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# Transient cell function disruption by low dose acute exposure of ionizing radiation

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Incubation of BMG-1 cells with thallium chloride ($^{201}$Tl) in the range of diagnostic dose did not show a smooth uptake curve and appeared to have an unsuspected deviation in initial phase. In the present study this unexpected phenomenon was explored, using commonly used radionuclides (viz., $^{201}$Tl and $^{131}$I). Comparison was made with technetium-99m pertechnetate ($^{99m}TcO_4^-$) and technetium-99m labeled methoxyisobutylisonitrile ($^{99m}$Tc-MIBI) that are known to show conventional 2 phase graph representing inflow and outflow segments. Serial *in vitro*, *ex-vivo* and *in vivo* gamma scintigraphy as well as NMR spectroscopy experiments were conducted to corroborate the results. BMG-1 cells demonstrated a four-phase uptake pattern with $^{201}$Tl as compared to a conventional biphasic pattern with $^{99m}$Tc-MIBI. Flow cytometry data however did not reveal any $^{201}$Tl induced cell injury. Further, mice tissue extracts injected with $^{201}$Tl also showed a transient depression in its uptake. Scintigraphy experiments in rabbits administered with diagnostic dose of $^{201}$Tl and $^{131}$I confirmed the *in vitro* and *ex vivo* findings. Further, proton NMR spectroscopy showed decrease in the level of choline at 3 h and 24 h in $^{201}$Tl treated animals as compared to control. Phosphoethanolamine peak firstly decreased at 3 h but reached normal level at 24 h time point. No significant change was observed in the level of betaine. This transient reduction in internalization of $^{201}$Tl and $^{131}$I may represent a hitherto unknown acute effect of low dose radiation, i.e., transient depression in $Na^+$-$K^+$ ATPase pump activity without any apparent evidence of cell damage, representing a transient cell membrane dysfunction. The phenomenon may present a mechanistical explanation of 'thyroid stunning' at cellular level and suggest that it may be more universal in nature than suspected till now.

**Keywords:** Thallium-201, Iodine-131, Thyroid stunning, $Na^+$-$K^+$ ATPase pump

$^{201}$Thallium ($^{201}$Tl) is a well-known radiotracer routinely used in humans as a diagnostic myocardial perfusion and tumour imaging agent[1-3]. The cellular uptake of thallium ions, like potassium ions, is an active process via the $Na^+$-$K^+$ ATPase pump[4]. This uptake is followed by its slow diffusion out of the cell passively, a phenomenon called 'redistribution phase'[5]. Since the pump is more active in myocites, particularly cardiomyocytes and tumour cells, there is a preferential uptake in these tissues resulting in a diagnostic image upon imaging by a gamma camera/ SPECT system. Based on initial time-activity curve (TAC) experiments in animals and humans for $^{201}$Tl, Bradely-Moore *et al*.[6] and other investigators[7] have assumed a smoothly rising curve for the isotope by heart and other tissues. This assumption may appear to be logical since handling of radiotracers by tissues, whether by zero, first or even second order kinetics, is governed by smooth physiological phase transitions, so much so that any deviation in the smooth running curves is taken to indicate the effect of another influencing factor.

However, the TAC data given by Bradely-Moore *et al*.[6] suggested that the pattern of $^{201}$Tl uptake is not smooth for several tissues and shows transient depression. It appeared to us that the authors did not attach much significance to the slight though consistent deviations and might have thought of it as a statistical error. Gamma camera performance and counting electronics in the past had appreciable standard error, and region-of-interest softwares were not as accurate as in presently available modern versions. No supporting *in vitro* studies for $^{201}$Tl are available in the literature that report TAC curves on the lines of *in vivo* observations.

In a bid to study agents and processes that may block or accelerate the flow of $^{201}$Tl in cells, *in vitro* experiments were conducted in which BMG-1 cell line was incubated with $^{201}$Tl to create base-line

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cell uptake data with respect to time. It was consistently noted that there appeared a perceptible transient dip in the uptake of $^{201}$Tl in the initial inflow phase, before the outflow or redistribution phase set in. This observation was also depicted clearly in the biodistribution data of $^{201}$Tl in rabbits as reported by Bradely-Moore *et al*.[6] supporting that the transient dip observed in the *in vitro* studies may not be merely statistical and could represent a hitherto unsuspected stimulus or phenomenon. Since diagnostic quantity of $^{201}$Tl was used it has been hypothesized that the cause of this transient dip in radiotracer uptake was radiation itself and not the metal toxicity because of the miniscule amount of thallium used. This hypothesis made the study more interesting and of larger interest because a) no observable acute cellular dysfunction is known to be associated with a single diagnostic dose of $^{201}$Tl, and b) this observation, if consistent, may not be limited to $^{201}$Tl only as radiation effect is non-specific. In this range of radiation exposure, only long term stochastic effects/genetic effects are known[8].

Objective of the study was therefore, to confirm the phenomenon in different experimental models using different radioisotopes, namely $^{201}$Tl, $^{131}$I and $^{99m}$Tc, and to understand the mechanism behind this transient cellular 'dysfunction'. The nearest related phenomenon regarding acute non-lethal effect of radiation given in diagnostic dose is that of thyroid stunning occurring with $^{131}$I where temporary suppression of iodine transporters has been suspected[9,10].

## Materials and Methods

Radiotracers, namely technetium-99m pertechnetate ($^{99m}TcO_4^-$), technetium-99m labeled methoxyisobutylisonitrile ($^{99m}$Tc-MIBI), thallous chloride-201 ($^{201}$TlCl) and iodine-131 ($^{131}$I) were obtained from BRIT, BARC in India. All other chemicals were of AR grade and were purchased from Merck Ltd. (Mumbai, India). All experimental animals were maintained at a constant temperature (24 ± 2°C) on a 12:12 h L: D cycle and provided with standard food (Lipton Ltd) and water *ad libitum*. All experiments were approved by duly constituted Animal Ethics Committee of the Institute.

Sequential *in vitro*, *ex vivo* and *in vivo* experiments were performed in different experimental models to confirm the phenomenon.

### Cell culture studies

*Uptake studies in BMG-1 cells*—BMG-1 cells were maintained on low glucose Dulbecco's Modified Eagle's Medium supplemented with 5% (v/v) heat-inactivated fetal bovine serum. These were trypsinized and seeded at a density of 1 million cells/ml in a petridish. After 2 days of seeding, 37KBq of $^{201}$Tl or 370KBq $^{99m}$Tc-MIBI was added to different petridishes and incubated for 15, 30, 45, 60, 90 120 and 180 min respectively. After incubation the media was removed and the cells were washed twice by decantation with ice-cold phosphate-buffered saline (PBS). Cells were trypsinized and centrifuged at 1000 rpm for 5 min to get the cell pellet. For washing, the pellet was resuspended in normal saline and centrifuged again. Supernatant was discarded and counts of the washed cell pellet were taken on a gamma counter (Capintec, USA) to estimate $^{201}$Tl or $^{99m}$Tc-MIBI uptake by cells (counts/million cells). Cell pellet of samples treated with $^{201}$Tl was stored in 80% ethanol (in PBS) at 4°C for cell cycle progression study and cell viability analysis.

*Cell cycle progression analysis*—Flow cytometer measurements were performed with 80% ethanol-fixed cells kept overnight at 4°C. Cell viability and cell cycle progression analysis were performed using propidium iodide (PI), a DNA intercalator[11]. Briefly, the cells (0.5-1 million) were washed with PBS after removing ethanol and treated with 200 µg/ml of RNase-A for 30 min at 37ºC. Subsequently cells were stained with 25µg/ml PI for 15 min at room temperature. Measurements were made with flow cytometer (FACS-Calibur Becton Dickinson San Jose, CA, USA) using the Argon laser (488 nm) for excitation. Distribution of cells in different phases of cell cycle was calculated from the frequency distribution of DNA content by using the Mod fit Programme (Variety Software, CA, USA).

### Nuclear medicine based animal studies

*Ex vivo radiometry study*—Balb/c mice (n=128; all males; 30 ± 2 g) obtained from Experimental Animal facility of the institute were divided equally into 4 groups, viz., A, B, C and D. Each group was further divided into 8 sub-groups of 4 mice each for time periods 15, 30, 45, 60, 90, 120, 150 and 180 min respectively. Four doses of $^{201}$Tl, viz., 0.740MBq, 1.11MBq, 1.48MBq and 1.85MBq to be injected in group A, B, C and D mice respectively were prepared at *p*H 7.0. Solution concentration was adjusted so that each mouse would receive the dose in 100 µl injected

volume. Injections were given via the tail vein. After administration of $^{201}$Tl, mice in each subgroup were sacrificed at respective time periods. Heart, liver and hind limb muscle were removed carefully, washed in chilled saline solution, weighed dried in oven for 5 min at 50°C to remove any extra water and their radioactive counts taken on a gamma counter (Capintec, USA). Tail counts were taken as a quality control procedure to validate the results. Mice showing more than 10% counts in the tail vein were rejected and replaced. For all others, count renormalization was done taking into account the tail vein counts. Results were expressed as percentage of $^{201}$Tl incorporated per mg of tissue.

*In vivo gamma scintigraphy study*—New Zealand white rabbits (n=22, 2.5 ± 0.3 kg) were divided into 4 groups, namely A, B, C and D receiving one-time diagnostic dose of $^{99m}$Tc-MIBI (74MBq), $^{131}$I (22.2MBq), $^{201}$Tl (18.5MBq), and $TcO_4^-$ (74MBq) respectively. Each rabbit received net desired dose of radioisotopes through the ear vein. Solution concentration was adjusted so that each rabbit would receive the dose in 200 µl injected volume (*p*H 7). $^{131}$I and $^{99m}$Tc-MIBI were used as positive controls for thyroid and muscle tissue respectively since these radiopharmaceuticals are internalized into these respective organs, while $^{99m}$Tc pertechnetate was used as a negative control for muscle tissue as it is not internalized in muscles.

Immediately after administration of the dose, rabbits of each group were imaged under the gamma camera (GE Millennium VG, USA), with appropriate energy settings and dynamic images were captured for initial 60 min (each frame of one minute). Static images were subsequently taken at a gap of 30 min till 3 h. Care was taken to avoid urinary contamination during the study period.

### NMR based metabolite study of mice serum injected with diagnostic dose of $^{201}$Tl

Since a number of metabolic pathways are altered due to radiation induced oxidative stress, NMR spectroscopy study was conducted to confirm any functional change in the cell membrane due to low dose of radiation.

*Animal handling and sample collection*—Male strain A mice (n=18, 30 ± 5 g) of 8 week of age were placed in polypropylene cages. Mice received tap water, were fed chow *ad libitum* and housed under a standard 12 h L:D cycle and at constant temperature of 25±2°C and relative humidity. All animal handling and experimental protocols were conformed to the guidelines stipulated by local animal ethical committee. Animals were divided into 3 groups with equal number of animals in each group (n=6) and blood of each group was collected to serve as control. Mice were left for 7 days with free access to food and water for blood recovery. At 7$^{th}$ day mice were injected with 1.85MBq of $^{201}$Tl i.p. and blood samples were collected at 3 h and 24 h post injection. Blood sample was allowed to clot for 30 min and serum was separated by centrifugation at 2665 g for 10 min. All serum samples were stored at -80°C until NMR measurement.

*Sample preparation and NMR Spectroscopy*—Serum sample of 200 µl was mixed with 400 µl of deuterium oxide ($D_2O$) and transferred to 5 mm NMR tubes containing 1 m*M* TSP (3-(trimethylsilyl) propionic-2,2, 3, 3-d4 acid sodium salt) closed capillary (Wilmad) as reference. All NMR spectra were acquired at 400.13 MHz, Bruker-Av spectrometer at 298K. Water suppressed Carr–Purcell–Meiboom–Gill (CPMG) spin echo pulse sequence (RD-90°-( $\tau$ -180°- $\tau$ ) $_n$acq) with a total spin echo (2 n$\tau$) of 200 ms was used to attenuate broad signals from proteins and lipoproteins. Typically 64 FIDs were collected into 32K data points over a spectral width of 8223.68 Hz with a relaxation delay of 2 sec and acquisition time of 3.98 sec. The FIDs were weighted by an exponential function with a 0.3-Hz line broadening factor prior to Fourier transformation. Peaks were assigned as described earlier[12].

*Statistical analysis*—Scintigraphy data were analyzed by inbuilt region-of-interest (ROI) software (Entegra$^{TM}$) drawn over dynamic images of heart, liver and thigh muscle area. Data was subjected to statistical analysis using one way analysis of variance (ANOVA). Value $P<0.05$ was considered statically significant.

## Results

#### Cell culture studies

*Uptake pattern in BMG-1 cells*— $^{201}$Tl uptake followed a complex four phase pattern. Rapid uptake in the initial 30 min was followed by a slow reduction in cellular counts, culminating in as much as 60% reduction in the initial counts by 90-120 min period. Thereafter, there was a consistent rise in cell counts again to the level of initial value, followed by a consistent and permanent fall till the end of study period. The four-phase pattern was seen in all

experiments run in triplicate as well as in the mean values at different time intervals. In contrast, the uptake of $^{99m}$Tc-MIBI in BMG-1 carcinoma cells showed a biphasic pattern. Maximum uptake was observed at 120 min followed by continuous elimination till the end of study (Fig. 1).

*Cell cycle analysis*—Cell cycle analysis was done to determine whether in the observed four-phase pattern of $^{201}$Tl uptake, any cell death or cell cycle block had occurred on continuous exposure of $^{201}$Tl or at the time points where the cells had maximum $^{201}$Tl uptake. Analysis by FACS showed that all the cell samples were in the same cell cycle phase. No significant cell death was observed in the samples of time points 90 min and 180 min on comparison with the control. Percentage of cells in G1, G2, S and M phases was similar at all time intervals studied (Fig. 2). Flow cytometry study suggested that continuous low exposure of $^{201}$Tl did not result in any significant cell death in the immediate post-exposure period. However, on continuous exposure to a higher dose of $^{201}$Tl to BMG-1 cells (370MBq; 180 min), a peak appeared before the G1 phase indicating 5% cell death.

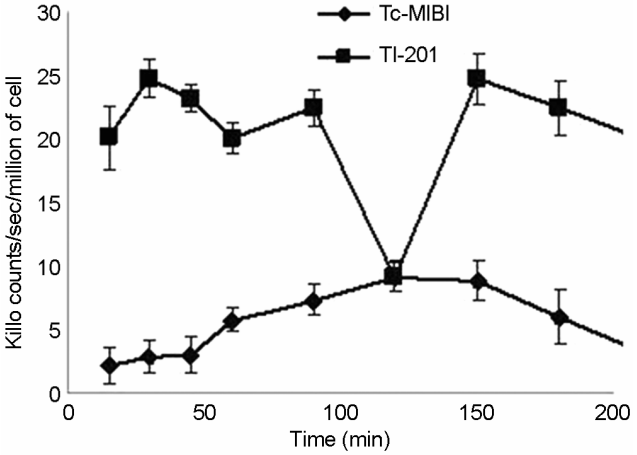


Fig. 1—$^{201}$Tl and $^{99m}$Tc-MIBI uptake by BMG-1 cells treated with 37KBq and 370KBq of $^{201}$TlCl and $^{99m}$Tc-MIBI (positive control) respectively, showing 4 phased uptake pattern for $^{201}$Tl as compared to conventional 2 phased pattern for $^{99m}$Tc-MIBI ($P$<0.05).

## Nuclear medicine based animal tissue studies

*Ex vivo radiometry study*—The transient depression in $^{201}$Tl uptake seen in BMG-1 cells was also seen in mice hepatic, skeletal and cardiac muscle *ex-vivo* models. The depth of the 'dip' phase appeared to be dose related, and was less deep as the dose was reduced from 1.85MBq to 0.74MBq. Time-activity curve of $^{201}$Tl in mice cardiac muscles at different doses (0.74MBq, 1.11MBq, 1.48MBq and 1.85MBq) is shown in Fig. 3A. At higher doses, uptake in cardiac muscle was double at 30 min as compared to that at 15 min; thereafter however, there was a significant dip till 80-100 min ($P$<0.05), before rising again, giving rise to a four-phase pattern as observed in *in-vitro* BMG-1 cell line experiments. A similar pattern was seen for hind limb muscle and liver,

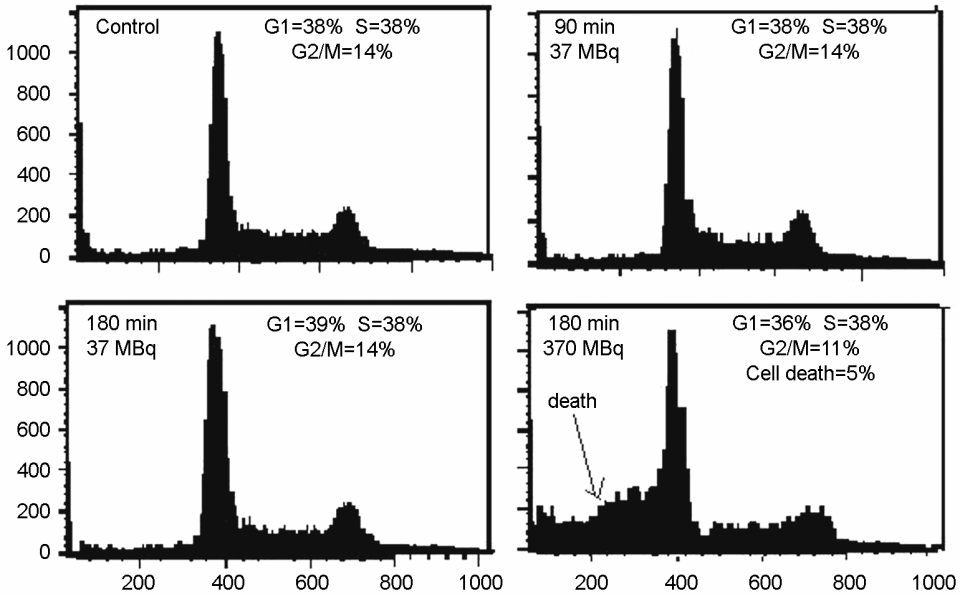


Fig. 2—Cell cycle checkpoints and cell death in exponentially growing BMG-1 cells on continuous exposure to low (37KBq) or high (370KBq) dose of $^{201}$Tl.

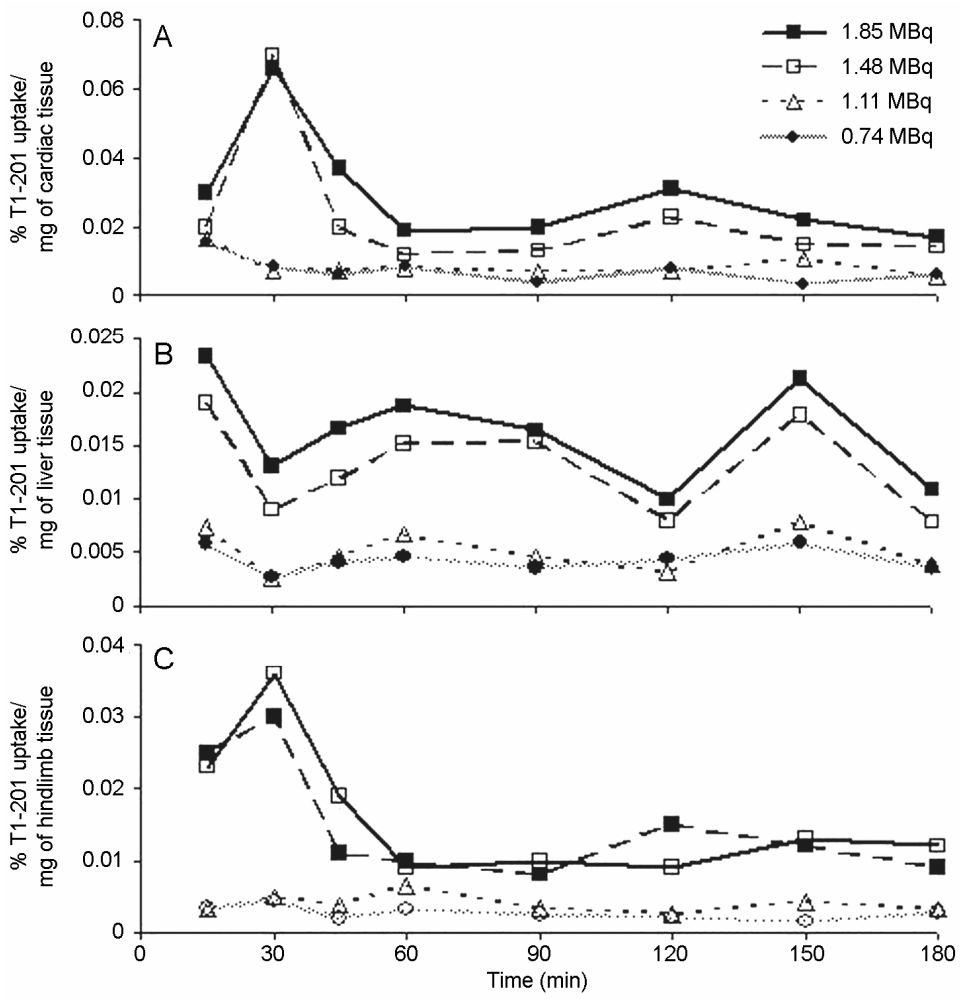


Fig. 3—Biodistribution of $^{201}$Tl in (A)-cardiac tissue, (B)-liver and (C)-hind limb muscle of mice administered with incremental doses of $^{201}$Tl.

though the time-points varied (Fig. 3A, B and C). This 4 phase pattern was seen at all doses of $^{201}$Tl, though the net uptake in tissues reduced proportionally to the dose. The curve appeared to get flattened with reducing dose but the transient depression was still significant at the lowest dose studied (0.74MBq).

*In vivo gamma scintigraphy study*—The time-activity curve (TAC) prepared over the heart and other target organs (liver and hind limb muscle) using dynamic and static gamma scintigraphy images provided a much higher data point density and consequently a better statistical accuracy. In rabbits injected with 18.5MBq of $^{201}$Tl, the curve drawn over cardiac muscle showed a smooth dip from 0-60 min followed by a peak before dipping again (Fig. 4A). The curve was therefore triphasic instead of four-phase considering that the initial rising curve starting from 'zero' radioactivity was missing because of the presence of radioactive cardiac blood pool activity in ROI area from the beginning.

Significant dip in tissue radioactivity was similarly evident in liver (maximum of 15% at 60 min) except that the curve kept on rising thereafter and was biphasic till the end of study at 3 h (Fig. 4B). In ROI drawn over thigh muscle, the initial uptake pattern was almost flat till about 60 min, followed by increase by 20%, giving a peak uptake at 80 min, followed by a slow outflow pattern (Fig. 4C).

Similar to the results obtained with $^{201}$Tl, TAC over thyroid in case of $^{131}$I also exhibited a four phased uptake pattern and a transient depression in uptake from 60-100 min, 'recovering' thereafter (Fig. 5).

$^{99m}$Tc-MIBI and $^{99m}$Tc-pertechnetate were used as positive and negative controls respectively (Fig. 6A and 6B). TAC of rabbit injected with 74MBq of $^{99m}$Tc-MIBI showed conventional biphasic pattern consisting of initial inflow and delayed outflow in all

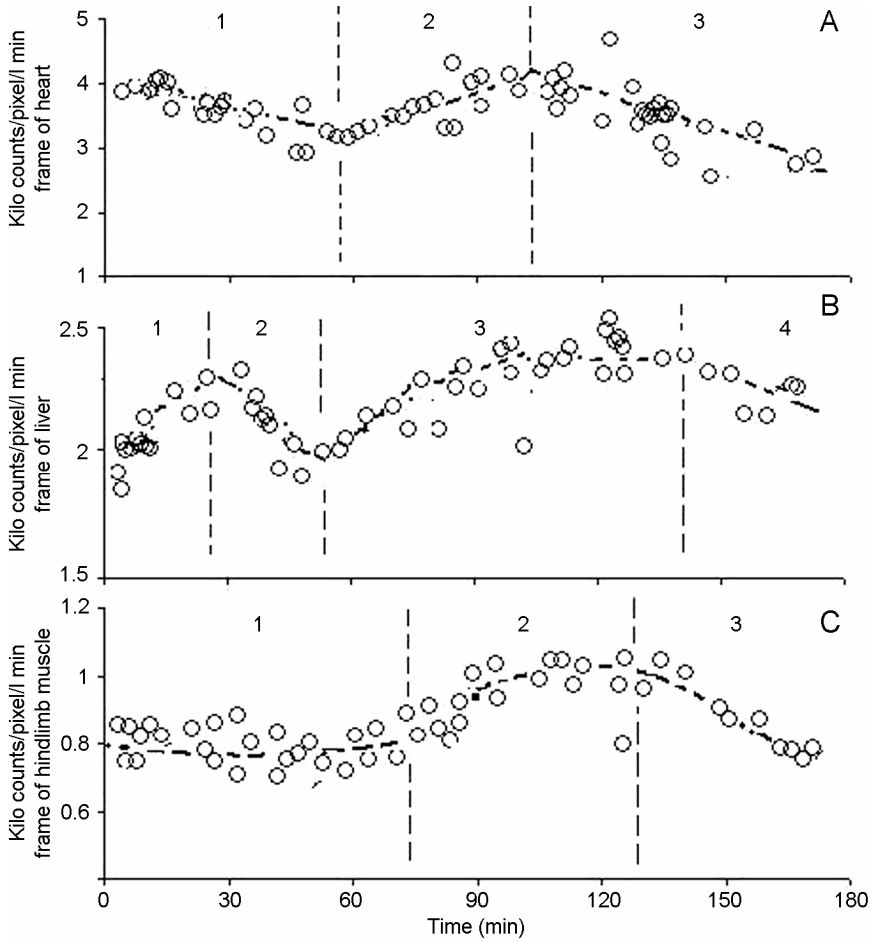


Fig. 4—Time activity curve (TAC) of $^{201}$Tl uptake in heart, liver and hind limb muscle of rabbits injected with 74MBq of $^{201}$Tl through ear vein. Each graph has been divided in to different phases by dash lines, the last phase representing distribution phase.

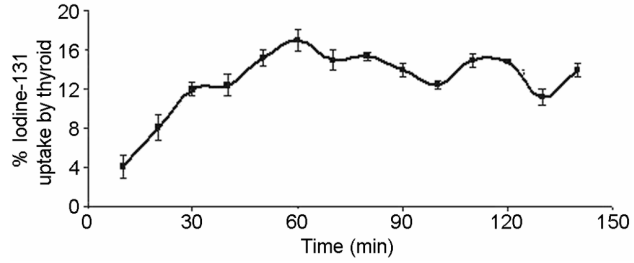


Fig. 5—TAC over thyroid in case of $^{131}$I showing a 4 phased uptake pattern and a transient depression in uptake from 60-100 min, 'recovering' thereafter.

the tissues studied, viz. heart, liver and thigh muscle. On the other hand, TAC of rabbit injected with 74MBq of $^{99m}$Tc-pertechnetate in heart, liver and hind limb muscle showed only an outflow pattern irrespective of the dose because the ion is not internalized in these tissues and slowly flows out of the extracellular fluid.

## NMR based metabolite study

Proton NMR spectroscopy showed decrease in the level of choline at 3 h and 24 h compared to the control. Whereas, membrane phospholipid phosphoethanolamine initially decreased at 3 h but reached normal level at 24 h time point (Table 1, Fig. 7). No significant change was observed in the level of betaine.

## Discussion

Results indicate that irrespective of the model system studied, from cells being incubated *in vitro*, or tissues receiving certain types of radiopharmaceuticals through perfusion and being assessed *ex-vivo*, or further, tissues receiving the radiopharmaceuticals and being assessed in living state through non-invasive imaging, uptake pattern of certain radiopharmaceuticals like $^{201}$Tl and $^{131}$I show a transient depression in initial phase in doses close to diagnostic doses before peak uptake occurs, followed

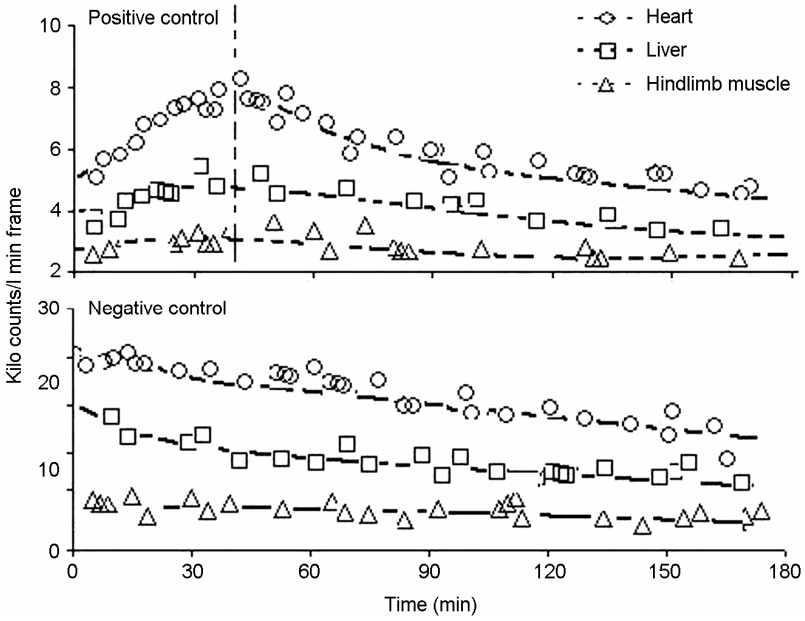


Fig. 6—(A)-TAC of heart, liver and hind limb muscle of rabbit injected with 74MBq of $^{99m}$Tc-MIBI (Positive control); (B)-TAC of heart, liver and hind limb muscle of rabbit injected with 74MBq of $^{99m}$Tc-pertechnetate (Negative control).

Table 1—NMR spectroscopy based Thallium-201 induced variations in serum metabolites as compared to controls

| Metabolites | Chemical shift with multiplicity (Bracket) | 3 h | 24 h |
|---|---|---|---|
| Choline | 3.21(s) | ↓↓ | ↓↓ |
| Phosphorylethanolamine | 3.23(t) | ↓ | ↑ |

↑ indicates relative increase in signal; ↓ and ↓↓, relative decrease in signal. Keys: s: singlet; t: triplet

by the classical outflow pattern.. This observation was however not made with $^{99m}$Tc based pharmaceuticals, represented by $^{99m}$Tc-MIBI.

This observation of a transient depression in uptake phase runs counter to the general assumption that uptake pattern in target tissue follows a classical biphasic pattern: an initial smooth uptake followed by a plateau or outflow phase depending on whether the system retains or expels a radiopharmaceutical with time. The observation made appears to be statistical in nature due to its repeatability and smoothness of curve, particularly evident in gamma scintigraphy *in vivo* dynamic studies that increased the reproducibility of data several folds. Findings of the study appear to represent a new phenomenon barely observed or recognized till now. The same pattern has also been reported in the in vivo experiments with $^{131}$I in thyroid tissue, its natural target organ[9,10].

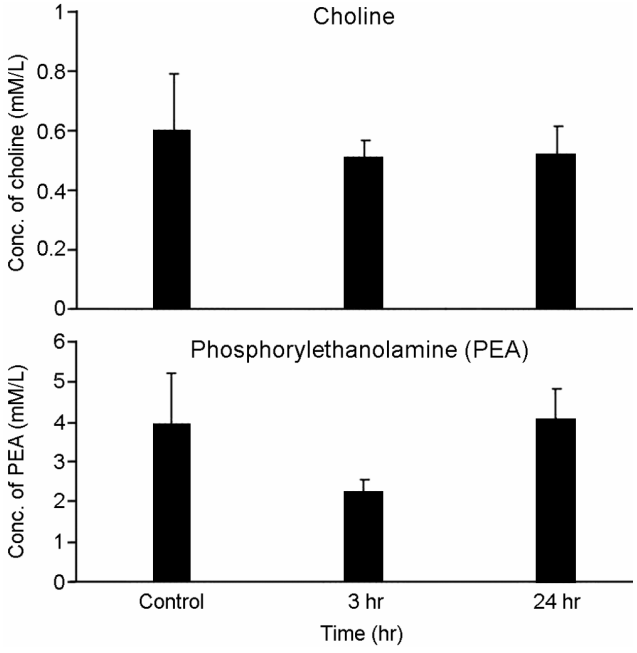


Fig. 7—NMR spectroscopy based $^{201}$Tl induced variations in serum metabolites showing effect on choline and phosphorylethanolamine levels at 3 h and 24 h as compared to control.

Flow cytometry studies of *in vitro* samples showed that the period of transient dip in uptake was not accompanied by any rise in injury or cell death parameters. In the *in vitro* samples, dip in uptake was quite high (even reaching 60% or more) compared to about 15% seen in the in vivo imaging protocols. This

difference may be explained by the fact that radiopharmaceutical dynamics tend to be under-reported on scintigraphy due to overlying and vascular activity.

Acute radiation effects are dose dependent and conventionally defined as lethal: when radiation disrupts DNA, or sub-lethal or repairable: when ionization radiation interacts with cellular water to produce free radicals causing damage to the cell organelles. In general however, it is believed that sub-lethal damage to organelle results in secondary functional disruptions. This study seems to suggest that at low doses, $^{201}$Tl and possibly $^{131}$I can cause temporary functional disruption without any apparent cell damage. $^{201}$Tl and $^{131}$I are internalized by an active energy process through $Na^+$-$K^+$ pump and specific transporter protein sodium/iodide symporter (NIS) respectively[13-15]. Temporary reduction in internalization of these radioisotopes suggests that this may represent an instantaneous but temporary disruption or suspension in some of the membrane functions as a result of interaction with low dose radiation. However, on the other hand, this does not seem to occur with diagnostic dose of $^{99m}$Tc-based compounds. This divergence in behaviour may be because $^{201}$Tl and $^{131}$I are known to give 10 and 1000 times more absorbed dose respectively than $^{99m}$Tc per microcuries[16]. $^{201}$Tl, upon entry into the cells, adheres to certain sulphur rich cytoplasmic and cell membrane proteins resulting in a more sustained radiation effect[17]. Moreover, it gives soft X-rays that are almost totally absorbed in a small space thus giving high LET radiation. Beta particles released by $^{131}$I also give much higher LET radiation as compared to 140KeV gamma photon emitted by $^{99m}$Tc. It is thus suggested that $^{201}$Tl and $^{131}$I in clinically relevant doses have a definitive interaction profile with cell membrane that may not injure it but may transiently disrupt pump and receptor functions.

The above-mentioned hypothesis has been further corroborated by NMR spectroscopy study, which showed a decrease in the level of choline at 3 h and 24 h compared to the control. A number of metabolic pathways get altered due to radiation induced oxidative stress. Radiation induced lipid peroxidation of cell membrane modifies membrane fluidity and the activities of some membrane enzymes[18,19]. Membranal damage results in release of phospholipids and choline compounds as choline is the major head of phospholipids. In mammalian system, choline has three main routes of metabolism: phosphatidylcholine (PC) synthesis, acetylcholine synthesis or oxidation to betaine[20]. The decrease in the level of choline in the NMR spectroscopy study might reflect its pathway towards the synthesis of phosphatidylcholine. The results also show no change in betaine level, which supports that more and more choline is being shifted towards formation of phosphocholine during membrane repair. The decrease in the level of phosphoethanolamine (PEA) at 3 h time point and its recovery at 24 h might be explained as a transient cell membrane dysfunction due to radiation.

Several pieces of information in literature support our observation that transient cell membrane dysfunction may occur on interaction with low dose radiation. Firstly, production of free radicals within the cytosol by radiation is known to be a dose-dependent effect, resulting in cell membrane function disruption depending upon the degree of oxidative stress[21,22]. Secondly, given the extreme dynamic nature of cell membrane, it is vulnerable to even minor insults, and cell membrane proteins responsible for pump and receptor functions are liable for easy disruption without any structural damage. Finally, oxidative stress is known to disrupt energy pathway that can temporarily reduce function of pumps requiring energy. It is suggested that a combination of these factors may be responsible for transient inhibition of $Na^+$-$K^+$ ATPase pump, which may be leading to the observed transient dip in internalization of thallium ions. It may be added here that low dose radiation is already known to cause potassium leak from cell systems[23]. Thus, it may be possible for diagnostic doses of radiopharmaceuticals as well to produce membrane dysfunctions without other evidences of cell injury. Naturally, the effect shall be more pronounced in cells that are internalizing the radioactivity compared to those that are not. Though it was not tested, it may be hypothesized that other membrane functions may also be disrupted temporarily as an immediate effect of low dose radiation and that high doses of $^{99m}$Tc-pertechnetate and $^{99m}$Tc-MIBI are likely to cause the same effect in their target tissues.

The 'depression' in $Na^+$-$K^+$ ATPase function normalizes in a matter of several minutes to a few hours in presence of continued radiation exposure suggested that the cell 'readjusts' to the presence of low dose radiation after an initial period of pump disruption. Instances of cellular adjustment to

different levels of ambient radiation have been reported in bacteria[24-26]. Cause of this relative radio-resistance is still a matter of conjecture, though it appears plausible that the cell responds by upgrading energy production or producing more membrane receptors to counter the effect of radiation. It may also represent the delay in optimal recruitment of cellular mechanisms for scavenging free radicals.

The only relevant clinical example of transient cellular function disruption by low dose radiation without causing cell injury is 'thyroid stunning' involving Iodine-131[27]. The observations with very low doses of $^{201}$Tl represents a broader extension of 'thyroid stunning' phenomenon, which may be applicable to isotopes other than $^{131}$I. Further, the study may used as a model for studying acute effects of low dose of radiation in tissues, including human models and can help in drug development process of radio-modifier group of drugs.

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