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Research Papers

THE GROWTH OF MICRO-ORGANISMS IN SOME PARENTERAL RADIOPHARMACEUTICALS

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SUMMARY

The ability of the non-radioactive components of 9 common technetium-labelled radiopharmaceuticals to support the growth of 5 different species of micro-organism has been investigated. Periods of incubation appropriate to shelf-life, and inoculum size appropriate to possible contamination during hospital assembly of the products, have been used. The effect of incubation at room and at refrigerator temperature on two organisms was studied in the two radiopharmaceuticals which most easily supported growth.

All of the radiopharmaceuticals tested were capable of sustaining the numbers of at least one microbial species but no consistent pattern of growth emerged in relation to organism or to radiopharmaceutical. In some cases, growth was similar at 4°C and at 25°C.

In view of the above results it has been recommended that radiopharmaceuticals be assembled under conditions compatible with the lowest possible microbial contamination.

INTRODUCTION

The commonest radiopharmaceutical dosage form (approximately 80% of all doses) is the parenteral injection (Bell and Horton, 1978). The radionuclide most frequently employed is technetium-99m (^{99m}Tc) which has a radioactive half-life of 6 h. It is obtained as sterile sodium (^{99m}Tc) pertechnetate solution by eluting a sterile chromatographic generator with sterile saline. An injection is then assembled by aseptic addition of sodium pertechnetate solution to the non-radioactive ingredients of the radiopharmaceutical in a sterile vial, usually commercially manufactured and often termed a 'kit' vial (Rhodes and Croft, 1978). This typically contains sufficient quantities of ingredients for up to 5 patient doses. The reconstituted doses are commonly administered without terminal sterilization either because the contents are thermolabile or because of timing

constraints. Due to their short radioactive half-life, they are administered on the day of preparation and sterility testing has to be a retrospective procedure. The addition of bactericides to such short-lived preparations is not customary (European Pharmacopoeia, 1975). For these reasons, radiopharmaceuticals are prepared under aseptic conditions (Hospital Physicists' Association, 1977).

The extent to which radioinjections can act as microbiological growth media is therefore important but unknown (Hupf, 1976). It has been proposed that radiopharmaceuticals in multi-dose containers without bactericide and without re-sterilization after each withdrawal may be safely used within 8 h of preparation provided they are stored at 4°C (British Institute of Radiology, 1975). However, experience with intravenous infusion fluids has shown that simple chemical solutions support the rapid multiplication of some microorganisms (Holmes and Allwood, 1979). Gram-negative bacteria are known to proliferate in distilled water (Carson et al., 1973).

MATERIALS AND METHODS

Micro-organisms

Three bacteria, *Escherichia coli* (NCTC 9001), *Pseudomonas aeruginosa* (NCTC 10332) and *Staphylococcus aureus* (NCTC 6571) and two fungi, *Candida albicans* (IMI 45348) and *Aspergillus niger* (IMI 17454) were used. Bacteria were incubated overnight at 31°C on nutrient Agar slopes (Mast Laboratories, Liverpool, England) *C. albicans* was incubated for 48 h and *A. niger* for one week, both at 25°C on malt Agar slopes (Mast Laboratories). Slopes were checked visually for purity and all were harvested with physiological saline, except those of *A. niger* which were harvested with 1% (v/v) polysorbate 80 in physiological saline. A suspension of each organism was prepared and standardized spectrophotometrically to a concentration of approximately 10^7 organisms/ml. Further dilutions were made as required with sterile saline.

Radiopharmaceutical agents

The non-radioactive components of radiopharmaceuticals used for this study are listed together with manufacturer in Table 1. They are amongst those most commonly used in nuclear medicine. For the purposes of the investigation, these were reconstituted by the aseptic addition of 5 ml of sterile physiological saline instead of the sodium (^{99m}Tc) pertechnetate solution normally added. Thus a 'cold' version of the radiopharmaceutical was produced. Aliquots of 1 ml from each vial were transferred by syringe to 5 10-ml sterile rubber-capped vials. These latter vials formed the incubation vessels for the following experiments.

(a) Five 1-ml aliquots of each preparation were inoculated with less than 100 viable organisms of the 5 species used. The resultant mixtures were shaken and then incubated at 25°C for 8 h. Samples were taken at 4 and 8 h to determine viable count.

(b) Four vials containing 1 ml nutrient broth (Mast Laboratories) were each inoculated with approximately 2000 viable organisms from the suspension of *P. aeruginosa.* Vials were then incubated for 6 h, two at 4°C and two at 25°C, and sampled hourly for the determination of the number of viable organisms.

(c) Ten vials of reconstituted MAA were each inoculated with approximately 10 viable

TABLE 1

RADIOPHARMACEUTICAL AGENTS

Radiopharmaceutical agents	Manufacturer	Purpose
Dimercaptosuccinic acid (DMSA)	1	Renal imaging
Tin colloid	2	Liver imaging
Diethylenetriamine pentaacetic acid (DTPA)	3	Brain or renal imaging
N-(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid (HIDA)	4	Liver function
Methylene diphosphonate (MDP)	3	Skeletal imaging
Gluceptate sodium	5	Brain or renal imaging
Human serum albumin (HSA)	4	Vascular marker
Sodium pyrophosphate	6	Skeletal imaging
Macroaggregated albumin (MAA)	2	Lung imaging

Notes: 1, I.R.E., 6220 Fleurs, Belgium; 2, The Radiochemical Centre, Amersham, England; 3, C.E. Frosst et cie, Division Pharmaco-Radioactive, Kirkland, Canada; 4, Sorin Biomedica-Gruppo Radiochimica, 13040 Saluggia, Italy; 5, New England Nuclear, Medical Diagnostics Division, North Billerica, Mass. 01862, U.S.A.; 6, C.E.A., Laboratoire des Produits Biomedicaux, Gif-sur-Yvette, France.

organisms from the suspension of *P. aeruginosa*. Vials were then incubated, 5 at 25°C and 5 at 4°C, for 8 h. Samples were taken at 4 and 8 h to determine the number of viable organisms present. The experiment was repeated with 10 vials of MAA each inoculated with approximately 10 viable organisms from the suspension of *E. coli* and 10 vials of HSA each inoculated with approximately 10 viable organisms from *P. aeruginosa* suspension.

Preparation of inocula

Slope cultures of the organisms were harvested and primary suspensions were standardized spectrophotometrically. These were stored at 4°C while viable counts, at 31°C for bacteria and 25°C for fungi, were made by a plate technique. Appropriate dilutions and volumes were calculated to deliver inocula of 100 and 10 organisms, respectively, in 0.1 ml and these were confirmed by plate count at the time of inoculation.

Sampling of test containers

Plate counts were carried out on duplicate 0.1-ml aliquots (hourly samples) or duplicate 0.2-ml aliquots (4-hourly samples) from the test containers. All vials contained 1.1 ml at zero time and the results were adjusted to give the total number of organisms which would have been present at the sampling times had the volume remained undisturbed.

RESULTS

Experiment a. The results are shown in Fig. 1. The response of each organism to the various radiopharmaceutical vehicles was variable. The preparation which was the least

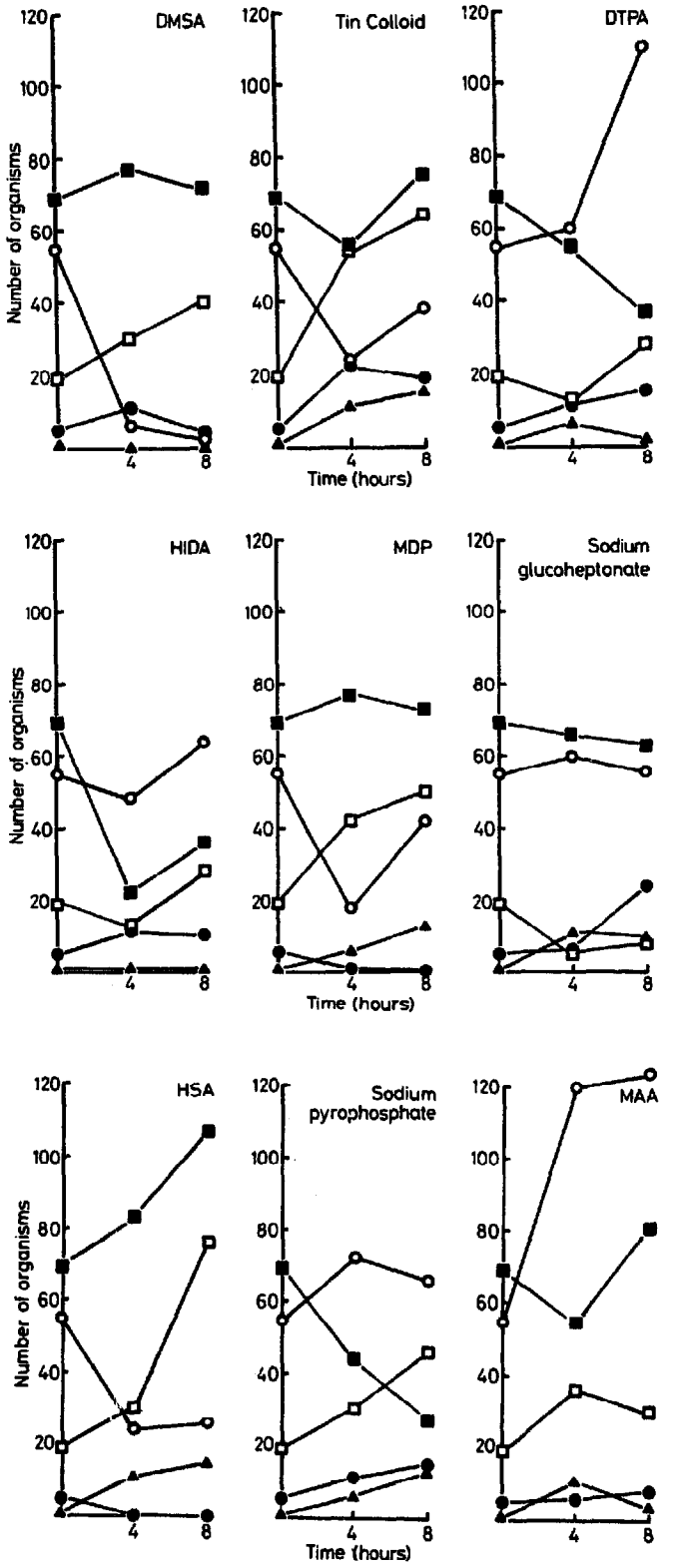


Fig. 1. The number of micro-organisms in 9 radiopharmaceutical preparations during incubation at 25°C after inoculation with *E. coli*, ○; *P. aeruginosa*, ●; *S. aureus*, ▲; *A. niger*, □; and *C. albicans*, ■. Each point is the mean of duplicate plate counts.

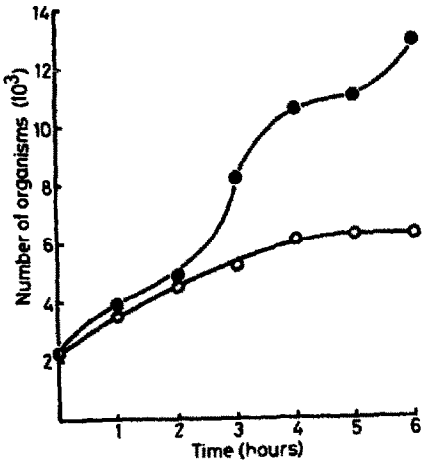
favourable for growth was DMSA, where *E. coli*, *P. aeruginosa* and *S. aureus* all decreased in numbers during 8-h incubations.

In contrast, MAA was the only preparation from which all 5 species were recovered after 8 h in larger numbers than were present at zero time.

A. niger increased in numbers in all the preparations except gluceptate sodium. *E. coli* failed to multiply in any of the preparations except DTPA and MAA. *C. albicans* failed to multiply in any preparation except HSA and MAA.

Experiment b. The results of the control incubation of about 2000 organisms of *P. aeruginosa* in 1 ml nutrient broth at 25 and 4°C for 6 h are given in Fig. 2. Incubation at 25°C produced, after 6 h, twice the number of organisms produced by incubation at 4°C.

Experiment c. Fig. 3 shows the results of incubating approximately 10 viable organisms of *P. aeruginosa* and *E. coli* in MAA and HSA suspensions at 25 and 4°C. *P. aeruginosa* showed a decrease in numbers when incubated in MAA at 4°C and in HSA at 25°C but in all other cases, an increase in numbers of organisms took place irrespective of whether incubation was at 25 or at 4°C.



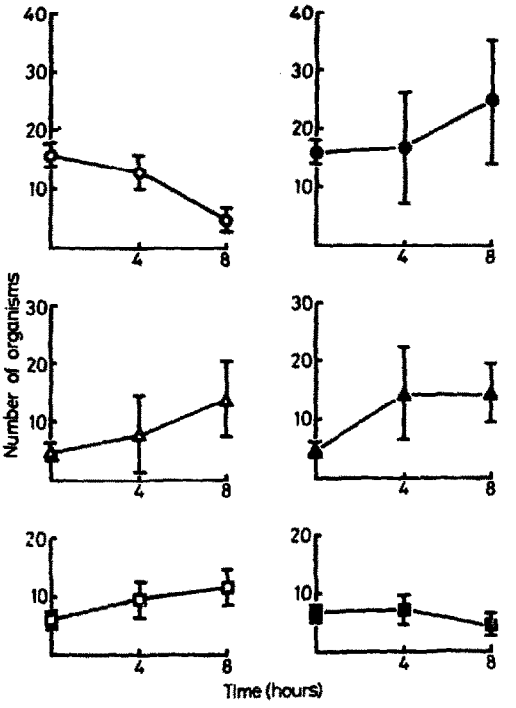


Fig. 2. Control data. The number of *P. aeruginosa* in nutrient broth during incubation at 4°C, ○; and 25°C, ●. Each point is the mean of duplicate plate counts.

Fig. 3. The number of *P. aeruginosa* in MAA during incubation at 4°C, ○; and 25°C, ●; of *E. coli* in MAA at 4°C, △; and 25°C; ▲; and of *P. aeruginosa* in HSA at 4°C, □; and 25°C, ■. Each point is the mean of 5 duplicate plate count determinations ±S.D.

DISCUSSION

Physiological saline was used in these experiments to reconstitute the radiopharmaceutical agents and therefore two factors must be taken into account when comparing the results with those to be expected from complete radiopharmaceuticals, namely the chemical and the radiation effects of sodium (^{99m}Tc) pertechnetate.

The chemical effect may be discounted in view of the fact that sodium pertechnetate in patient doses of radiopharmaceuticals occurs only in nanogram quantities (2×10^{-8} M).

The radiation effect within a vial containing 555 MBq will amount, in a period of 8 h, to 10 Gy. Depending on species and suspending medium, micro-organisms vary greatly in their susceptibility to ionizing radiations and decimal reduction doses from 40 Gy for *P. aeruginosa* and 240 Gy for *E. coli* to more than 15 kGy for *Sarcina rubens* have been quoted (Stapleton and Engel, 1960). Therefore the organism most susceptible to irradiation (*P. aeruginosa*) would suffer at most a 44% reduction in numbers from the present results.

The 5 organisms used were chosen to be representative of the main microbial groups and are those recommended as test organisms for the Antimicrobial Preservatives Effectiveness Test of the United States Pharmacopoeia (1975).

The results show that all the radiopharmaceuticals tested are capable of sustaining the numbers of at least one microbial species. It is noteworthy that an incubation temperature of 4°C failed to prevent an increase in numbers of *E. coli* in MAA and *P. aeruginosa* in HSA suspensions. Otherwise, the results are seen to be very variable. The control experiment (Fig. 2) demonstrates that aeration of the contents of a vial is not a limiting factor as *P. aeruginosa* was able to increase in numbers in a temperature-sensitive manner when incubated with nutrient broth.

Gram-negative organisms in general, and pseudomonas species in particular are known to grow in media containing a minimum of nutrients (Carson et al., 1973). *C. albicans* has also been reported as capable of growing in intravenous infusion fluids of simple chemical composition (Wilkinson et al., 1973). Gram-positive organisms, alternatively, are said to be more fastidious in their nutritional requirements. In as much as radiopharmaceuticals may be regarded as minimal growth media, the present experiments did not support this generalization.

It appears that the conditions governing multiplication of an organism in a given radiopharmaceutical are complex, depending upon organism type, inoculum size and chemical nature of the radiopharmaceutical. The fact that the inocula were small, not well defined in terms of physiological age, and were undergoing transfer to a different medium may have led to extended lag phases during the experimental incubations thereby contributing to the variability observed.

Many hospital radiopharmacies have no special aseptic facilities (Abra et al., 1980). In the context of microbial contamination, such environments might equate to the hospital ward conditions in which Maki et al. (1974) found that accidental contamination usually amounted to less than 100 organisms per container. This is the range of inoculum numbers employed in these experiments. It is clear from the present results that radiopharmaceuticals, despite the fact that they are administered in quantities too small to give

rise to a pharmacological response (Horton, 1975), contain sufficient nutrients to sustain at least some species of micro-organisms at this level of contamination.

Most radiopharmaceuticals are assembled without terminal sterilization due to time constraints. They should therefore be produced under conditions which reduce the chance of contamination to a minimum. Clearly the fact that ^{99m}Tc-labelled radiopharmaceuticals are used on the day of assembly reduces the possibility of gross multiplication of micro-organisms. In the light of the results of this paper, storage at 4°C between the times of assembly and administration (British Institute of Radiology, 1975) may do little to modify the growth of contaminants which are unacceptable in any numbers, however small.

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REFERENCES

Abra, R.M., Bell, N.D.S. Horton, P.W. and McCarthy, T.M., A survey of radiopharmaceutical manufacture in United Kingdom hospitals. J. Clin. Hosp. Pharm., 5 (1980) in press.

Bell, N.D.S. and Horton, P.W., Radiopharmaceuticals – present and past. J. Clin. Pharm., 2 (1978) 137–154.

British Institute of Radiology, Guidelines for the Preparation of Radiopharmaceuticals in Hospitals. Special Report No. 11, London, 1975, Section 4.5.5.

Carson, L.H., Favero, M.S., Band, W.W. and Peterson, N.J., Morphological, biochemical and growth characteristics of *Pseudomonas apacia* from distilled water. Appl. Microbiol., 25 (1973) 476–483.

European Pharmacopoeia, Vol. III, Maisonneuve, France, 1975, pp. 371–387.

Holmes, C.J. and Allwood, M.C. The microbial contamination of intravenous infusions during clinical use. J. Appl. Bact., 46 (1979) 247–267.

Horton, P.W., The dispensing of radiopharmaceuticals. In Greig, W.R. and Gillespie, F.C. (Eds.) Advances in Clinical Nuclear Medicine, Churchill Livingstone, Edinburgh, 1975, pp. 1–43.

Hospital Physicists' Association, Hospital Preparation of Radiopharmaceuticals. Scientific Report Series No. 16, London, 1977.

Hupf, H.B., Production and purification of radionuclides. In Tubis, M. and Wolf, W. (Eds.) Radiopharmacy, Wiley, New York, 1976, pp. 225–253.

Maki, D.G., Anderson, R.L. and Shulmann, J.A., In-use contamination of intravenous fluids. Appl. Microbiol., 28 (1974) 778–784.

Radiochemical Centre, The, Instruction leaflet for Technetium (MAA) Agent for lung scintigraphy. Product Licence No. PL 0221/0070, Amersham, England, 1978.

Rhodes, B.A. and Croft, B.Y., Basics of Radiopharmacy, C.V. Mosby, St. Louis, 1978.

Stapleton, G.E. and Engel, M.S., Cultural conditions as determinants of sensitivity of *Escherichia coli* to damaging agents. J. Bacteriol., 80 (1960) 544–551.

United States Pharmacopoeia XIX, U.S.P. Convention Inc., U.S.A., 1975, p. 587.

Williamson, W.R., Flores, L.L. and Pagones, J.N., Growth of micro-organisms in parenteral nutritional fluids. Drug Intell. Clin. Pharm., 7 (1973) 226–231.

Wolf, W., Radiochemistryy, In Tubis, M. and Wolf, W. (Eds.) Radiopharmacy, Wiley, New York, 1976, pp. 203–223.