Rev. Inst. Med. trop. São Paulo
31 (3): 135-138, maio-junho, 1989

LABELING OF Salmonella typhymurium WITH IODINE-131 TO STUDY PHAGOCYTIC FUNCTION IN RATS.

Maria K. SATO(1), Aldo J. RODRIGUES JUNIOR(2) & Edwaldo E. CAMARGO(1)

SUMMARY

The present study describes a method for labeling **Salmonella typhymurium** with iodine-131 to evaluate both the morphological and the functional characteristics of the reticulo-endothelial system.

A suspension containing 2 x 10^9 bacteria per ml was labeled with carrier-free $Na^{131}I$ without reductor, with a labeling yield of 46.5 ± 3% and 3.5 ± 1.3% of free Iodine-131.

The biodistribution of the labeled bacteria in rats was studied with a large-field-of-view scintillation camera equiped with a pinhole collimator. Whole body images were obtained 15 and 30 minutes after intravenous injection of the labeled microorganisms. Images showed accumulation of bacteria in the liver and both normal and transplanted spleens of the animals. Autoradiographs of liver and spleen demonstrated labeled bacteria within the cells of the reticulo-endothelial system.

The method described is easy to perform, has a good labeling yield and allows the functional evaluation of the reticulo-monophagocytic system, including transplanted spleens.

KEY WORDS: Salmonella typhymurium; Iodine-131; Transplanted spleen.

INTRODUCTION

Several microorganisms have been labeled with radioactive isotopes for studying the phagocytic function of the reticulo-endothelial system (RES), the mecanism involved in inflammatory processes or the blood clearance of bacteria[1, 2, 4, 6].

Although these phenomena can be studied with non-radioactive colloidal particles, by using labeled bacteria deposition is prevented.

This investigation describes a labeling procedure for **Salmonella typhymurium** with iodine-131 to study the phagocytic function of the RES.

MATERIAL AND METHODS

Preparation of Bacteria

The microorganisms, grown on simple agar medium, were obtained from the Bacteriology

(1) Centro de Medicina Nuclear, Complementar do Departamento de Radiologia da Faculdade de Medicina da Universidade de São Paulo. São Paulo, SP, Brasil.
(2) Disciplina de Cirurgia Geral, Faculdade de Medicina da Universidade de São Paulo. São Paulo, SP, Brasil.
Address for correspondence: Dra. Maria Kasue Sato. Centro de Medicina Nuclear da Universidade de São Paulo. Caixa Postal 22022. CEP 01499 São Paulo, SP, Brasil.

Section, Instituto Adolfo Lutz, São Paulo, Brazil, and identified as **Salmonella typhymurium** 4,12:i:2 strain. An aliquot of the bacteria was scraped from the agar plate and transferred to another simple agar plate and incubated for 18 to 24 hours at 37 C. The bacteria were then homogenized and suspended with sterile saline solution and diluted to a final concentration of 2 x 10^7 bacteria per ml, according to MacFarland barium sulfate standards.

Labeling Technique

A modification of the method of GREEN WOOD et al[3], was used for labeling the microorganisms. Three ml of the final **Salmonella typhymurium** suspension were transferred to a conic tube and centrifuged at 1.000 x g for 10 minutes. The supernatant was discarded and to bacterial pellet were added 0.2 ml of phosphate buffer pH 7.3, 0.01-0.02 ml of carrier-free $Na^{131}I$ (222.0 MBq/mg or 6.0 Ci/mg) and 2.0 mg of chloramine T (Merck). The suspension was gently mixed and allowed to reaction at room temperature for 15 minutes. The reaction was then stopped with 4.0 mg of sodium metabisulphite. The suspension was again centrifuged at 1,000 x g for 10 minutes and the supernatant discarded. The bacteria were resuspended in 5.0 ml of saline and the protein-free iodide removed by centrifugation with three consecutive saline washes. After the third wash, the bacteria were transferred to another tube and the washing procedure repeated twice with 2.0 ml of saline solution. Bacterial viability was not studied and the tracer contaminant amount was carried by free ^{131}I quantification in ascending chromatography.

Biodistribution

The adequacy of labeled bacteria for studying the phagocytic function of the RES was evaluated in 14 normal Wistar rats (183-259 g). The investigation was then extended to include the phagocytic function of transplanted spleen of 27 Wistar rats (182-243 g). Each animal was injected with 70 μCi (2.59 MBq) of bacterial suspension into the tail vein 15 to 30 minutes prior to whole body imaging[5]. Images with 50,000 counts were obtained with a large-field-of-view scintillation camera equiped with a pinhole collimator.

Twelve hours after the injection the animals were sacrificed and the liver and spleen removed for histologic and autoradiographic studies.

RESULTS

The labeling yield of 10 consecutive experiments averaged 46.5 ± 3% and the amount of free radioactive iodide 3.5 ± 1.3%. Whole body imaging demonstrated selective uptake of **Salmonella typhymurium**-^{131}I by the liver and spleen (Fig. 1).

Autoradiographs of the liver showed silver granules precipitation over the Kupffer cells, which indicated phagocytosis of the bacteria. Similarly, autoradiographs of both normal and transplanted spleens showed silver granules precipitation over the mononuclear cells of the red pulp, indicative of bacterial phagocytosis (Figs. 2, 3 and 4).

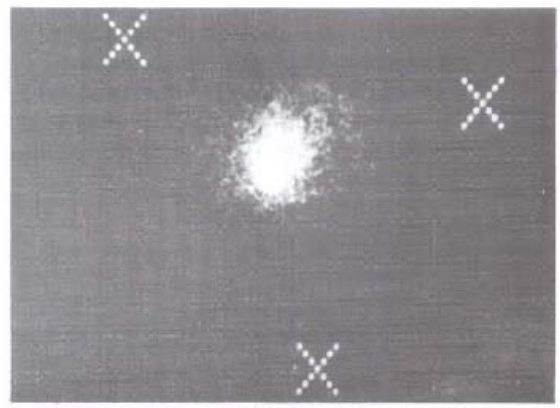

Fig. 1 — Whole body imaging of a normal rat 15 minutes after intravenous injection of **Salmonella typhymurium**-^{131}I. The radioactive area represents uptake of the bacteria by the liver and spleen.

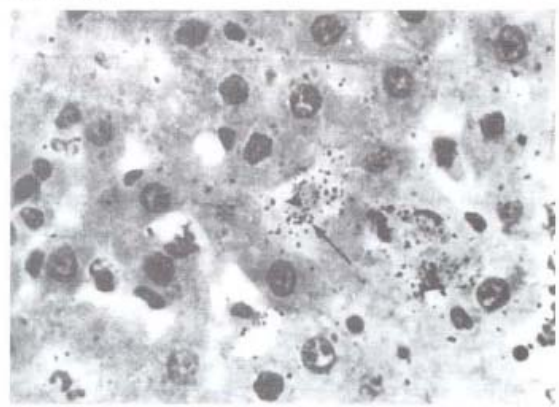

Fig. 2 — Autoradiogram of a rat liver showing labeled bacteria (arrow) in the cytoplasm of the Kupffer cells (1,200 X).

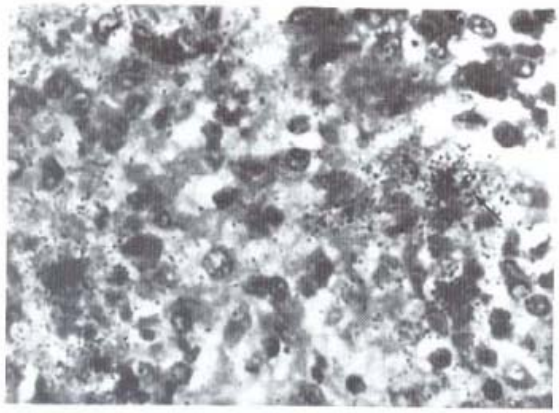

Fig. 3 — Autoradiogram of a rat spleen showing labeled bacteria (arrow) in the cytoplasm of reticulo-monophagocytic system cells (1,200 X).

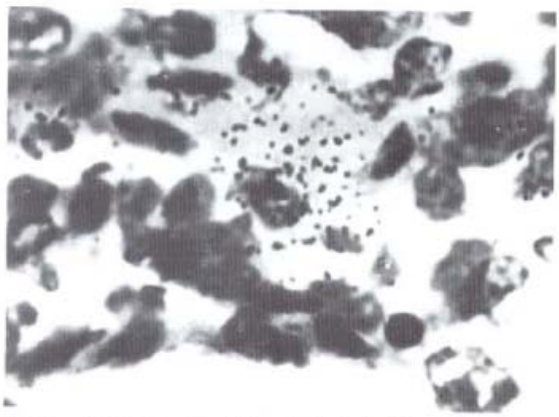

Fig. 4 — Autoradiogram of a transplanted spleen showing labeled bacteria in the cytoplasm of the reticulo-monophagocytic system cells (3,000 X).

DISCUSSION

Because of its tropism to splenic tissue and the ease of maintenance in the laboratory, **Salmonella typhymurium** is the microorganism of choice for assessment of the phagocytic function of the transplanted spleen.

It was noticed, in this investigation, that when the bacteria were not freshly grown prior to labeling the radiochemical yield of the procedure was very low, increasing the amount of free iodide and therefore making the microorganisms unsuitable for use.

To improve the radiochemical yield, a few modifications were introduced in the chloramine T iodation method[6]. Iodide-131 incorporation into bacterial protein is strongly dependent upon the reaction volume. Therefore, the reaction volume was reduced to a minimum by centrifugation of the bacterial suspension and addition of chloramine T in the crystalline form, with no dissolution, to the bacterial pellet. Since chloramine T is a strong oxidant agent, an excess of microorganisms was used to minimize cell damage. The pellet was washed with saline solution only, to minimize bacterial loss. With these modifications the microorganisms could be used for up to 24 hours after labeling.

Since the description of the hazards in overwhelming post splenectomy syndrome there have been an increased attention in splenic conservative maneuvers mainly directed to splenic trauma.

In this way, the omental pouch splenic heterotopic autotransplant has been reported. Meanwhile some controversies do exist on the filtering hability of these regenerated splenic tissue.

RESUMO

Marcação de Salmonella typhymurium **com iodo-131 para estudo da função fagocitária em ratos.**

O presente trabalho descreve um método para marcação de **Salmonella typhymurium** com iodo-131, útil para avaliar tanto os aspectos morfológicos como funcionais do sistema retículo-endotelial.

Uma suspensão contendo 2 x 10^9 bactérias por ml foi marcada com $Na^{131}I$ livre de carregador e de redutor, resultando em um rendimento de marcação de 46,5 ± 3% e 3,5 ± 1,3% de iodeto-131 livre.

Estudou-se a biodistribuição das bactérias marcadas em ratos, obtendo-se imagens de corpo total aos 15 e 30 minutos após injeção em uma câmara de cintilação de campo de visão amplo, equipada com um colimador "pinhole". As imagens mostraram captação das bactérias pelo fígado e pelo baço normal ou transplantado, dos animais estudados. Os cortes autoradiográficos de baço e fígado mostraram bactérias mar-

cadas dentro das células do sistema retículo-endotelial.

O método de marcação da **Salmonella typhymurium** com iodo-131 se mostrou de fácil execução com rendimento adequado, possibilitando a avaliação funcional de órgãos do sistema retículo-monofagocitário como o baço transplantado.

ACKNOWLEDGEMENT

The authors are indebted to Gilde C. D. Hemielevski for her assistance in the preparation of the references cited.

REFERENCES

1. BETTIN, K.; ALLEN, O'C. M.; GERDING, N. D.; FORSTRENN, L & SHAFER, R. — In 111 **Pseudomonas aeruginosa**: a simple method of labeling live bacteria with a gama emitting radioisotope. **Europ. J. Nucl. Med., 12**: 277-279, 1986.

2. BIOZZI, G.; HOWWARD, J. G.; HALPERN, B. N.; STIFFEL, C. & MOUTON, D. — The kinetics of blood clearance of isotopically labeled Salmonella enteritidis by the reticuloendothelial system in mice. **Immunology, 3**: 74-89, 1960.

3. GREENWOOD, F. C.; HUNTER, W. M. & GLOVER, J. S. — The preparation of ^{131}I labeled human growth hormone of high specific radioactivity. **Biochem. J., 89**: 114-123, 1963.

4. MELBY, K. & MIDVEDT, T. — Brief report: a study of the elimination phase of phagocytosis of ^{32}P labeled **Escherichia coli** by human polymorphonuclear cells. **Acta path. microbiol scand., 89**: 37-39, 1981.

5. RODRIGUES Jr., A. J. **Auto transplante heterotópico do baço: estudo experimental.** São Paulo, 1985 (Tese de Doutoramento — Faculdade de Medicina da Universidade de São Paulo).

6. SPECTOR, W. G.; REICHHELD, N. & RYAN, G. B. — Degradation of granuloma inducing microorganisms by macrophages. **J. Path., 101**: 339-354, 1970.

Recebido para publicação em 5-4-88.