BR8519461

ISSN 0101-3084

CNEN/SP

ipen Instituto de Pesquisas Energéticas e Nucleares

STANDARDIZATION OF THE BIOLOGICAL CONTROL OF ^{67}Ga-CITRATE IN RATS AND MICE

Emiko Muramoto, Marycel Figols de Barboza, Setsuko Sato Achando, Nilda Petrona Sosa de Pereira e Constância Pagano Gonçalves da Silva

IPEN - PUB -- 209.

PUBLICAÇÃO IPEN 209

SETEMBRO/1988

SÃO PAULO

PUBLICAÇÃO IPEN 209 SETEMBRO/1988

STANDARDIZATION OF THE BIOLOGICAL CONTROL OF ^{67}Ga-CITRATE IN RATS AND MICE

Emiko Muramoto, Marycel Figols de Barboza, Setsuko Sato Achando, Nilda Petrona Sosa de Pereira e Constância Pagano Gonçalves da Silva

DEPARTAMENTO DE PROCESSAMENTO

CNEN/SP
INSTITUTO DE PESQUISAS ENERGÉTICAS E NUCLEARES
SÃO PAULO - BRASIL

Série PUBLICAÇÃO IPEN

INIS Categories and Descriptors

C21.20

GALLIUM 67
CITRATES
RADIONUCLIDE KINETICS
STANDARDIZATION

IPEN - Doc - 3077

Aprovado para publicação em 30/06/87.

STANDARDIZATION OF THE BIOLOGICAL CONTROL OF ^{67}Ga-CITRATE IN RATS AND MICE*

Emiko Muramoto, Marycel Figols de Barboza, Setsuko Sato Achando, Nilda Petrona Sosa de Pereira e Constância Pagano Gonçalves da Silva

ABSTRACT

^{67}Ga-citrate is frequently used in Nuclear Medicine for tumor diagnosis. This study established the biological distribution parameters in rats with experimental abscesses in the left thigh. The biological model choosen made possible the routine control of ^{67}Ga-citrate giving a realiable result in a relative short-time (60 min.) after the radionuclide administration.

PADRONIZAÇÃO DO CONTROLE BIOLÓGICO DE CITRATO - ^{67}Ga EM RATOS E CAMUNDONGOS

RESUMO

A fim de estabelecer os parâmetros do controle biológico do citrato- ^{67}Ga, estudaram-se animais induzidos com processo inflamatório experimental na perna esquerda (E) considerando a perna direita (D) como controle. Para o controle biológico de rotina foi possível estabelecer que a captação do citrato- ^{67}Ga, pelo tumor, se dá aos 60 minutos após a administração da dose.

INTRODUCTION

Several papers had been published on the use of ^{67}Ga-citrate in Nuclear Medicine for the localization of a variety of malignant human tumors.

Over twenty years ago radiogallium was investigated as a prospective agent fot the diagnosis and treatment of neoplasm involving bone as suggested by Dudley, Maddox and La Rue, who discovered concentration of ^{72}Ga at sites of osteogenesis in animals (2). This radionuclide was produced in nuclear reactor by a (n,γ) reaction on stable Ga-67.

After that event, clinic studies were made with ^{67}Ga essentially carrier-free gallium (cyclotron produced by a (p,2n) interation with

---

(*) Paper presented at the "XI Congreso de la Asociación Latino Americana de Sociedades de Biologia y Medicine Nuclear" - Cidade de México - México, 16 a 21 de novembro de 1987.

Zn-68)revealed that the amount of stable gallium administered had a profound tissue distribution in rats.

Nelson et al. made studies in rats using ^{67}Ga prepared with 0.2mg gallium carrier per Kilograma of body weight (5).

Experiments in rats have shown no important different between carrier-free doses and carrier levels up to 0.25 mg/kg (3).

Larson et al. (4) verified in "vitro" studies that secondary effects such as competition with other ligands present in the tissue-culture medium, are important in determining the degree of transferrin binding of carrier-free ^{67}Ga.

The biological distribution of ^{67}Ga after intravenous administration depends on the plasma protein migration, mainly on the transferrin migration, to the tissues and organs. The uptake mechanism and the affinity of the ^{67}Ga for the tumor is not yet known.

In tracer quantity the gallium acts as ferric ion, none the less, there are some differences between them. The gallium has less affinity to the transferrin than iron and it does not bind to the "heme" or to other biological proteins.

The purpose of this study was to establish the biological distribution parameters of the ^{67}Ga prepared at the "Instituto de Pesquisas Energéticas e Nucleares - São Paulo (IPEN-SP), for a routine quality control and also to establish the uptake levels in animals with experimental abscesses in the left thigh.

The results were compared with those obtained with pretreated (rats) using ^{67}Ga citrate from Mallinckrodt, Inc U.S.A.

MATERIAL AND METHODS

1. ^{67}Ga-citrate (carrier-free).

   ^{67}Ga citrate was prepared at the Instituto de Pesquisas Energéticas e Nucleares - São Paulo (IPEN-SP). ^{67}Ga is obtained in cyclotron through (p,2n) interaction with Zn-68. The compound was found 99.21% mean rate radiochemical purity. The ^{67}Ga-citrate from Mallinckrodt, Inc was found with 99.5% radiochemical purity.

2. Animals.

   Adults male "Wistar" rats weighting 150-250g (average weight of 200g), and male "Swiss" mice with weight varying between 30-38g (average weight 34g) were used.

3. Pre-treatment.

   Sterile bacterial abscesses were induced by the intramuscular injection of 0.2ml (rats) and 0.1ml (mice) of Bacto Adjuvant Complete Freund into left thigh of the animal is usefull within two-five days after injection. The right thigh was used as control.

4. Biological Distribution.

   The animal were anaesthetized with "Uretana" solution (100mg/100g body

weight) or diethyl ether, and ^{67}Ga-citrate administered intravenously or intraperitoneally, with a dose of 180 μCi (rats) and 75 μCi (mice).

The animals were sacrified 60 min. and 72h. after administration of the dose. The following organs were taken off: left thigh, right thigh, kidney, heart, lung, stomach, rib, liver, intestine, cervical column and blood. Each organ was individually weighted and counted in well scintillation counter (type Automatic Counts "Nuclear Chicago") and compared with a standard aliquot of ^{67}Ga.

Another group of animals were administered 500 μCi of ^{67}Ga-citrate i.v., A linear Scintillography, 60 min. after the injection, were made in a "PHO-DOT" (Nuclear Chicago) linear scintillographer.

The percent of uptake dose in each organ was estimated by the following expression:

$$\% \text{ dose} = \frac{\text{cpm in each organ}}{\text{cpm of standard (67-Ga Citrate)}}$$

$$\% \text{ total} = \frac{\text{cpm in each organ}}{\Sigma \text{ in each organ}}$$

RESULTS

All results are expresssed as percent of the administered dose and as percent of total radioactivity, with the mean and standard deviation calculated from data of 60 min. and 72h. (rats) and 90 min. (mice), with experimental abscesses.

Table I and II show the results of % dose and % total administered dose in different organs after 60 min. and 72h in rats with experimental abscesses.

A rapid and significant clearance in blood was observed (blood = 0.06 ± 0.04% dose and 0.83 ± 0.18% total) in 72h after the ^{67}Ga-citrate when compared with data from 60 min. (1.73 ± 0.64% dose and 8.89 ± 1.34% total).

The uptake of ^{67}Ga by the abscesses was immediate, i.e., soon after its administration (data not reported for 15 min.). However, although the activity (% of administered dose) is lower after 72h. than 60 min. (F = 1.38), the factor observed between the abscesses thigh against the normal is more than F = 2.21.

$$F = \frac{\text{left thigh activity}}{\text{righ thigh activity}}$$

Table III - Presents the results from biological distribution of ^{67}Ga-citrate in mice with abscesses. A higher uptake in the left thigh was verified with a factor F = 1.41 regarding the righ thigh.

The total organ uptake values in rats and mice were higher for the liver and intestine when compared with those of other tissues. They are in agreement with the results reported by Larson et al 1973 (4) and Saha et. al. 1983 (6).

Table IV - Shows the results in rats with experimental abscesses after intraperitoneal injection of ^{67}Ga from Instituto de Pesquisas Energéticas e Nucleares - São Paulo and Mallinckrodt. Similar were observed in all organs, except in the rib where a higher uptake was observed in those animals injected with ^{67}Ga from the Mallinckrodt, Inc.

The maximum activity (tumor peak) is observed at 24h. After this time the uptake decreases.

From figure 1 and 2, we can verify that the data for organs such as liver, intestine, cervical column and rib remain inaltered.

Figures 3, 4 and 5 illustrate the linear scintillography in rats with experimental abscesses in left thigh, 60 min. 24h. and 72h. after injection of 500 μCi of ^{67}Ga-citrate, showing a higher accumulation of radioactivity in the left thigh

Figure 6 - illustrates the linear scintillography in mice 90min. after the administration of 200 μCi ^{67}Ga.

CONCLUSION.

The biological study of ^{67}Ga-citrate in normal rats with experimental abscesses, shows no difference in organs distributions in 1, 24 and 72h after tracer administration. However, the experimental model choosen make possible the biological routine control of ^{67}Ga-citrate, giving a reliable result, a hight uptake in the left thigh F = 1,38 comparing with the normal thigh, in a relative short time (60 min.).

ACKNOWLEDGMENT.

The authors thank the hepfull aid during the development of this work to technician Marina Leonel da Silva.

TABLE I - BIOLOGICAL DISTRIBUTION OF ^{67}Ga-CITRATE (% DCSE ± S.D.) IN RATS*.

ORGAN \ TIME	60 min.	72h
Left Thigh	4.88 ± 1.62	2.26 ± 1.39
Right Thigh	3.52 ± 0.94	1.02 ± 0.35
Kidney	1.67 ± 0.91	0.93 ± 0.28
Heart	0.36 ± 0.11	0.10 ± 0.04
Lung	0.62 ± 0.16	0.26 ± 0.11
Stomach	0.85 ± 0.38	0.91 ± 0.33
Rib	2.28 ± 0.60	2.15 ± 1.27
Liver	4.57 ± 1.37	5.35 ± 1.93
Intestine	4.01 ± 1.18	3.16 ± 1.01
C. Column	2.09 ± 0.67	1.96 ± 0.75
Blood	1.73 ± 0.64	0.06 ± 0.04

*N = 14

TABLEII - BIOLOGICAL DISTRIBUTION OF ^{67}Ga-CITRATE (% TOTAL ± S.D) IN RATS*.

ORGAN	60 min.	72h
Left Thigh	22.69 ± 3.64	8.92 ± 5.30
Right Thigh	16.71 ± 3.14	4.06 ± 1.33
Kidney	7.37 ± 3.14	3.66 ± 0.51
Heart	1.70 ± 0.43	0.38 ± 0.11
Lung	3.22 ± 1.03	0.99 ± 0.27
Stomach	4.01 ± 1.04	3.68 ± 1.43
Rib	10.85 ± 1.65	8.21 ± 2.49
Liver	23.50 ± 5.39	29.07 ± 7.11
Intestine	13.78 ± 5.12	12.49 ± 3.86
C. Column	6.23 ± 1.37	7.41 ± 1.76
Blood	8.89 ± 1.34	0.23 ± 0.18

*N = 14

TABLE III - BIOLOGICAL DISTRIBUTION OF ^{67}Ga-CITRATE 90 MINUTES AFTER ADMINISTERED DOSE IN MICE*.

ORGAN	% DOSE ± SD	% TOTAL ± SD
Left Thigh	5.44 ± 1.53	21.49 ± 2.17
Right Thigh	3.85 ± 0.49	13.72 ± 1.88
Kidney	1.11 ± 0.25	4.13 ± 0.63
Heart	0.31 ± 0.04	1.19 ± 0.24
Lung	0.69 ± 0.07	2.61 ± 0.41
Stomach	0.82 ± 0.21	3.04 ± 0.73
Rib	1.90 ± 0.26	7.14 ± 0.96
Liver	4.30 ± 1.10	15.75 ± 2.15
Intestine	4.50 ± 1.08	16.48 ± 2.02
C. Column	1.69 ± 0.21	6.91 ± 1.35
Blood	1.48 ± 0.27	6.06 ± 0.96

*N = 7

TABLE IV - COMPARATIVE DISTRIBUTION STUDIES IN RATS WITH ^{67}Ga-CITRATE FROM IPEN-CNEN/SP AND MALLINCKRODT. INC U.S.A.

% organ	Left Thigh	Right Thigh	Kidney	Heart	Lung	Stomach	Rib	Liver	Intestine	Blood	
% DOSE	5.23 ± 1.62	3.64 ± 0.35	0.19 ±2.23	0.14 ± 0.05	0.47 ± 0.25	1.81 ± 0.46	1.75 ± 0.24	8.27 ± 2.17	6.29 ± 3.30	0.17 ± 0.02	IPEN
	6.62 ± 1.06	4.76 ± 1.31	1.58 ±0.12	0.24 ± 0.09	0.42 ± 0.13	1.06 ± 0.52	3.15 ± 0.24	9.50 ± 0.98	6.95 ± 1.09	0.32 ± 0.20	MALLINCKRODT
% TOTAL	17.91 ± 6.14	12.42 ± 1.00	4.06 ±0.61	0.37 ± 0.02	2.17 ± 0.55	6.14 ± 1.20	5.98 ± 0.77	26.61 ± 3.38	18.02 ± 5.92	0.58 ± 0.08	IPEN
	19.53 ± 2.71	15.91 ± 1.60	4.37 ±0.51	0.67 ± 0.18	1.43 ± 0.03	6.16 ± 1.75	7.75 ± 1.83	26.70 ± 3.61	18.90 ± 1.11	0.87 ±0.46	MALLINCKRODT

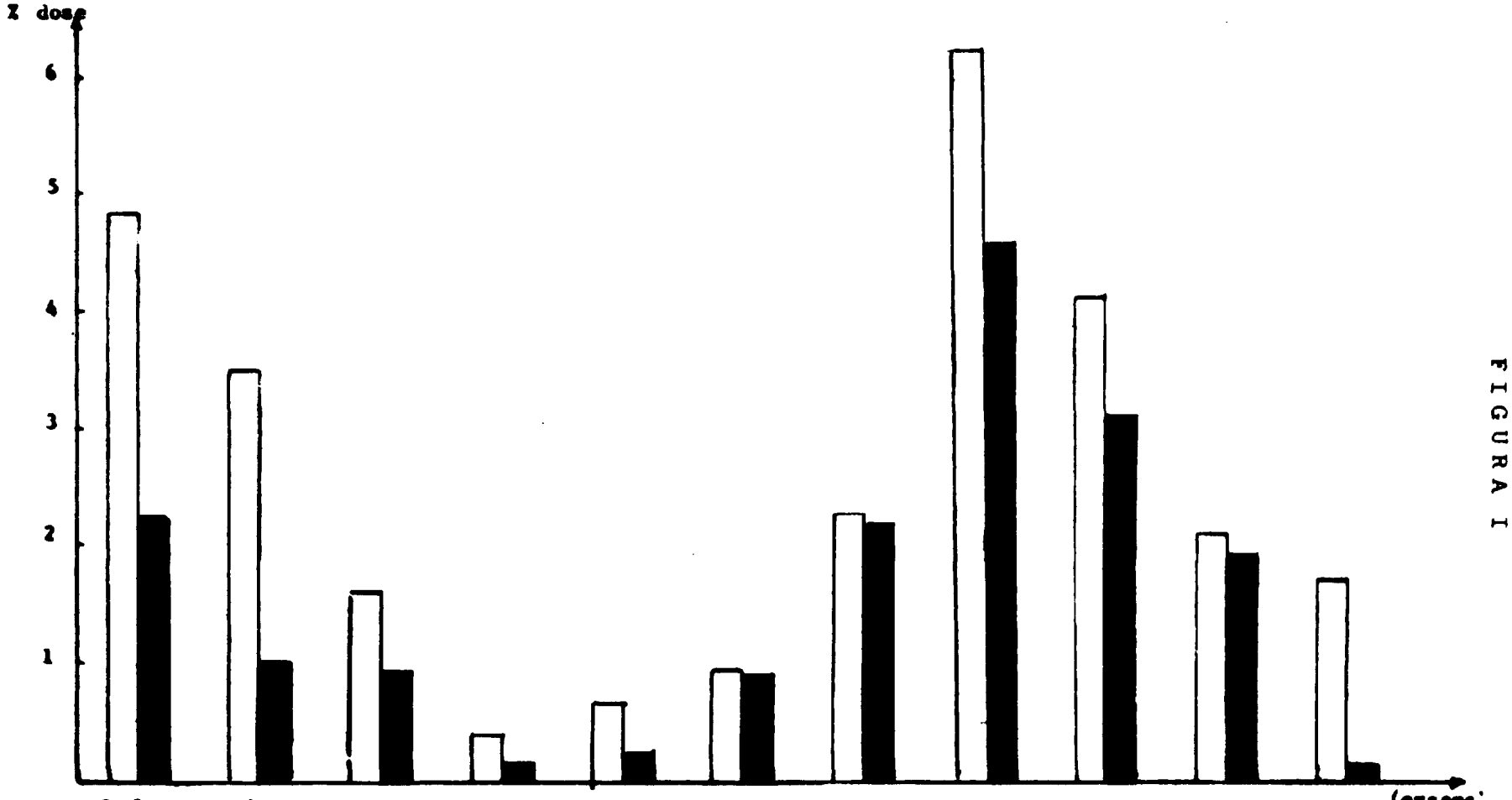


FIGURA I

BIOLOGICAL DISTRIBUTION OF ^{67}Ga-CITRATE

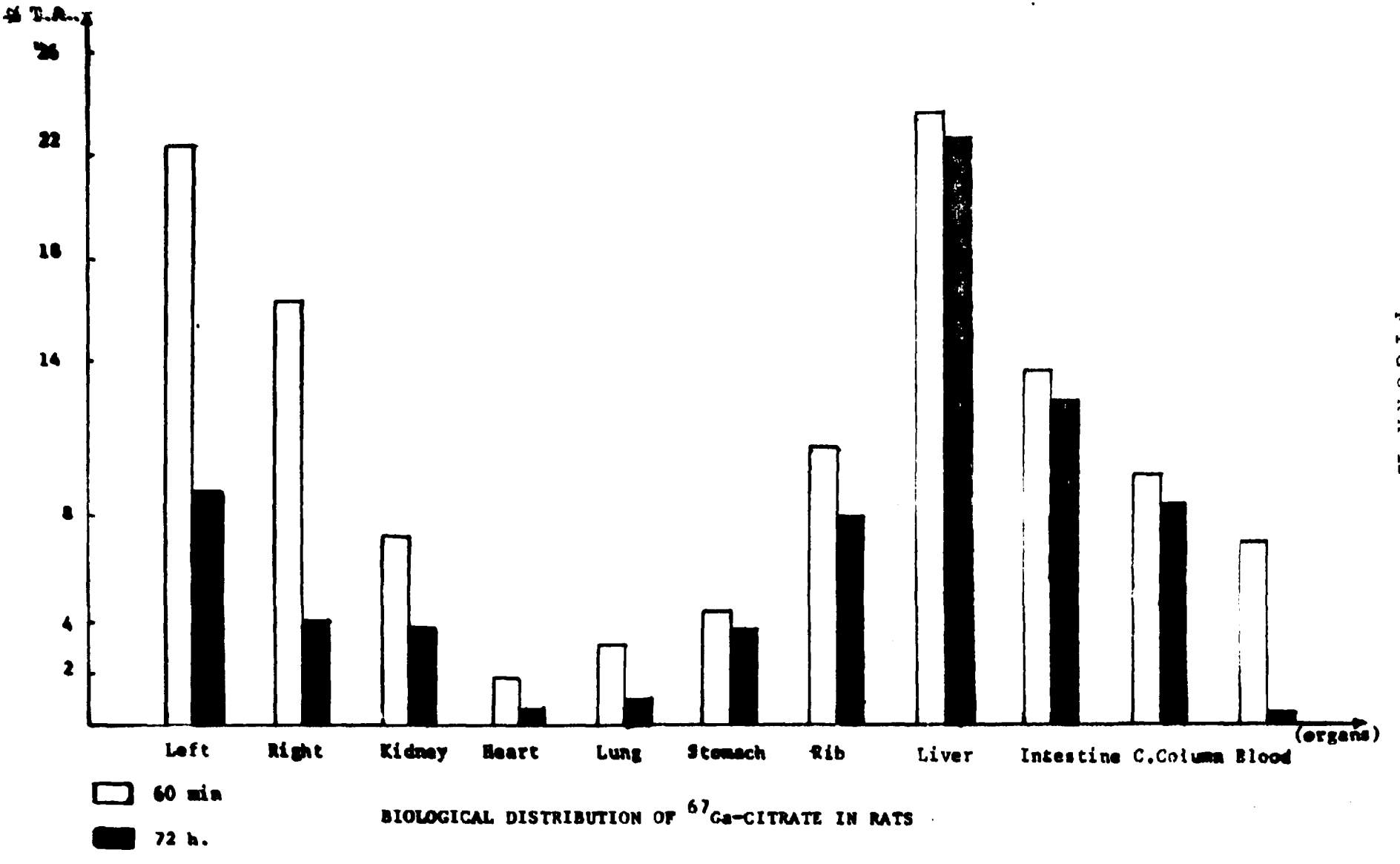


FIGURA II

BIOLOGICAL DISTRIBUTION OF ^{67}Ga-CITRATE IN RATS

FIGURE 3 - LINEAR SCINTILOGRAM IN RATS WITH ABSCESSES IN LEFT THIGH (VENTRAL POSITION) 60 min. AFTER THE INJECTED DOSE.

FIGURE 4 - LINEAR SCINTILOGRAM IN RATS WITH ABSCESSES IN THE LEFT THIGH (DORSAL POSITION) 72h AFTER THE ADMINISTERED DOSE.

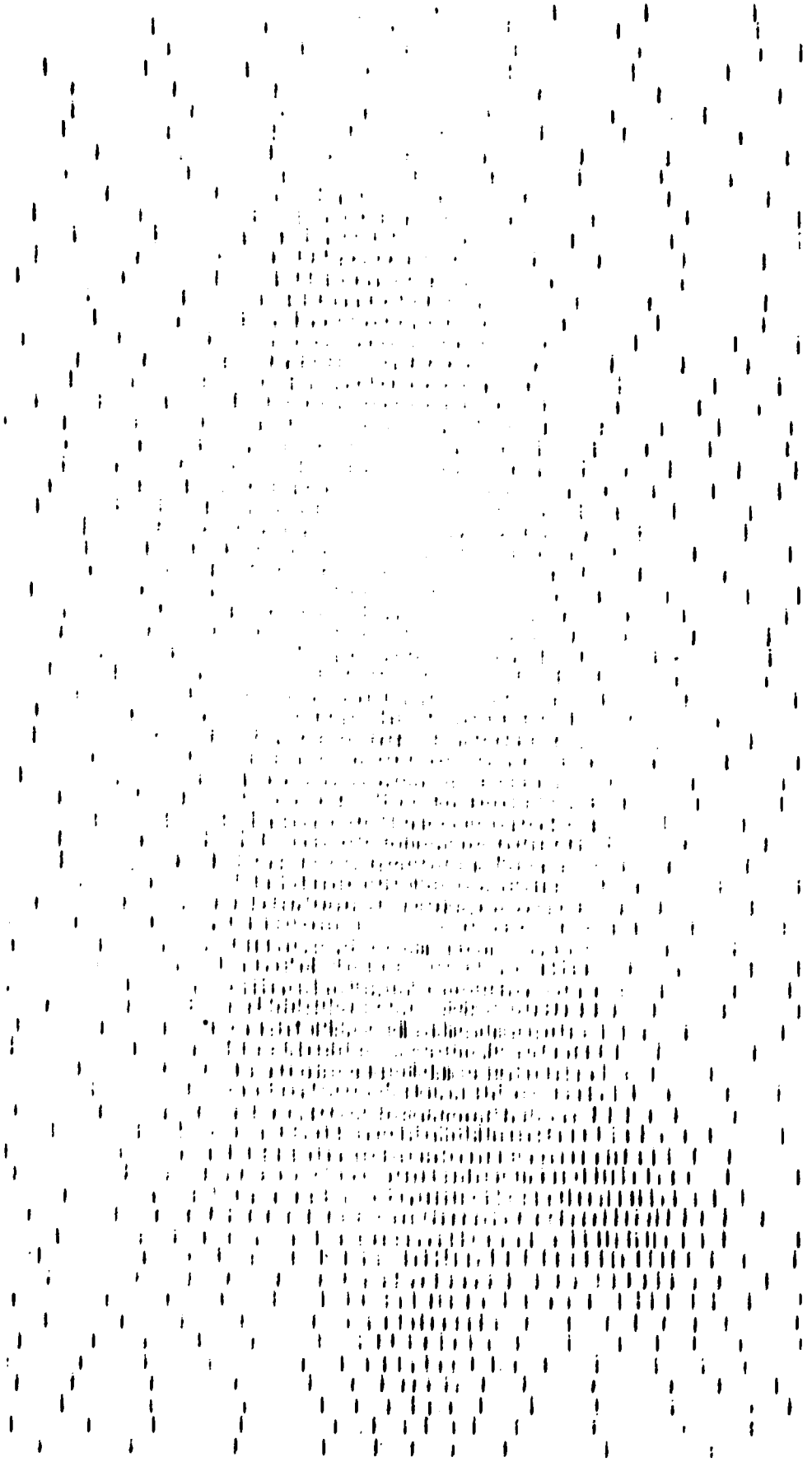


FIGURE 5 - LINEAR SCINTILOGRAM IN RATS WITH ABSCESSES IN LEFT THIGH (VENTRAL POSITION) 24h. AFTER THE INJECTED DOSE.

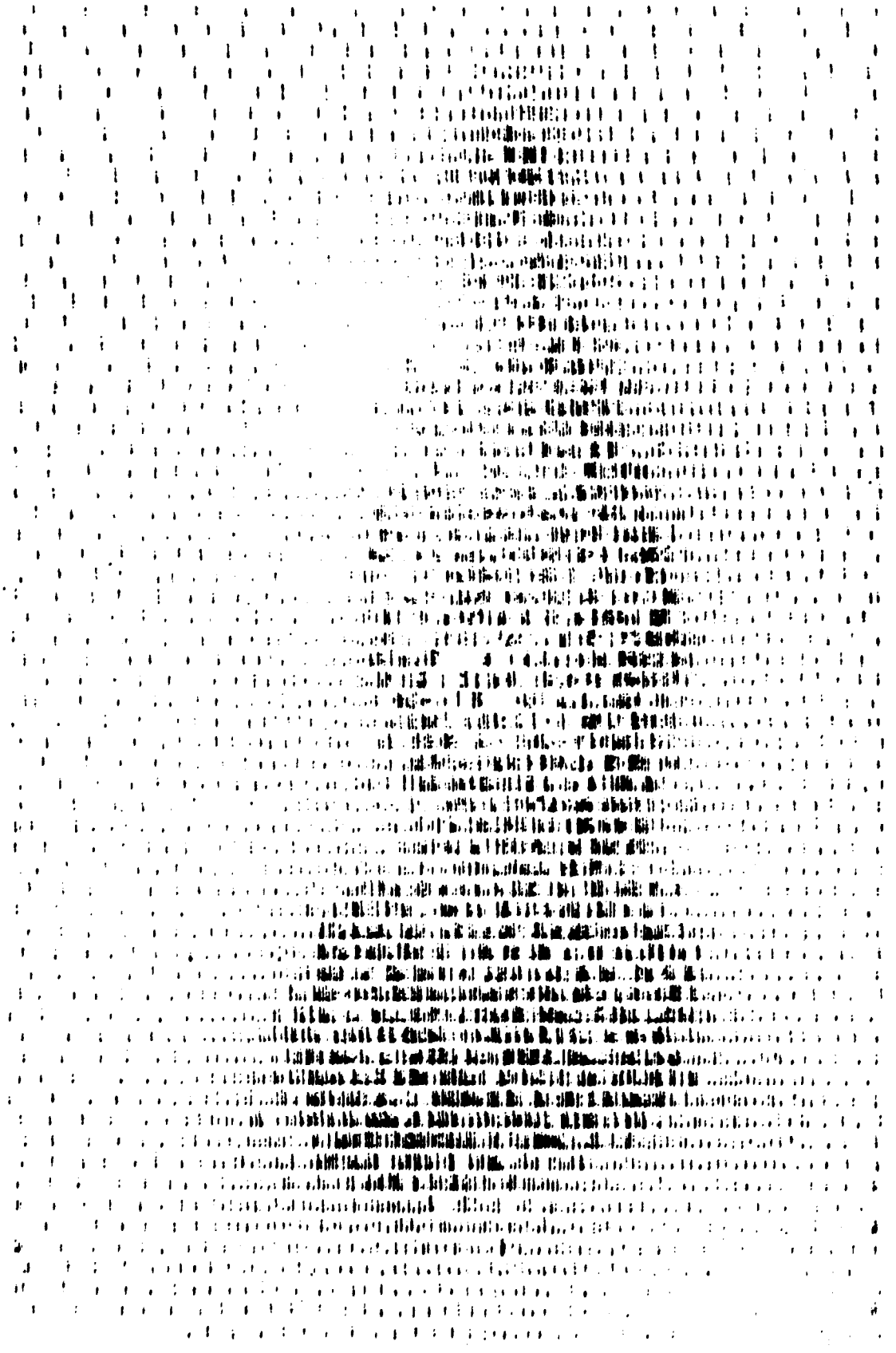
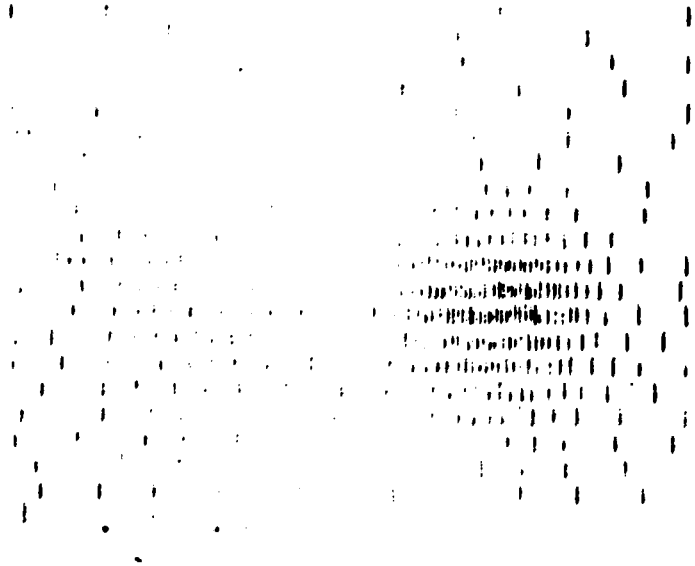


FIGURE 6 - LINEAR SCINTILOGRAM IN MICE WITH ABSCESSES IN LEFT THIGH 90 min. AFTER THE INJECTED DOSE.



REFERÊNCIAS BIBLIOGRÁFICAS

1. BRUNER, H.D.; HAYES, R.L.; PERKISON, J.D. A study of gallium (^{72}Ga) X. Preliminary data on gallium. Radiology, 61:602-11, 1953.

2. DUDLEY, H.C.; MADDOX, G.E.; LA RUE, H.C. Studies of the metabolism of gallium. J. Pharmacol. Exp. Ther, 96:135-8, 1949.

3. HAYES, R.L.: CALTON, I.E.; BYRD, B.L. Bone scanning with gallium-68 a carrier effect. J.Nucl. Med., 6:605-10, 1965.

4. LARSON, S.M.; ALLEN, D.R.; RASEY, I.; GRUNBAUM, J. Kinetics of binding of carrier-free Ga-67 to human transferrin. J.Nucl.Med., 19:1245-9, 1978.

5. NELSON, B.; HAYES, R.; EDWARDS, L.; KNISELEY, R.; ANDREWS. G.S. Distribution of gallium in human tissues after intravenous administration. J. Nucl. Med., 13:92-100, 1972.

6. SAHA, G.B. & BOYOD, C.M. Tissue distribution of ^{67}Ga-Citrate and $^{111}InCl_3$ in mouse with adenocarcinoma. Int. J. Nucl. Med. Biol., 10(4):223-5, 1983.