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MICROBIAL AND PYROGENIC CONTAMINATION OF MOLYBDENUM 99/TECHNETIUM 99m RADIONUCLIDE GENERATORS

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SUMMARY

Radionuclide generators employed for the production of short-lived radionuclides may be contaminated with microorganisms during use, although this may not result in significant microbial contamination of the eluate. The mechanism by which this reduction was achieved was examined, using technetium 99m generators deliberately contaminated with known numbers of *Escherichia coli*. Results indicated that a small proportion of the inoculum was eluted from loaded ('active') or unloaded columns suggesting that organisms were entrapped in the alumina bed. Cell death occurred and could be caused by significant exposure to radiation, and the toxicity of aluminium and molybdate ions. Loss of viability led to an increased release of pyrogenic material into the eluate.

INTRODUCTION

Radionuclide generators are employed for the production of short-lived radionuclides such as technetium 99m. The eluate, which contains the radionuclide in the form of a simple salt, may be used directly or be employed in the preparation of other labelled pharmaceuticals. Where such materials are intended for injection, the eluate must be both sterile and free from pyrogens.

Molybdenum 99/technetium 99m generators can be eluted up to twice daily and have a maximum working life of 17 days. The generator consists of a double-ended sealed sterile (autoclaved) column of alumina onto which is adsorbed radioactive ammonium molybdate (^{99}Mo). Following decay of the molybdenum, the technetium 99m (^{99m}Tc) can be separated from the parent by elution with sodium chloride injection BP. The column is shielded in lead and access to the inlet and outlet ports achieved via a system of needles and narrow bore tubing. Following initial elution, the column and associated tubing remain moist during the working life of the generator. Although the generator is supplied

sterile and is maintained as a closed system, it may not be housed in an aseptic environment and contamination during use is always possible.

A previous study (Sorensen et al., 1977) demonstrated that gross contamination of generators does not result in significant contamination of the eluate, the number of organisms in the eluate being reduced from those applied to the generator by a factor of 10^4 to 10^6. However, no indication was given of the mechanism by which this reduction was achieved nor of the viability of organisms retained on the column. These points were further investigated. Regardless of whether the column is detrimental to cell survival, pyrogenic material could be released into the eluate following bacterial contamination. This possibility was also investigated.

MATERIALS AND METHODS

Radionuclide generators

In an attempt to separate the chemical and radiological effects of ammonium molybdate from the effects of the column-packing material on cell viability, 3 different types of column were used.

Unloaded columns. These were prepared by the Radiochemical Centre, (Amersham, Bucks, England), in exactly the same way as normal generator columns but without the addition of ammonium molybdate.

Loaded columns. Molybdenum–technetium generators (Radiochemical Centre, Code MCC4) in various stages of decay following routine use in the Department of Nuclear Medicine, Addenbrooke's Hospital, Cambridge. The residual molybdenum 99 activity in all cases was less than 1×10^{-6} mCi and the final elution of the generators shown to yield a sterile eluate.

Active columns. Molybdenum–technetium generators containing at least 100 mCi of residual molybdenum 99 activity on the day of use but in all other respects similar to those described as 'Loaded'.

A supply of alumina, prepared and treated ready for packing into generator columns was also obtained from The Radiochemical Centre.

Elution was accomplished using pressurized vials of 0.9% w/v Sodium Chloride Injection B.P. (without bactericide) using the method recommended by the manufacturer. All vial tops were swabbed with chlorhexidine acetate and empty elution and collection vials left in position between elutions. Generators were stored at room temperature in a laboratory environment for the duration of the study.

Organisms and contamination of generator columns

Escherichia coli. Manchester University Collection Type 35 was grown in Tryptone soya broth (Oxoid) for 18 h at 37°C in Erlenmeyer flasks. The suspension was centrifuged, the pellet washed 3 times with water and finally resuspended in sterile distilled water to give about 1×10^8 viable cells/ml. Generator columns were contaminated by elution with a pressurized eluent vial containing 15 ml saline previously inoculated with 0.1 ml cell suspension (about 10^7 viable cells).

Assessment of viable counts

Viable bacterial numbers were assessed by the surface-plate method. Quadruplicate

0.25 ml or 0.5 ml aliquots were spread on overdried Tryptone soya Agar (Oxoid) plates and incubated for 48 h at 37°C. Representative colonies were subcultured onto Macconkeys Agar (Oxoid) plates to confirm that the organism was a coliform, and not a contaminant from the environment. No such contaminants were recovered throughout the experiments.

Recovery of viable Escherichia coli from generator columns

To determine if viable cells were present in generator columns after the final elution, columns were removed from the generator housing, carefully opened and samples of alumina scraped off with a sterile spatula into 10 ml 0.9% sodium chloride. Each suspension was shaken thoroughly, allowed to settle for 20 min and viable counts determined as previously described.

Pyrogen content of eluates

The pyrogen content of the eluates from generators contaminated with *E. coli* and from two decayed generators which had been intentionally contaminated with 20 ng and 2 μg of *E. coli* endotoxin (Pyrogent Test Kit), respectively, was assessed by the Limulus Amoebocyte Lysate (LAL) test. Qualitative results were obtained using Pyrogent Test Kits (B.Y.K. Mallinkrodt, Heathrow Airport, Slough, Bucks, England). Quantitative determinations were performed by Travenol Laboratories, Thetford, using their own 'in-house' method. After collection and sampling for microbial contamination, the vial contents were heated to 98–100°C for 10 min to kill viable microorganisms. Vials were then stored at 3°C until tested for the presence of pyrogens.

RESULTS

Repeated daily elution of loaded and unloaded generator columns after initial contamination indicated that a very small proportion of the inoculum was eluted from any of the columns tested (Table 1). The greatest numbers of viable organisms were eluted during the first and second elutions. Subsequent elutions removed decreasing numbers.

These results also suggest that fewer viable bacteria were eluted from loaded compared with unloaded columns. However, since in both cases the numbers eluted represented so small a proportion of the inoculum the significance of such differences is difficult to substantiate.

Four columns were examined for the presence of viable bacteria after the final elution. Results are presented in Table 2 and indicate that a greater loss of viability occurred on the loaded columns. Highest counts were obtained from alumina samples taken from the upper section of the alumina bed, viable counts of samples from the grid and filter pad or bottom of the column being much lower. The processes whereby organisms may be prevented from being eluted from columns are by entrapment and filtration or by adsorption onto the alumina bed. However, when a suspension containing 10^7 viable *E. coli* was added to a slurry of alumina (10 g in 20 ml 0.9% w/v sodium chloride), less than 10% of the initial inoculum were adsorbed onto the alumina. Therefore, entrapment and filtration are probably the most important processes involved.

The ability of viable bacteria to survive on generator columns is of great importance.

TABLE I

THE NUMBER OF VIABLE *ESCHERICHIA COLI* RECOVERED FROM CONTAMINATED RADIONUCLIDE GENERATORS BY CONSECUTIVE DAILY ELUTIONS

ND, none detected; NE, not eluted.

	Total viable count/eluate vial			
	Experiment 1		Experiment 2	
Column	Loaded	Unloaded	Loaded	Unloaded
Column age relative to reference data (days)	471		114	
Residual molybdenum 99 activity	3×10^{-49} mCi		1×10^{10} mCi	
Days after inoculation				
0	15	4680	15	90
1	210	1320	ND	375
2	150	960	ND	105
3	45	360	ND	15
4	NE	NE	NE	NE
5	NE	NE	NE	NE
6	ND	30	ND	ND
7	ND	30	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	ND
10	ND	ND	ND	90
11			NE	ND
12			NE	NE
13			ND	30
14			ND	ND
15			ND	15

TABLE 2

THE NUMBERS OF VIABLE *ESCHERICHIA COLI* REMAINING ON RADIONUCLIDE GENERATORS AFTER REPEATED DAILY ELUTIONS

ND, none detected.

Sample origin	Viable count/ml supernatant after shaking with sample			
	Experiment 1		Experiment 2	
	Loaded column	Unloaded column	Loaded column	Unloaded column
Plastic grid on top of column	ND	2	44	18
Filter pad	84	490	2	>1000
First 10 mm of alumina	42	>1000	ND	>1000
Middle 10 mm of alumina	6	104	ND	830
Last 20 mm of alumina	10	4	ND	34

TABLE 3

THE NUMBER OF VIABLE *ESCHERICHIA COLI* ELUTED FROM CONTAMINATED RADIONUCLIDE GENERATORS BY CONSECUTIVE HOURLY ELUTIONS

	Total viable count/eluate vial		
Column	Loaded	Loaded	Unloaded
Column age relative to reference date (days)	4	77	
Residual molybdenum 99 activity	100 mCi	1.5×10^{-6} mCi	
Hours after inoculation			
0	2130	2970	960
1	240	1260	1860
2	180	900	2640
3	90	390	2120
4	30	750	1920
5	60	420	1260
6	60	810	780

The finding that very low and decreasing counts were obtained in daily eluates suggests that some loss of viability may occur during the 12–16 day period. This is further supported by results in Table 3. Eluates obtained by repeated elution at hourly intervals contain greater numbers of viable bacteria compared to successive daily elutions (Table 1). Therefore, the time period between each elution, rather than the number of elutions, leads to the greatest reduction in numbers of viable organisms eluted. This is true for loaded and unloaded columns. Eluates from the only 'active' (100 mCi) column studied contain fewer organisms than from the decayed column. This could be because of the exposure of organisms to radiation on the active column (see Discussion).

Moist alumina columns are not an ideal growth environment for Gram-negative bacteria. However, such organisms can survive in simple aqueous environments (Holmes and Allwood, 1979). The difference in numbers of viable organisms eluted from decayed, loaded and unloaded columns could be the result of chemical toxicity due to molybdate absorption or the presence of aluminium ions released from the alumina due to radiation effects. It is not possible to reproduce the exact chemical environment existing in a loaded column, since the concentrations of molybdate or aluminium in solutions are difficult to estimate. The maximum permissible concentrations of aluminium ions and molybdenum are 2 μg/ml and 0.1% respectively. It was found that the addition of aluminium chloride, equivalent to 2 μg/ml, or ammonium molybdate, equivalent to 0.1% w/v molybdenum, to a cell suspension in 0.9% saline, caused increased loss of viability. For instance, after 24 h storage at room temperature, cell viability in aluminium- and molybdate-containing solutions had fallen to 9% and 0.6% respectively, compared with 80% survival in saline alone. Although the conditions used do not exactly represent those pertaining on a column, results suggest that any differences in survival on loaded or unloaded columns could be influenced by inorganic ions. It is not possible to quantify this effect.

The significance of cell survival derives from the fact that cell death leads to increased

TABLE 4

THE APPEARANCE OF PYROGENIC MATERIAL IN ELUATES FROM RADIONUCLIDE GENERATORS CONTAMINATED WITH *ESCHERICHIA COLI* AFTER CONSECUTIVE DAILY ELUTION

Pyrostat test result: + = stable gel; – = no gel; ± = unstable gel. NE = generator not eluted on this day. Quantitative levels of *E. coli* endotoxin equivalent per ml as determined by the Travenol Limulus Lysate method are given in brackets.

Column	Pyrogen test result				
	Experiment 1 Loaded	Experiment 2		Experiment 3	
		Loaded	Unloaded	Loaded	Unloaded
Days after inoculation					
0	–	–	–	–	–
1	–	–	–	–	–
2	–	–	–	NE	NE
3	–	–	–	NE	NE
4	NE	NE	NE	–	–
5	NE	NE	NE	–	–
6	–	±(5 pg)	–	±	±
7	–	±(0 pg)	–	±	±
8	–	+(80 pg)	–	±	+
9	+	+(200 pg)	±(5 pg)	NE	NE
10	+	+(130 pg)	+(12 pg)	NE	NE
11		NE	NE	±	–
12		NE	NE	±	+
13		+(25 pg)	+(12 pg)	±	+
14		–	–	±	+
15		–	–		

TABLE 5

ESTIMATED RADIATION DOSES TO MICROORGANISMS TRAPPED IN THE UPPER SECTIONS OF GENERATOR COLUMNS CONTAMINATED AT VARIOUS TIMES AFTER THE REFERENCE DATE AND REMAINING ON THE COLUMN THROUGHOUT THE SHELF LIFE [a]

Time of contamination after reference date (h)	Radiation dose (Mrad) (200 mCi column)	
	Maximum 0–10 mm	Minimum 0–15 mm
0 = ref. date	3.9	2.9
33	2.5	1.8
67	1.7	1.3
134	0.7	0.5

[a] Shelf life column = 11 days from reference date. For convenience this is taken as 268 h or 4 × half-life of Molybdenum.

pyrogen release. Since most of the inoculum is held in the column, release of pyrogens is possible. Therefore, eluates were examined for the presence of pyrogens. Results are shown in Table 4. The first few eluates from loaded and unloaded columns produced negative results. Later eluates, commencing between 6 and 9 days after inoculation, contained pyrogenic material. There was no consistent difference between the first appearance of pyrogenic material for loaded and unloaded columns although for one experiment greater quantities of pyrogenic material were released over a longer period from the loaded column.

Tests on eluates obtained by repeated elution of both loaded and unloaded columns at 30-min intervals for up to 6 h indicated that no samples contained pyrogens. Similarly, repeated elutions (15) at 5-min intervals of loaded generators inoculated with 20 ng and 2 μg of *E. coli* endotoxin, respectively, also yield eluates containing less than 5 pg/ml of endotoxin, this being the limit of detection of the method. It may, therefore, be that not only do the generator columns retain microorganisms but also endotoxins such as that derived from *E. coli.*

DISCUSSION

Gross contamination of radionuclide generators does not lead to comparable contamination of eluates (Sorensen et al., 1977). Generators could possibly be contaminated during use by airborne microorganisms as a result of poor handling or from contaminated vial closures. Results of sterility tests in the Department of Nuclear Medicine have, however, never shown contamination of final generator eluates throughout a 4-year period (400 samples) despite the fact that the generators are housed in a laboratory environment. Nevertheless, the possibility that columns could become colonized with microorganisms must be considered. Viable microorganisms on the column could then contaminate the eluate. Contamination with pyrogens would also be possible, particularly if cell death occurs.

This study confirms the work of Sorensen et al. (1977) that few microorganisms from a column are removed into eluates. Examination of columns indicated that most of the cells were to be found in the upper sections of the column. Since few cells are adsorbed by alumina, it is suggested that cells are retained by entrapment and filtration. Repeated elution does not dislodge cells to a significant extent so that they move very slowly further down the column. This conclusion was supported by the observation that repeated hourly elution on the same day did not lead to increased contamination of eluates. The observation that lower numbers of viable organisms were recovered from eluates after repeated daily elution than from repeated elution on the same day also suggests that loss of viability of the inoculum occurs during the working life of the column. Further, viable counts of eluates from loaded columns were in every case lower than from unloaded columns. One cause of cell inactivation could be the release of toxic compounds from loaded columns. The most likely ion released is aluminium. Loss of viability of cell suspensions in contact with aqueous solutions of aluminium chloride suggests that this ion is detrimental to cell survival. Molybdenum is also toxic to cells. Further evidence for the toxicity of these column components is provided by eluate contamination rates from columns decayed below 1×10^{-6} mCi molybdenum as opposed to unloaded columns (Table 1).

Organisms trapped in the alumina beds of such decayed columns would be exposed to negligible radiation doses which would not be expected to have any influence on cell viability. Therefore, the difference in viability on loaded and unloaded columns suggests that chemical inactivation plays a significant role in reducing the viability of contaminants.

Results in Table 3 indicate that, during the normal working life of a generator, microbial contaminants may be further inactivated by the significant doses of radiation to which they are exposed due to their proximity to ^{99}Mo on the column. Although it is impossible to calculate the exact radiation dose to the microorganisms without knowing the distribution of both organism and radioactive molybdenum along a column, an estimate of the order of magnitude of this dose can be obtained. The largest component of the radiation dose to the microorganisms will come from non-penetrating radiations (particulate radiation and low energy (<10 keV) X- or γ-rays emitted as a result of decay). The path length of these radiations will only be of the order of 1 mm in alumina and therefore, only microorganisms in close proximity to the alumina particles on to which the radioactive species are adsorbed will be subject to significant radiation dose. In the case of a ^{99}Mo/^{99m}Tc generator, the major contribution to the radiation dose will result from decay of molybdenum 99. In order to calculate the actual radiation dose to which organisms are subjected it is necessary to know the activity of molybdenum present at the time of contamination, the period of exposure, the equilibrium dose rate constraints and the mass being irradiated. The most difficult of these terms to define is the mass being irradiated since neither the distribution of the molybdenum nor the organisms throughout the alumina bed is known accurately. It has, however, been shown that, in the case of 200 mCi generator, all of the molybdate is adsorbed onto alumina contained within the top 10–15 mm of the alumina bed (distances being measured from the disc placed on the top of column of the alumina) (personal communication, The Radiochemical Centre). Assuming, therefore, an even distribution of the molybdate throughout the alumina contained in this section of the column, and knowing the volume and density, the minimum and maximum radiation dose to organisms trapped in this section of the column has been calculated and is shown in Table 5. The figures show the radiation dose to which the organisms are exposed during the working life of the generator (11 days from reference date) when contamination occurs at various intervals after the reference date. (For convenience, times have been chosen which are multiples of the half-life of molybdenum 99). The maximum doses have been calculated assuming that the molybdate is evenly distributed throughout alumina in the first 10 mm of the column, and the minimum doses, assuming distribution throughout the first 15 mm of the column. It is emphasized that only those microorganisms entrapped in the section of the alumina in which the molybdenum is adsorbed will be subject to significant radiation dose.

However, in those experiments in which sections of unloaded columns were removed for assessment of viable bacteria, most organisms were found in the upper sections with decreasing numbers along the length of columns. Therefore, a high proportion of contaminants are entrapped in the upper areas of columns which is where they will be exposed to the highest radiation dose. This suggests that contaminants of columns may well be exposed to lethal doses of radiation, depending on the activity of the generator and the time of exposure. However, any bacteria gaining access through the upper sections to lower parts of the alumina bed will not be exposed to lethal doses of radiation regardless

of the activity of the column or the duration of exposure. Generators can, therefore, be described as extremely hostile environments for microorganisms but not self-sterilizing.

Pyrogens are bacterial endotoxins which may be released during growth or death of microorganisms. Since bacteria are retained on generator columns, which are themselves hostile to the survival of microorganisms, the possibility of pyrogen release into eluates was investigated. The eluates from columns loaded with *E. coli* endotoxin contained less than 5 pg/ml endotoxin in the eluate after repeated elutions. However, when columns were inoculated with microorganisms, positive limulus lysate responses were occasionally elicited in eluates collected 5 or more days after inoculation. The maximum level of *E. coli* endotoxin found was 200 pg/ml. It is probable that pyrogen release in this case is a consequence of loss of viability and subsequent cell lysis leading to release of cell wall components including lipopolysaccharide. Only in one case, however, was the level of pyrogen release equated with loss of viability. It is, therefore, difficult to draw definite conclusions concerning the mechanisms involved in pyrogen release. Considering, however, the inoculum size, the low levels of pyrogens detected and the small injection volume of all radiopharmaceuticals (<10 ml), it can be assumed that pyrogen release from generator columns should not present a clinical problem.

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