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SOME EFFECTS OF GAMMA-RADIATION ON *TRYPANOSOMA CRUZI*, CULTURE AND BLOOD FORMS

E. CHIARI (1), E. MANSUR Neto (2) and Z. BRENER (3)

SUMMARY

In order to study the effects of gamma radiation on *T. cruzi*, culture and blood forms of this flagellate were irradiated with Cobalt-60 different dosages. MR strain culture forms kept in Yaeger's "LIT" liquid medium were submitted to different gamma-ray dosages and then inoculated in albino mice. All animals inoculated with flagellates submitted to 15 and 30 krad have got infected; with doses of 60 and 90 krad, infection was achieved in 60% and 10% of the animals, respectively; the animals did not get infected at all when inoculated with culture forms irradiated with 120 krad.

Study of the growth curve and metacyclic percentage of the cultures after irradiation has shown the number of flagellates to be closely correlated with the increase of dosage.

Attempts at immunization have been made by injecting mice with culture forms irradiated with 120 krad, the presence of protective antibodies having then not been observed. Blood forms have displayed greater susceptibility to irradiation and 30 krad was enough to hinder their infectivity in mice; these forms, however, did not afford any immunity to re-inoculation.

No morphological changes nor any interference in the reproduction "in vitro" have been observed after the irradiation of blood forms with 30 krad. A 60 krad dosage has hindered the growth of blood forms in "LIT" liquid medium. No radiolysis has been observed.

INTRODUCTION

The present paper deals with some effects of gamma radiation on culture and blood forms of *T. cruzi*, such as lethal doses, loss of infectivity and morphological changes as well as the effects of the mentioned radiation on growth curves of culture-forms. Immunization with irradiated culture forms has also been tried. Some of these aspects had been previously reported by EMMETT [6] and SILVA et al. [9].

MATERIAL AND METHODS

T. cruzi strains

MR and FL strains were isolated by BRENER & CHIARI [4] from naturally infected *Triatoma infestans* collected in different localities of Rio Grande do Sul, Brasil. Y strain was

(1, 3) Centro de Pesquisas René Rachou, Instituto Nacional de Endemias Rurais, C. P. 1743, Belo Horizonte, Minas Gerais, Brasil

(2) Instituto de Pesquisas Radioativas da Universidade Federal de Minas Gerais, Belo Horizonte, Brasil

isolated by PEREIRA DA SILVA & NUSSENZWEIG [8] through xenodiagnosis from a patient with acute Chagas' disease.

Maintenance of parasites and culture medium

All the strains were maintained in mice by regular blood passage. The Y strain has been kept in the laboratory for about 12 years; the other strains were isolated three years ago (BRENER [2], BRENER & CHIARI [4]).

The culture medium used was Yaeger's "LIT" liquid medium described by CAMARGO[5]. MR and Y strains have been maintained in "LIT" medium through serial passages, every 10 days, for about two years.

Irradiation of parasites

Culture and blood forms were placed into pyrex-glass tubes and then irradiated into Gamacell 220. This apparatus displays the following characteristics: Cobalt-60, half life-5,2 years with a dosage of 287 kilorad per hour in irradiation position, thus giving an average of 4,717 roentgen per minute. The present activity of the apparatus is 3,457 curie. Roentgen unit is considered similar to rad with a variation of about 5%.

Growth experiments

Flagellates were grown in 125 ml Erlenmeyer flasks containing 30 ml of "LIT" medium. Inocula from 9-day-old cultures were irradiated with varying gamma-ray dosages and then diluted in fresh medium to give and initial density of 5×10^6 flagellates per ml. The incubation temperature was 28°C.

The counting of flagellates was carried out in a Sanborn-Frommer Cell Counter model 75. The parasites were properly suspended in saline, which was then homogenized and rapidly poured into the reservoir of the apparatus cartridge cell for direct reading.

Determination of the percentage of metacyclic forms

The percentage of crithidiae and metacyclic trypanosomes was determined by microscopically examining and counting, in daily culture smears stained by May-Grünwald-Giemsa method, at least 1,000 unselected forms.

Inoculation of irradiated culture and blood forms

About 0.5 ml of 9-day-old irradiated culture forms (MR strain) were intraperitoneally injected in white mice weighing 18-20 g, just after having being exposed to varying dosage of gamma radiation. Fresh blood examinations were accordingly performed.

Infected blood was collected from mice inoculated with Y, FL and MR *T. cruzi* strains and then immediately submitted to different dosages of gamma radiation. Just after radiation the trypanosomes were injected intraperitoneally into albino mice weighing 18-20 g. Non-irradiated blood forms from the same pool were inoculated into controls. Fresh blood examination and counting of parasites were performed according to the previously described technique (BRENER [1]).

Sub-inoculation, blood culture and re-inoculation

In order to detect sub-microscopical infections, mice were killed and their blood intraperitoneally inoculated in two normal albino mice weighing 16 g. With the same purpose, blood of sacrificed animals was inoculated into two "LIT" medium tubes. With the purpose of finding out whether the infection of irradiated forms give origin to a sub-patent chronic infection non-detected by fresh blood examination or whether the animals did not actually become infected, some of the animals were re-inoculated with about 4,000 blood-forms/gramme by intraperitoneal route and examined for blood trypanosomes.

Vaccination experiments

Thirty albino mice weighing 18-20 g were weekly injected, per subcutaneous route, with about 0.5 ml of 9-day-old culture forms (MR strain) irradiated with 120 krad, for three consecutive weeks. Three weeks after the last injection, the animals injected with the doses of irradiated culture forms were thus divided:

a) 10 animals to be challenged with about 4,000 blood-forms/gramme of *T. cruzi* MR strain;

b) 10 animals to be challenged with about 0.5 ml of 14-day-old culture forms of MR strain;

c) 10 animals to be killed and their blood inoculated into "LIT" medium tubes.

RESULTS

Figure 1 shows the growth curves of flagellates in "LIT" liquid medium and the metacyclic percentages of culture forms (MR strain) irradiated with different dosages of

TABLE I

Effect of varying gamma-rays dosages on the virulence of *T. cruzi* culture forms (MR strain). Number of positive animals/Total number of inoculated animals

Gamma-rays dosages	Days after inoculation				
	13	18	27	33	47
15 krad	10/10	—	—	—	—
30 krad	2/10	10/10	—	—	—
60 krad	0/10	1/10	6/10	—	—
90 krad	0/10	0/10	1/10	—	—
120 krad	0/10	0/10	0/10	0/10	0/10
Control	10/10	—	—	—	—

TABLE II

Number of trypanosomes in 5 cmm of blood from animals reinoculated with blood forms after receiving a single dose of irradiated culture forms (120 krad) and from controls. All animals examined 12 days after inoculation of blood forms

Number of animals	Animals inoculated with irradiated culture and reinoculated with blood forms	Controls of reinoculation with blood forms
1	980	3,500
2	8,400	4,200
3	4,900	11,200
4	3,500	10,500
5	9,800	6,300
6	2,800	—
7	3,500	—
8	8,400	—
9	7,700	—
Mean number of parasites	6,670	7,100

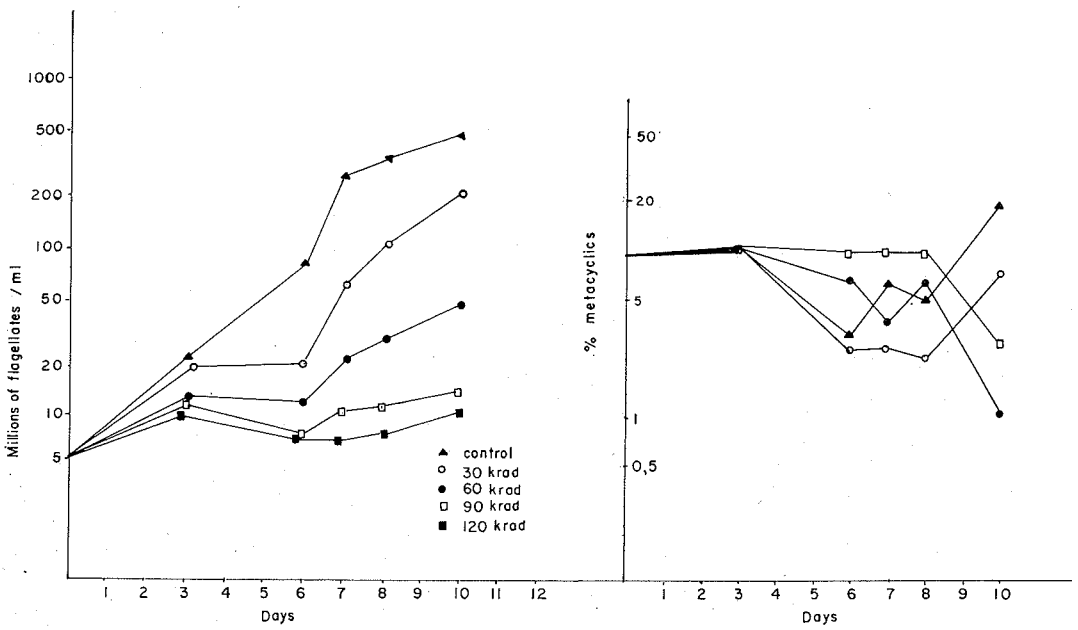

Fig. 1 — Growth curves and percentage of metacyclics trypanosomes (MR strain) of culture forms submitted to varying gamma-rays dosages and cultivated in "LIT" liquid medium

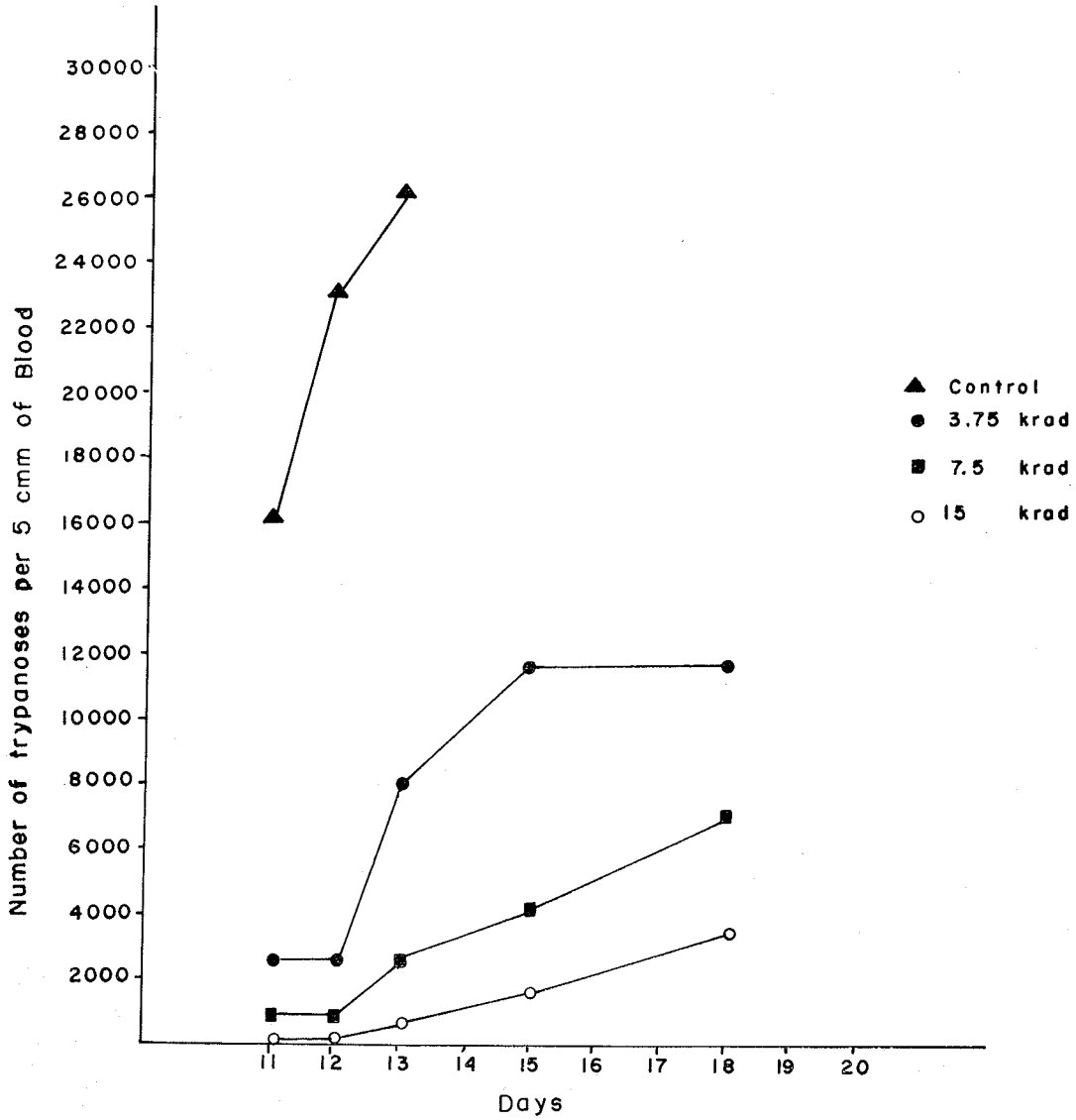

Fig. 2 — Number of trypanosomes in 5 cmm of blood from animals inoculated with blood forms irradiated with different dosages of gamma radiation

gamma radiation. A clear correlation between the gamma-ray dosages and the decrease of growth rate has been observed. Table I shows the effects of different gamma-ray dosages on the culture-form virulence studied through mice inoculation with irradiated flagellates. There has been observed a close correlation between increase of gamma-ray dosages and loss of infectivity. These results have been based on repeated fresh blood examination and blood culture or sub-inoculation. Re-inoculation of animals previously inoculated with irradiated culture forms (120 krad) shows that no immunity could be detected and that the animals were not infected with the irradiated forms (Table II). Similar results have been obtained after inoculation of irradiated blood forms (Table V). Tables III and IV show that mice inoculated with three doses of irradiated culture forms (120 krad) were not protected against challenge infection of *T. cruzi* blood and culture forms. Figure 2 shows the number of trypanosomes in 5 cmm of blood from mice inoculated with blood forms previously exposed to different gamma-ray dosages.

TABLE III

Number of trypanosomes in 5 cmm of blood from animals reinoculated with blood forms after receiving 3 doses of irradiated culture forms (120 krad) and from controls. All animals examined 12 days after inoculation of blood forms

Number of animals	Animals inoculated with irradiated culture and reinoculated with blood forms	Controls inoculated with blood forms
1	2,800	4,900
2	11,200	4,970
3	1,050	770
4	1,190	3,150
5	2,520	980
6	1,400	630
7	19,600	700
8	3,640	—
9	70	—
10	2,520	—
Mean number of parasites	4,535	2,300

TABLE IV

Number of trypanosomes in 5 cmm of blood from animals reinoculated with culture forms after receiving 3 doses of irradiated culture forms (120 krad) and from controls. All animals examined 11 days after challenge with culture forms

Number of animals	Animals inoculated with irradiated culture and challenged with culture forms	Controls inoculated with culture forms
1	420	1,330
2	560	140
3	490	4,900
4	280	1,050
5	0	770
6	140	980
7	770	910
8	280	1,050
9	—	980
10	—	350
Mean number of parasites	367	1,246

TABLE V

Number of trypanosomes in 5 cmm of blood from animals reinoculated with blood forms after receiving a single dose of irradiated blood forms (30 and 60 krad) and from controls. All animals examined 12 days after challenge with blood forms

Number of animals	Animals with irradiated blood (30 krad) and challenged with blood forms	Animals with irradiated blood (60 krad) and challenged with blood forms	Controls inoculated with blood forms
1	2,100	2,100	1,400
2	3,150	3,150	2,100
3	7,140	7,140	3,500
4	2,100	2,100	2,800
5	2,030	2,030	2,100
6	21,000	21,000	—
7	10,500	10,500	—
8	3,500	3,500	—
Mean number of parasites	6,440	6,000	2,380

DISCUSSION

According to EMMETT [6], culture forms irradiated with 51,000 r to 100,000 r lose their infectivity to mice. In our experience most of the animals inoculated with culture forms irradiated with 90 krad did not get infected whereas no infection at all could be detected after inoculation of flagellates irradiated with 120 krad. EMMETT [6] has reported that sub-cultures made on the day of irradiation (100,000 r) were still unable to infect mice, although the viability and morphology of these organisms, seemed to be normal. According to our experiments, sub-cultures made on the day of irradiation (60, 90 and 120 krad) have partially recovered their infectivity and inoculation of mice with these forms, performed one week after the culture passage, has infected 80%, 40% and 10% of the animals respectively. It is difficult to account for such disagreement in these results but it should be remembered that the material and methods used in the two experiments are quite different.

Blood forms are rather more sensitive to gamma radiation than culture forms and these data seem to be in accordance with those of HALBERSTAEDTER [7] who showed that *T. gambiense* blood forms are rendered non-infective after being submitted to as low a radiation as 12,000 r. Although it was not intended to perform a comparative study of the susceptibility of different strains of *T. cruzi* to gamma radiation, this paper can show that blood forms of MR, FL and Y behave similarly after being exposed to this kind of radiation. Growth of culture forms in "LIT" medium has been clearly hindered by gamma radiation and in this respect our data are quite in accordance with those of SILVA et al. [9] who reported that the mobility and reproductivity of the parasites had been lost under doses of or higher than 100 krad.

It has been observed that a certain degree of gamma radiation destroys the infectivity of the parasites but not their viability. Mice repeatedly inoculated with these living irradiated flagellates could not get infected as shown by negative fresh blood examination, subinoculation and blood culture; likewise, chicken-embryo tissue cultures, which are readily infected with *T. cruzi* culture forms (BRENER [3]), were not infected by irradiated flagellates either. An attempt at using irradiated culture forms as a kind of living vaccine showed that no protection against a challenge infection was achieved this way. The fate of the injected irradiated parasites as well as the possible alteration of their antigenic structure have not been investigated.

A quantitative study of the action of gamma rays on culture forms showed that the mobility of the flagellates was very rapidly affected by 466 krad; employing doses of 350, 245 and 155 krad, mobility could be easily observed up to 72 hours after irradiation, after which it gradually decreased. Doses over 155 krad have a lethal effect on the culture forms.

RESUMO

Efeitos da irradiação gama sôbre as formas sangüíneas e de cultura do Trypanosoma cruzi

Com a finalidade de conhecer melhor os efeitos da irradiação gama sôbre o *T. cruzi*, formas de cultura e sangüíneas foram irradiadas com diferentes doses usando-se Cobalto-60. Formas de cultura da amostra MR cultivadas em meio líquido "LIT" de Yaeger foram submetidas a diferentes doses de irradiação gama e inoculadas em camundongos albinos. Com doses de 15 e 30 krads todos os animais se infetaram; com doses de 60 e 90 krads, respectivamente, 60% e 10% dos animais se infetaram e, finalmente, com 120 krads os animais não se infetaram. Uma maior percentagem de formas metacíclicas nas culturas não influe nos resultados. Êsses resultados foram comprovados por exames de sangue a fresco, hemoculturas, subinoculações e reinoculações com formas virulentas. Os estudos das curvas de crescimento e percentagem de metacíclicos após irradiação das culturas mostram um gradiente de redução do número de flagelados diretamente proporcional ao aumento da dose. Foi feita uma tentativa de imunização injetando-se culturas irradiadas com 120 krads, não sendo assinalada a presença de anticorpos protetores. Foi observada maior suscetibilidade das formas sangüíneas à irradiação já que a dose de 30 krads mostrou-se suficiente para impedir a infecção de animais; estas formas também não conferem proteção à reinoculação. Não se observaram alterações morfológicas nem bloqueio da reprodução *"in vitro"* após a ir-

radiação das formas sangüíneas com dose de 30 krads. A dose de 60 krads impede o desenvolvimento de hemoculturas de formas sangüíneas em meio "LIT". Não foi observada radiólise.

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