1958.

5. HANKS, J.H. & WALLACE, R.E. — Relation of oxygen and temperature in the preservation of tissues by refrigeration. *Proc. Soc. Exp. Biol. Med.* **71**:196-200, 1949.

6. KIMURA, E. & FERNANDES, J.F. — Personal communication, 1973.

7. KOPRIVA, B.M. & LEBLOND, C.P. — Improvements in the coating technique of Radioautography. *J. Histochem. Cytochem.* **10**:269-284, 1962.

8. KUBICEK, M.T. — Tissue culture studies of Proliferative capacity of cervical carcinoma and normal Epithelium. *Cancer Res.* **12**:264-265, 1952.

9. LERHØY, J. — Effect of Rubella virus in a Rabbit cornea cell line. *Science* **149**:633-634, 1965.

10. LITTLE, P.A. & OLESON, J.J. — The cultivation of *Trypanosoma cruzi*. *J. Bact.* **61**:709-714, 1951.

11. MESSIER, B. & LEBLOND, C.P. — Preparation of coated radio-autograph by Dipping Sections in Fluid Emulsion. *Proc. Soc. Exp. Biol. Med.* **96**:7-10, 1957.

12. MOORE, A.M. & BOYLEN, J.B. — Utilization of uracil by a strain of *Escherichia coli*. *Arch. Biochem. Biophys.* **54**:312-317, 1955.

13. REY, L. & FERNANDES, J.F. — Nucleotide and Polynucleotide synthesis in *Trypanosoma cruzi*. VII. Precursors of the Pyrimidine Nucleotide. *Exp. Parasit.* **12**:55-60, 1962.

14. SILVA, L.H.P. & NUSSENSZWEIG, V. — Sobre uma cepa de *Trypanosoma cruzi* altamente virulenta para o camundongo branco. *Folia Clin. Biol.* **20**:191-207, 1953.

15. WRIGHT, L.D.; DRISCOLL, C.A.; MILLER, C.S. & SKEGGS, H.R. — Dihydroorotic acid in nutrition of lactic acid Bacteria (20763). *Proc. Soc. Exp. Biol. Med.* **84**: 716-719, 1953.

16. WRIGHT, L.D.; MILLER, C.S.; SKEGGS, H.R.; HUFF, J.W.; WEED, L.L. & SILSON, D.W. — Biological precursors of the pyrimidines. *J. Amer. Chem. Soc.* **73**:1898-1899, 1951.

17. WRIGHT, L.D. & MILLER, C.S. — Uracil in growth and pyrimidine nucleotide synthesis of *Lactobacillus bulgaricus* 09. *Proc. Soc. Exp. Biol. Med.* **81**:131-134, 1952.

18. YONEDA, S. — Some aspects of the purine metabolism of *Trypanosoma cruzi* in tissue culture. *Rev. Brasil. Pesquisas Med. Biol.* **4**: 205-218, 1971.

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