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# Evaluation of a novel decorporation approach to prevent radioactivity uptake by using acidosis in experimental animals

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With an aim to devise a prophylactic and/or therapeutic approach for preventing internalization of radiothallium ($^{201}$Tl), and more importantly by implication, its chemical analogue radiocesium ($^{137}$Cs) during any nuclear emergency, different *ex vivo* and *in vivo* animal models were created to determine the role of *p*H in absorption of $^{201}$Tl across jejunum/muscle tissue and whole body retention of $^{201}$Tl respectively. Movement of $Tl^+$ under simulated *p*H conditions proved that *p*H had direct influence on its absorption. Oral intake of acidified water or parenteral administration of lactic acid was able to reduce the body burden of $^{201}$Tl by up to 12 and 50% respectively. The results indicate that acidification of gut, within physiological range may be used as an option for decorporation/inhibition of incorporation of radiothallium and radiocesium, particularly in cases of mass casualty.

**Keywords:** Acidosis, Cesium-137, Decorporation, Radioactivity, Radiometry, Thallium-201

There has been a phenomenal increase in the use of radionuclides in areas like healthcare and energy production, thereby increasing vulnerability to potential nuclear accidents such as that seen in Fukushima, Japan. In addition, an ever-increasing threat of the use of a dirty bomb or nuclear explosion by rogue states or terrorist organisations can not be ruled out, which if happens shall affect a large number of people who are exposed to radioactive fallout.

Besides inhalation, gastrointestinal tract (GIT) is the other major portal of entry of radioactivity into the body[1]. Any effort to reduce absorption of ingested radionuclides from GIT can therefore be a major preventive and/ or therapeutic approach for managing radioactivity toxicity. Unfortunately, the focus of research till now has primarily been on decorporation of internalized radioactivity after it has entered the system, and not as much on preventive aspects.

Cesium-137($^{137}$Cs; $t_{1/2}$ =30 years) is one of the major radionuclide that would be present in a radioactive fallout scenario and is amongst the most difficult ones to remove from the body once internalized posing significant health hazards, including cancers and genetic mutations[2,3]. The main treatment for $^{137}$Cs decorporation is oral administration of Prussian blue (PB)[4,5]. However, if internalized, $^{137}$Cs gets preferentially incorporated into muscles in 5-7 days. Presently there is no standard protocol to remove $^{137}$Cs from muscles. Another glaring problem with cesium decorporation is the availability of PB. It is manufactured by a single source in Germany, resulting in very high cost and lack of easy availability. Advantages of other treatments like activated charcoal and diuresis have not been conclusively proved in humans. Therefore there is need to devise a strategy to reduce body burden of $^{137}$Cs during a radiation induced emergency. Since these metal ions are expected to be absorbed through the intestine, intervention at GIT level will have obvious advantage. Such a treatment shall also be useful in non-radioactive cesium/thallium metal toxicity, which may be more common but less dramatic.

Thallium (Tl), a chemical analogue of cesium and potassium (K) shares the same biodistribution and medical management as cesium[6-8]. Radioactive thallium ($^{201}$Tl; $t_{1/2}$=3 days) is used as a routine radiopharmaceutical in nuclear medicine for

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myocardial scintigraphy in humans. Thallium-201 therefore offers an opportunity to plan and set up *in vitro* and *in vivo* experiments by acting as a model for radioactive cesium.

In the present investigation different *ex vivo* and *in vivo* models were selected to analyse whether *p*H plays a crucial role in passive and energy dependent absorption of $^{201}$Tl. The objective is to ascertain effect of acidification on uptake of radioactivity across GIT and muscle by using $^{201}$Tl as the source of radioactivity. The radiometric findings may help in providing leads for devising a preventive/ therapeutic approach so as to circumvent any undesired internalization of $^{201}$Tl, and more importantly by implication, $^{137}$Cs into the human system**.**

## Materials and Methods

The experiments were conducted on male strain 'A' mice (weighing 26±2 g) and Sprague Dawley rats (weighing 200±20 g), obtained from experimental animal facility of the Institute. All animal experiments were approved by the Institutional Animal Ethical Committee and confirmed to general national guidelines on the care and use of laboratory animals. The animals were kept for one week acclimatization and maintained at controlled temperature and hygiene conditions.

*Chemicals and reagents*: Thallous chloride ($^{201}$TlCl) was obtained from Board of Radiation & Isotope Technology (BRIT), Bhabha Atomic Research Center (BARC), India. All other chemicals were of AR grade and were purchased from Sigma Chemical Company, St. Louis, MO, USA. Ringer and Tyrode solutions were prepared as per Bressler and Matsuba[9] and Henning *et al.*[10] respectively.

*Ex vivo models to study the effect of acidosis on decorporation/ uptake of $^{201}$Tl*

*(a) Absorption of $^{201}$Tl from jejunum at different pH:* Sprague Dawley rats (200±20 g; n=5) were euthanized with chloroform and sacrificed. Their jejunum (6-7 cm long) were removed and washed immediately in cold isotonic solution. Jejunum was fixed on infusion assembly and incubated in serosal solution (Tyrode solution *p*H 7.4). Mucosal solutions [consisting of Tyrode solution + tracer quantity (0.37 MBq) of $^{201}$Tl] of varying *p*H (4.5, 5.5, 6.5, 7.4 and 8.5) were run through jejunum while *p*H of serosal solution was kept constant. Flow rate was kept constant at 1mL/min and the mucosal solutions were gassed with a mixture of 95% $O_2$ and 5% $CO_2$. Outflow of $^{201}$Tl ion from mucosal fluid to the serosal fluid was measured by measuring the radioactive counts in serosal solution using a gamma counter (Capintec, USA). Counts of 100 µL serosal solution ($Cp_f$) were taken at different time intervals, viz. 5, 10, 15, 30, 45, 60, 75, 90 and 120 min. Initial counts of 100 µL of mucosal solution ($Cp_i$) were also taken to calculate the percentage release of $^{201}$Tl from intestine, as defined by $Cp_i / Cp_f * 100$.

*(b) Uptake of thallium in hind limb muscle at different pH:* Hind limb muscle of the same animals mentioned above was removed carefully and washed with cold ringer solution before incubating in radioactive ringer solution [containing tracer quantity (0.37 MBq) of $^{201}$Tl] for different time periods (5, 10, 20, 30, 45 and 60 min respectively), and continuously gassed with a mixture of 95% $O_2$ and 5% $CO_2$. Three different *p*H of ringer solution (6.8, 7.4 and 7.8) were used for the study. After incubation, samples were removed and washed with non-radioactive chilled ringer solution. The percentage uptake of $^{201}$Tl per mg of muscle tissue was defined by measuring the radioactive counts with the help of a gamma counter (Capintec, USA).

*In vivo models to study the effect of acidosis on decorporation/ uptake of $^{201}$Tl*

*In vivo* radioactive decorporation studies were carried out by using two approaches. In the first case acidosis was induced by oral administration of ammonium chloride, while in second case physiological acidosis was induced by administration of lactic acid by parentral route.

*(a) Oral acidosis:* Based on the results of *ex vivo* experiments described above as well as evidence from literature, wherein oral acidosis has been induced to lower the physiological *p*H so as to enhance the elimination of strontium from bones[11], experiments were set up to study the effect of oral intake of acidic water (*p*H 2.0 and 5.0) on elimination of $^{201}$Tl.

Eighteen mice (30±2 g) were administered 1.11 MBq of $^{201}$TlCl. The mice were then randomly divided into 3 groups, namely I, II and III. Group I and II mice were given water having a *p*H of 2.0 and 5.0 respectively, while group III mice drank water of *p*H 7.4 and served as control. Urine was collected at 3, 5 and 24 h post exposure and whole body counts of each group were taken using gamma counter (Capintec, USA).

*(b) Parenteral acidosis:*

*(i) Through intramuscular dose of lactic acid at different pH*: Mice (n=54) were equally divided into

3 groups of 18 each, namely Group I, II and III. Each mouse received an im injection of 1.11 MBq $^{201}$TlCl. Subsequently, 6 mice each in Group I were given an im injection of 100 µL lactic acid (24 µg/mL; *p*H 5.0) at 24, 30 and 48 h post $^{201}$Tl exposure respectively. Similarly, 6 mice each in Group II were given an im injection of 100 µL lactic acid (24 µg/mL; *p*H 3.0) at the same time intervals as mentioned above. Group III served as control and 6 mice each from the group were given an im injection of 100 µL normal saline at 24, 30 and 48 h post $^{201}$Tl exposure. During the experiment food and water was given *ad libitum*. Whole body counts were taken at 0, 3, 5, 24, 30 and 48 h after administration of lactic acid/ normal saline to assess the effect of lactic acid at *p*H 3.0 and 5.0 on whole body elimination of $^{201}$Tl using gamma counter (Capintec, USA).

*(ii) Through intramuscular dose of different concentration of lactic acid at pH 3:* Based on the results of previous experiment mentioned above, 54 mice were again divided into 3 groups of 18 mice each, namely Group I, II and III. Each mouse was given an im injection of 1.11 MBq $^{201}$TlCl. Subsequently, 6 mice each in Group I was given an im injection of 100 µL lactic acid (24 µg/mL; *p*H 3.0) at 24, 30 and 48 h post $^{201}$Tl exposure respectively. Similarly, 6 mice each in Group II were given an im injection of 100 µL lactic acid (48 µg/mL; *p*H 3.0) at the same time intervals as mentioned above. Group III served as control and 6 mice each from the group were given an im injection of 100 µL normal saline at 24, 30 and 48 h post $^{201}$Tl exposure. During the experiment food and water was given *ad libitum*. Whole body counts were taken at 0, 3, 5, 24, 30 and 48 h after administration of lactic acid/ normal saline to assess the effect of varying conc. of lactic acid (*p*H 3.0) on whole body elimination of $^{201}$Tl using gamma counter (Capintec, USA).

*Statistical analysis*—The data from individual experiments were presented as mean ± SD and analyzed using analysis of variance (ANOVA). A minimum criterion for statistical significance was set at $P < 0.01$ for all comparisons.

## Results

*Ex vivo models to study the effect of acidosis on decorporation/ uptake of $^{201}$Tl*

*(a) Absorption of $^{201}$Tl from jejunum at different pH:*Movement of $^{201}$Tl from mucosal (*p*H 4.5, 5.5, 6.5, 7.4 and 8.5) to serosal (*p*H 7.4) solution was investigated as a function of time. Release of $^{201}$Tl from jejunum had a linear relationship to *p*H (Fig. 1). Total outflow of $^{201}$Tl during the first 2 hours at physiological *p*H from jejunum was 54%. It decreased significantly at *p*H 6.5 (37%), *p*H 5.5 (31%) and *p*H 4.5 (13%) as compared to physiological *p*H of 7.4. Unidirectional flow of $^{201}$Tl across jejunum from mucosal to serosal solution at *p*H 8.5 was 90%, indicating that increase in *p*H leads to increase in absorption of $^{201}$Tl ion from the intestine, or conversely, reducing the *p*H of intestinal content results in decreasing $^{201}$Tl absorption (a factor of up to 4 times).

*(b) Uptake of thallium in hind limb muscle at different pH:* The effect of varying *p*H (6.8, 7.4 and 7.8) on uptake of $^{201}$Tl was studied in hind limb muscle at different time periods (Fig. 2). Initially at

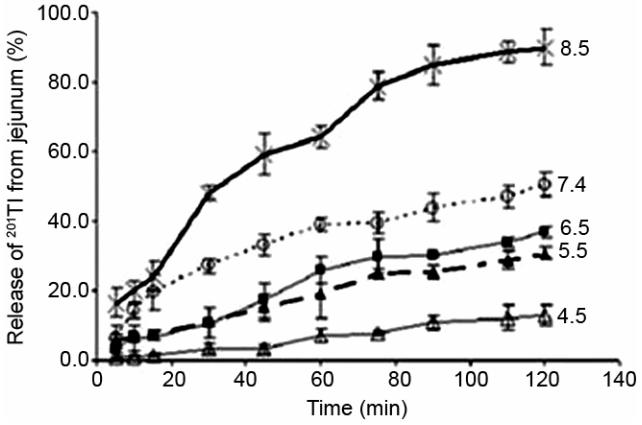


Fig. 1—Unidirectional outflow of $^{201}$Tl (0.37 MBq) across rat jejunum from mucosal solution (*p*H=8.5, 7.4, 6.5, 5.5 and 4.5) to serosal solution (*p*H=7.4) measured at different *p*H (n=5, $P<0.01$) over a period of 120 min.

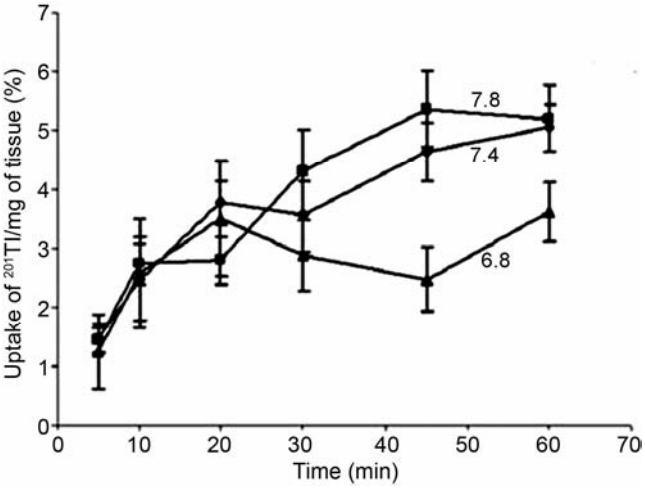


Fig. 2—Uptake of $^{201}$Tl (0.37 MBq) as a function of time at different *p*H (6.8, 7.4 and 7.8) in rat hind limb muscle (n=6, $P<0.01$) over a period of 60 min.

5 and 10 min intervals, uptake of $^{201}$Tl in muscle tissues was similar at all *p*H studied. However, with time it was observed that at physiological *p*H of 7.4 there was an increase in $^{201}$Tl uptake whereas at pH 6.8 a continuous efflux of internalized $^{201}$Tl could be seen. At 60 min, the muscle tissue incubated in ringer solution of *p*H 7 .8 had significant uptake of $^{201}$Tl compared to the samples at *p*H 6.8. These results confirmed that *p*H has direct correlation with $^{201}$Tl uptake in muscle tissue.

*In vivo models to study the effect of acidosis on decorporation/ uptake of $^{201}$Tl*

*(a) Oral acidosis:* Per cent retention of $^{201}$Tl was similar in all the groups at 3 h (Fig. 3). However, at 6 and 24 h, elimination of $^{201}$Tl increased by 5 and 12% respectively in Group I mice, which were fed water of *p*H 2.0 as compared to control animals. There was a decrease in food and water consumption among Group I animals by 12 and 25% respectively in comparison to control, whereas such an effect on Group II animals was much less. This may explain the decrease in per cent decorporation observed with *in vivo* studies in comparison to *ex vivo* models. Animal survival was 100% with no residual effect after one day.

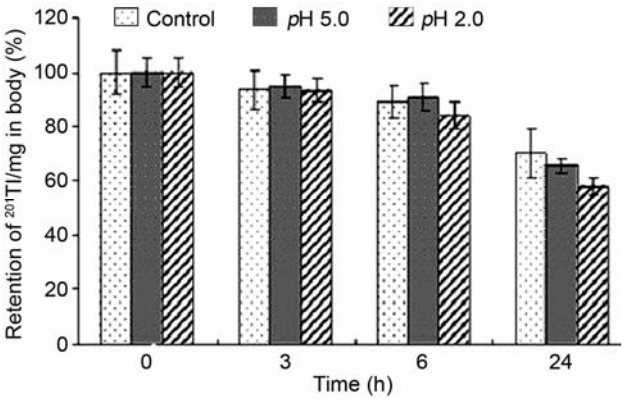


Fig. 3—The effect of oral acidosis (intake of acidified water of *p*H 2.0 and *p*H 5.0) on whole body retention of $^{201}$Tl (1.11 MBq) in experimental mice (n=6; $P<0.01$) over a period of 24 h.

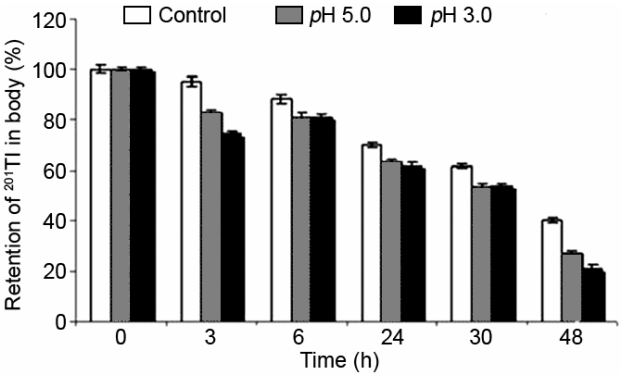


Fig. 4—Effect of intramuscular administration of lactic acid (*p*H 5.0 and *p*H 3.0) on whole body retention of $^{201}$Tl (1.11 MBq) as a function of time in experimental mice (n=6, $P<0.01$) up to 48 h.

*(b) Parenteral acidosis:*

*(i) Through intramuscular dose of lactic acid at different pH:* Per cent retention of $^{201}$Tl in mice after an intramuscular injection of 100 µLlactic acid (24 µg/mL) is presented in Fig. 4. At 3 h, there was a significant decrease in whole body $^{201}$Tl retention in mice that were treated with lactic acid as compared to control. The effect was more significant when *p*H of lactic acid was kept at 3.0 in comparison to *p*H 5.0.

*(ii) Through intramuscular dose of different concentration of lactic acid at pH 3:* Although initially at 3 h, per cent elimination of $^{201}$Tl was more in the treated group given low dose of lactic acid (Group I; 24 µg/mL; *p*H 3.0), it increased with time in animals who were administered a higher lactic acid dose (Group II; 48 µg/mL; *p*H 3.0). There was a significantly higher elimination of $^{201}$Tl in Group II animals at 48 h ($P<0.01$), with 50% more elimination of $^{201}$Tl seen as compared to Group I animals (Fig. 5). Animal survival was 100% and no residual effects were seen after 48 h except local inflammation at the site of administration of lactic acid.

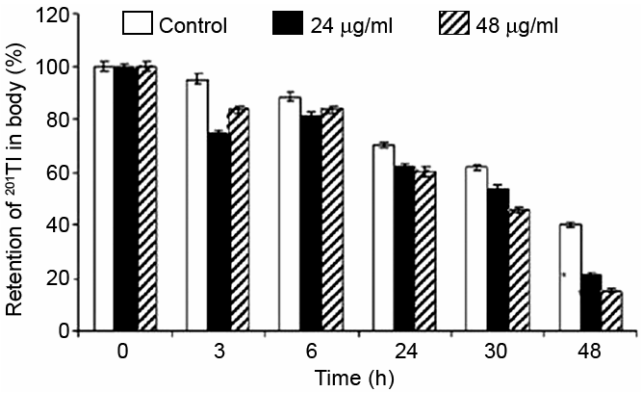


Fig. 5—Effect of varying conc. of lactic acid (*p*H 3.0) on whole body retention of $^{201}$Tl (1.11 MBq) as a function of time in experimental mice (n=6; $P<0.01$) up to 48 h.

## Discussion

This study was performed to confirm whether the reported decrease in intestinal absorption of $K^+$ at low *p*H[12] can be manipulated for $^{201}Tl/^{137}Cs$ as a mass therapeutic approach during any nuclear emergency. Metal ions can enter the system from the gut through paracellular pores. The size of these pores as well as ionization level of the metal ions determines the absorption potential of these metal ions. It is therefore logical to suggest that *p*H adjustments of the gut milieu should have a significant influence on their internalization. Several pieces of evidences in literature illustrate that *p*H and $[H^+]$ has significant role on absorption of $K^+$ in small intestine[12-14]. However, there is no experimental *in vitro* and *in vivo* data demonstrating the influence of *p*H on the absorption of other metal ions such as $Tl^+$ till date. Objective of the present study was to explore this possibility in sufficient detail with respect to thallium so as to reach a point of conclusion.

Being chemical analogues, $Cs^+$ and $Tl^+$ have the ability to mimic the biological behaviour of $K^+$. The *ex vivo* experiments showing movement of $Tl^+$ across jejunum in simulated *p*H conditions prove that *p*H has direct influence on the efflux of $^{201}Tl$ from the intestine, much like as is reported for $K^+$. Lowering the *p*H in intestinal lumen significantly increases the mucosal membrane potential, resulting in decrease in net voltage difference across intestinal membrane, thereby inhibiting the efflux of potassium[15]. Similarly decrease in absorption of $^{201}Tl$ from jejunum on lowering the *p*H strongly suggests the role of transmural potential difference. The present results are corroborated by the findings of Scafer *et al.*[16], who have shown a strong influence of potential difference across mucosal membrane on outflow of $^{204}Tl$. The present findings, substantiated by other works in literature strongly suggests that acidification of the gut can be a viable option to reduce $^{201}Tl/^{137}Cs$ absorption or reabsorption into the human system. Since $^{201}Tl/^{137}Cs$ is excreted through hepatobiliary system in the duodenum, acidification of upper GIT will help reduce reabsoption and enhance fecal excretion. Acidification of gut (within physiological range) can be used as a prophylactic or therapeutic measure for significant decorporation/inhibition of incorporation of radiothallium and radiocesium.

An experimental model comprising hind limb muscle was thought to be necessary because muscle is the major target organ for $^{137}Cs$ post internalization. Movement of $^{137}Cs$ /$^{201}Tl$ in hind limb muscle is an active process and is governed by functioning of the $Na^+/K^+$ ATPase pump[6,7]. In one of the experiments, addition of 5% dextrose in ringer solution increased $^{201}Tl$ uptake in hind limb tissue by 3.5 folds at 120 min as compared to control under *ex vivo* conditions (unpublished data). This experiment was done to confirm the viability of muscle cells in *ex vivo* conditions and to reinforce the fact that movement of $^{201}Tl$ ions in skeletal muscle was an energy dependant process as reported elsewhere[6]. In the present experiments, initially the concentration of $^{201}Tl$ in muscle tissue was zero. Once compartmentalization of $^{201}Tl$ occurred in tissue, the effect of *p*H on its uptake could be seen. Effect of *p*H on $^{201}Tl$ uptake in muscle can be predicted on the basis of Donnan equilibrium[17], i.e.

$$[Tl_i]/[Tl_e]=[H_i]/[H_e]$$

where, $Tl_i = [^{201}Tl]$ in hind limb muscle, $Tl_e = [^{201}Tl]$ in Ringer solution, $H_i = [H^+]$ in hind limb muscle and $H_e = [H^+]$ in Ringer solution.

On decreasing the *p*H of Ringer solution there was an increase in hydrogen ion concentration in Ringer solution ($H_e$) as compared to its intracellular concentration in the tissue ($H_i$). Therefore, to maintain ideal $H_i/H_e$ concentration, the tissues had to either efflux or inhibit the influx of thallium ion to maintain homeostasis. Similar findings but having different implications have been reported where intravenous administration of $^{201}Tl$ along with sodium bicarbonate increased the uptake of $^{201}Tl$ in heart of human volunteers undergoing stress thallium myocardial scintigraphy[18]. Reduction in $^{201}Tl$ (and therefore $^{137}Cs$) uptake in cells under slightly acidic microenvironment could probably be because of the fact that $H^+$-$K^+$ pump shall favour extermination of potassium (and therefore radiocesium/radiothallium) so that hydrogen ion could be internalized to reduce $H^+$ burden in extra cellular fluid (ECF). Conversely, alkalinity shall favour internalization of potassium ion/its analogues and this may be reason for significant enhancement in $^{201}Tl$ deposition in muscles seen at higher *p*H in the present experiments.

*In vivo* models of acidosis showed conclusively that oral intake of acidified water or parenteral administration of lactic acid were able to reduce the body burden of radiothallium significantly. The fact that the animals drank acidified water

voluntarily, though with some reduced intake, which was expected, suggests that the approach can be extrapolated and employed to humans e asily since a number of routine foods and beverages can be used to provide an acidic microenvironment safely and continuously to maintain sub-clinical acidosis on a short-term or medium-term basis. Short-term acidosis can help the rescue teams entering a radioactive zone as a prophylactic while medium-term acidosis can be useful for affected inhabitants who would be reluctant to vacate their lands in a radioactive fall out scenario. Though we have used ammonium chloride or lactic acid for inducing acidosis in animals, citric acid could probably be another suitable choice because it is part of a number of palatable foods taken routinely. Since primary requirement is the amount of $H^+$ ion quantum, oral route provides a very wide range of options to a physician.

The present experiments reinforce the effect of acidification in delaying the uptake of radiothallium from GIT and extracellular fluid. This may help in devising better strategies for medical management of ingested radioactivity, particularly of monovalent ions such as cesium and thallium. The present approach has the potential to be a cost effective, easy and ubiquitous option for large scale medical management of nuclear exigencies, and be a viable lifesaving option particularly in case of mass casualty.

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