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# Effect of *n*-propylthiouracil or thyroxine on arsenic trioxide toxicity in the liver of rat

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## Abstract

Involvement of thyroid gland in the hepatotoxic manifestations of arsenic trioxide ($As^{III}$) has been studied in rat. The effects of *n*-propylthiouracil (PTU) (a thyrotoxic compound) and L-thyroxine (a thyroid hormone) have been studied with reference to $T_3$ and $T_4$ values in the serum, arsenic concentration in the liver, $Ca^{2+}$ accumulation in the liver, aspartate transaminase, alanine transaminase and bilirubin values as the indicators of liver function, histopathological observations and finally the ultrastructural studies. It is concluded that hypothyroid condition protects against $As^{III}$ toxicity. Scavenging of reactive oxygen species (ROS) that significantly contribute in $As^{III}$ toxicity, by high intracellular concentration of reduced glutathione, as a consequence of PTU treatment is proposed as the plausible protective mechanism.


***Keywords:*** Arsenic trioxide; Hepatotoxicity; *n*-propylthiouracil; L-thyroxine; Rat

## Introduction

Arsenic is an ubiquitous metalloid. It is well known for its toxicity and carcinogenicity in humans [1,2]. Arsenic may exist in several forms, including inorganic forms – arsenic, arsenite and arsenate as well as methylated species (monomethylarsonous acid and dimethylarsinic acid). While both forms are toxic, they exhibit distinct biochemical properties. Trivalent arsenic ($As^{III}$) has a high affinity for sulfydryl groups [3], whereas pentavalent arsenic ($As^{V}$) mimics phosphate as uncoupler of oxidative phosphorylation [4]. Thus elucidation of the effects of arsenic is complicated owing to speciation of the metalloid. However, both *in vivo* and *in vitro* studies show that trivalent arsenical form is more toxic than pentavalent form [5,6] In biological systems, pentavalent arsenicals are reduced to trivalency before methylation. Earlier, methylation was considered a detoxification pathway, however, recent studies indicate that methylated arsenicals are more toxic than inorganic arsenic [7,8]. Nevertheless, it has been agreed that exposure to arsenic generates reactive oxygen species (ROS) *in vivo* such as dimethylarsenic radical ($(CH_3)_2As^{\bullet}$), dimethylarsenic peroxyl radicals ($(CH_3)_2AsOO^{\bullet}$), superoxide anion, singlet oxygen and hydroxyl radicals. Free radical theory of arsenic toxicity/carcinogenicity has been largely accepted [9].

Further, metabolism of arsenic has been found to be affected by several factors. Selenite is known to influence the disposition of arsenate and arsenite in rats [10]. Ascorbate is also known to modulate arsenic toxicity [11]. An earlier study from our laboratory has shown that thyroid hormones ameliorate oxidative stress induced by $As^{III}$ in liver and kidney of rat [12]. Recent

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investigations have suggested that several environmental contaminants affect thyroid gland [13]. Thyroid status in turn might affect toxic manifestations of these chemicals [14]. Influence of thyroxine and *n*-propylthiouracil (PTU) on nephrotoxicity of inorganic arsenic has also been studied in our laboratory [15]. Therefore, a study on hepatotoxicity of arsenic trioxide in rats made hyper and hypothyroidic by administering L-thyroxine ($T_4$) and PTU, respectively, was considered important.

The present communication describes histopathological and ultrastructural effects of arsenic in the liver of hyper and hypothyroidic rats. Serum transminases, alkaline phosphatase and bilirubin have also been estimated to access the liver function. In addition, total calcium ($Ca^{2+}$) has been estimated in the liver to denominate hepatocellular injury.

## Materials and methods

### Chemicals

$As^{III}$ was procured from Loba Chemie (Mumbai). $T_4$ was purchased from Glaxo Laboratories (Mumbai) and PTU was supplied by Sigma Chemical Company (USA). RIA kits for $T_3$ and $T_4$ were procured from Diagnostic Products Corporation (LA, USA). Glutaraldehyde, osmium tetraoxide, Epon 812, uranyl acetate and lead citrate were procured from Sigma Chemical Company (USA).

### Animals and diet

Adult male Wistar rats weighing $150 \pm 30$ g were maintained under standard laboratory conditions (room temperature $20 \pm 5$ °C and relative humidity $= 50 \pm 10\%$). The rats were acclimated to laboratory housing conditions under 12 h light and dark cycle for 2 weeks. Each rat was housed separately in a polypropylene cage and offered pelleted food (Golden Feeds, New Delhi) and tap water *ad libitum*. All experiments were performed after the approval of the Institutional Ethical Committee according to the standards laid down by Ministry of Social Justice and Empowerment, Government of India.

### Experimental procedure

After acclimatization, rats were divided at random into six groups of five rats each. Rats of groups B and E were injected $T_4$ (25 μg/100 g body weight) intramuscularly on every 4th day for 3 weeks. Similarly, rats of groups C and F were injected PTU (2.5 mg/100 g body weight) intramuscularly twice a week for 30 days. Rats of group D were administered $As^{III}$ only (4 mg/100 g body weight) by gavage whereas rats of group A were offered saline to serve as controls. After these experiments, rats of groups E and F were further subjected to arsenic treatment. Each rat of these groups was administered a predetermined sublethal dose, i.e., 4 mg/100 g body weight of arsenic trioxide dissolved in saline through gavage on each alternate day for 30 days [12]. Gain or loss in the body weight of each rat was recorded daily. Suitable care was taken to maintain general health and hygiene of experimental rats. No mortality occurred during these investigations.

On the 31st day, all the rats were starved overnight and sacrificed next morning to make following observations.

#### Determination of $T_3$ and $T_4$

Blood was collected through cardiac puncture. Serum was separated by centrifugation and used for $T_3$ and $T_4$ estimations. RIA kits were used in this study. Coat-A-count procedure is a solid-phase radioimmunoassay wherein labeled $T_3/T_4$ competes with $T_3/T_4$ present in the sample for antibody sites. This reaction takes place in the presence of blocking agents that serve to liberate bound $T_3/T_4$ from carrier proteins. Hence the assay measures total $T_3/T_4$ since both free and protein bound $T_3/T_4$ from the sample are able to compete with respective radiolabelled antibody sites. After the tubes were decanted and counted, the concentration of hormones was read from a standard curve.

#### Estimation of arsenic

Liver was carefully removed from the rats. One gram of liver sample was digested in 10 mL of concentrated nitric acid at 80 °C for 16 h. The digests were stored at 4 °C before analysis. A 2 mL aliquot of the digest was analyzed for arsenic by hydride generation at pH 6 using sodium borohydride as the reducing agent. The hydride was collected on a cryogenic column before quantification by inductively coupled plasma-emission spectrophotometry (ICP-ES), using equipment supplied by GBC (Australia). These samples were analyzed at University Science Instrumentation Centre, Indian Institute of Technology, Roorkee (India).

#### Liver function tests

Serum concentrations of aspartate transaminase (AST) and alanine transaminase (ALT) were determined using a commercial kits [16]. Serum alkaline phosphatase was determined by the method of Kind and King [17]. Phosphate concentration was determined as suggested by Fiske and Subbarao [18]. Serum bilirubin was analyzed using the method of White et al. [19].

#### Calcium estimation

A total of 10% (w/v) homogenates of liver samples were prepared in saline. Calcium in the liver sample was

estimated by the spectrophotometric method of Wootton [20]. The absorbance was recorded at 450 nm.

### Histopathological observations on liver and thyroid

Small pieces of liver and thyroid glands were removed from the rats and fixed in 10% neutral formaline for 24 h. These samples were embedded in paraffin. Six to eight μm thick sections were stained with hematoxylin and eosin and examined under research microscope (Nikon, Japan).

### Ultrastructure of liver and thyroid

Very small pieces (1 $mm^3$) of liver and thyroid were fixed in 2.5% glutaraldehyde in 0.1 g/mol sodium phosphate buffer (pH 7.4), post fixed in 1.0% osmium tetraoxide, dehydrated through graded series of ethanol and embedded in Epon 812 after several changes in propylene oxide. Ultra thin sections stained with uranyl acetate and lead citrate were examined under a Philips CM10 transmission electron microscope. These observations were made at DST Facility at All Indian Institute of Medical Sciences, New Delhi (India).

## Statistical analysis

Statistical analyses were carried out applying Fisher's test for multiple comparisons [21]. Differences were considered statistically significant at $p<0.05$. Results were analyzed using analysis of variance (two-way) using SPSS software.

## Results

Present results show that administration of $As^{III}$ altered the status of $T_3$ and $T_4$ in the serum. A non-significant increase in $T_3$ and $T_4$ was recorded in $T_4$ and $As^{III}$-treated rats. However, in rats pretreated with PTU and subjected to arsenic treatment, a significant decrease in $T_3$ and $T_4$ values was observed. A comparison with $As^{III}$-treated group showed significant differences in $T_3$ values only (Table 1).

Arsenic concentration in the liver was affected by pretreatments of rats with $T_4$ and PTU. Its concentration increased in the liver of PTU pretreated rats, as compared to $As^{III}$-treated group. However, it decreased in the liver of rats treated with arsenic after $T_4$ treatment (Table 2).

Liver dysfunction in $As^{III}$-treated rats was indicated by high values for serum AST. Pretreatment with PTU decreased AST in $As^{III}$-treated rats. Non-significant inhibition of AST was recorded in $T_4$ and $As^{III}$-treated rats. A non-significant decline in ALT values was observed in both PTU and $T_4$ pretreated rats. Effect of $As^{III}$ on alkaline phosphatase activity was not significant. Pretreatment with $T_4$ and subsequent administration of $As^{III}$ increased alkaline phosphatase activity. However, its activity declined in PTU pretreated and then $As^{III}$ administered rats. Present results further showed that arsenic treatments did not cause hyperbilirubinemia. Contrarily, serum bilirubin declined after arsenic treatment. Non-significant decline was recorded in PTU as well as $T_4$ and $As^{III}$ treated rats (Table 3).

Concentration of calcium increased in the liver of $As^{III}$-treated rats. Administration of PTU as well as $T_4$ to $As^{III}$-treated rats decreased calcium concentration in the liver (Fig. 1).

**Table 1.** Total triiodothyronine ($T_3$) and thyroxine ($T_4$) in the serum of arsenic-treated rats

| Group | Treatment | $T_3$ (*n* moles/L) | $T_4$ (*n* moles/L) |
|---|---|---|---|
| A | Saline | $1.45\pm0.15$ | $30.14\pm1.86$ |
| B | $T_4$ | $5.8\pm0.30^{*}$ | $54.4\pm4.87^{*}$ |
| C | PTU | $0.76\pm0.07^{*}$ | $22.4\pm0.78^{*}$ |
| D | Arsenic | $6.9\pm0.27^{*}$ | $52.0\pm3.28^{*}$ |
| E | $T_4$ and arsenic | $7.0\pm0.50^{*,\bullet,b}$ | $72.0\pm4.48^{*,\#,a}$ |
| F | PTU and arsenic | $3.48\pm0.30^{*,\#.d}$ | $44.4\pm2.78^{*,\bullet,c}$ |

Results are expressed as mean ± SE ($n = 5$) significant at $p<0.05$:
*Significant when compared with saline-treated group.
#Significant when compared with arsenic-treated group.
•Non-significant when compared with arsenic-treated group.
[a]Significant when compared with $T_4$ group.
[b]Non-significant when compared with $T_4$ group.
[c]Significant when compared with PTU group.
[d]Non-significant when compared with PTU control group. *F*-value: $T_3 = 137.9$, $T_4 = 62.10$.

**Table 2.** Influence of thyroid hormones on accumulation of arsenic (μg/g wet weight) in liver of arsenic-treated rats

| Group | Treatment | Liver |
|---|---|---|
| A | Saline | $0.011\pm0.002$ |
| B | $T_4$ | $0.015\pm0.003^{+}$ |
| C | PTU | $0.012\pm0.001^{+}$ |
| D | Arsenic | $0.234\pm0.019^{*}$ |
| E | $T_4$ and arsenic | $0.104\pm0.014^{*,\#,a}$ |
| F | PTU and arsenic | $0.204\pm0.015^{\bullet,*,c}$ |

Results are expressed as mean ± SE ($n = 5$) significant at $p<0.05$:
*Significant when compared with saline-treated group.
+Non-significant when compared with saline-treated group.
#Significant when compared with arsenic-treated group.
•Non-significant when compared with arsenic-treated group.
[a]Significant when compared with $T_4$ group.
[c]Significant when compared with PTU group. *F*-value: 74.49.

## Histopathological observations on liver

Cellular architecture of liver of a healthy rat is shown in Fig. 2. Liver being the target organ was severely

**Table 3.** Influence of thyroid hormones on serum transaminases (Karmen units/L) in arsenic-treated rats

| Group | Treatment | AST (Karmen units/L) | ALT (Karmen units/L) | Serum alkaline phosphatase (KA units) | Serum bilirubin (mg%) |
|---|---|---|---|---|---|
| A | Saline | $44.4 \pm 4.40$ | $40.0 \pm 15.09$ | $11.62 \pm 1.79$ | $0.597 \pm 0.03$ |
| B | $T_4$ | $58.8 \pm 14.22^{+}$ | $123.6 \pm 12.62^{*}$ | $25.62 \pm 0.47^{*}$ | $0.632 \pm 0.09^{+}$ |
| C | PTU | $40.0 \pm 2.19^{+}$ | $86.40 \pm 24.18^{*}$ | $11.86 \pm 4.46^{+}$ | $0.632 \pm 0.06^{+}$ |
| D | Arsenic | $127.2 \pm 22.50^{*}$ | $145.0 \pm 2.64^{*}$ | $31.37 \pm 2.82^{*}$ | $0.398 \pm 0.04^{*}$ |
| E | $T_4$ and arsenic | $82.8 \pm 8.98^{*,\bullet,b}$ | $135.6 \pm 10.50^{*,\bullet,b}$ | $29.62 \pm 2.43^{*,\bullet,b}$ | $0.631 \pm 0.09^{+,\#,b}$ |
| F | PTU and arsenic | $62.8 \pm 10.27^{+,\#,d}$ | $110.5 \pm 17.70^{*,\bullet,d}$ | $18.62 \pm 2.57^{+,\#,d}$ | $0.569 \pm 0.08^{+,\#,d}$ |

Results are expressed as mean $\pm$ SE ($n = 5$) significant at $p < 0.05$:
[*]Significant when compared with saline-treated group.
[+]Non-significant when compared with saline-treated group.
[#]Significant when compared with arsenic-treated group.
[•]Non-significant when compared with arsenic-treated group.
[b]Non-significant when compared with $T_4$ group.
[d]Non-significant when compared with PTU group. $F$-value: AST = 6.514, ALT = 6.437, ALP = 10.72, bilirubin = 2.128.

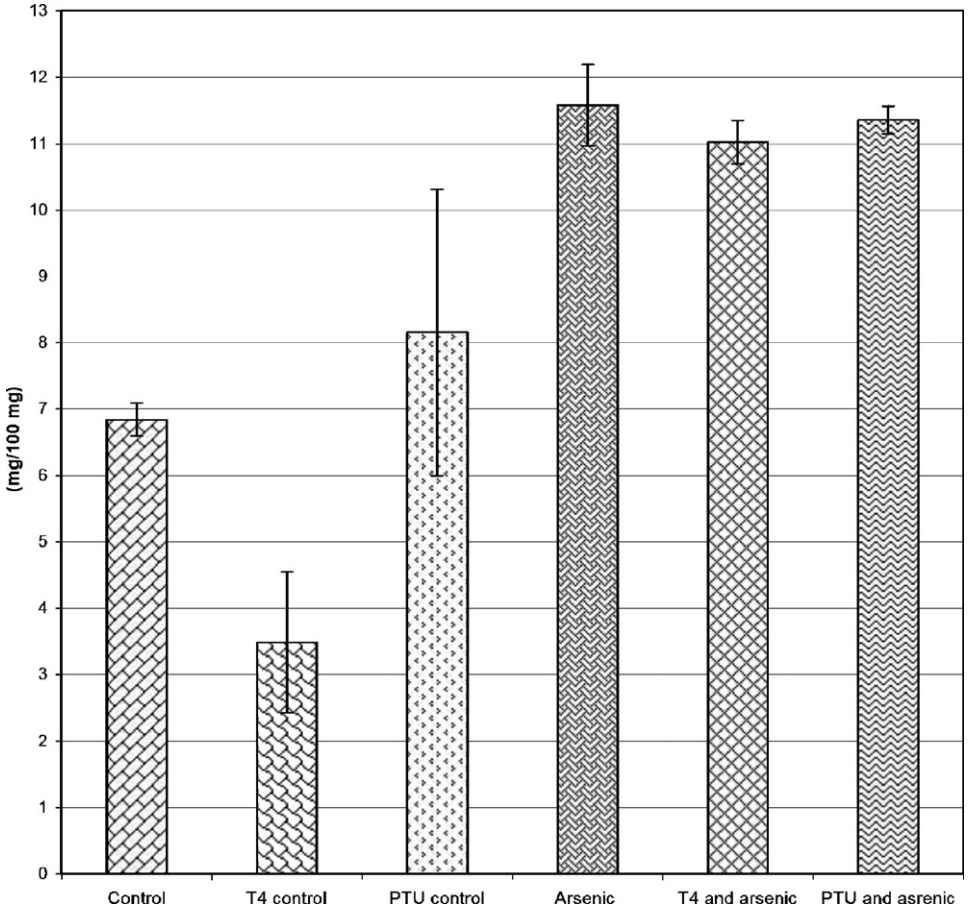


**Fig. 1.** Influence of thyroid hormones on calcium (mg/100 mg) in the liver of arsenic-treated rats.

affected by arsenic. It caused mild focal necrosis, hyperplasia and vascular lesions. Binucleated cells indicated increased proliferative activity (Fig. 3).

In PTU and arsenic treated rats, pathological lesions included inflammed hepatocytes and enlarged bile canalicular spaces. However, necrotic spaces were wanting (Fig. 4). In the liver of $T_4$ and arsenic-treated rats, no vascular lesions were observed. However, focal necrosis was observed at certain zones (Fig. 5).

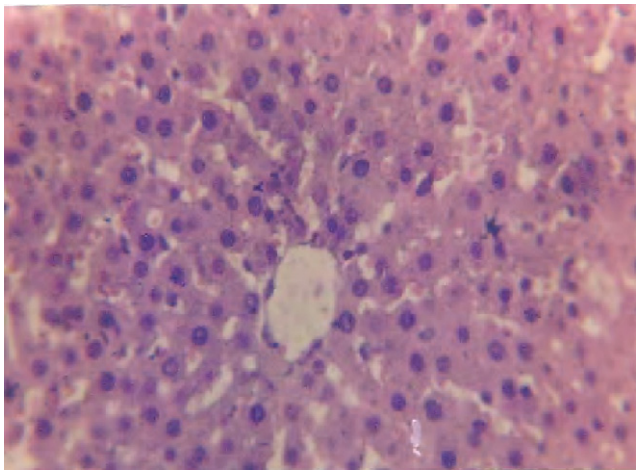

**Fig. 2.** Representative section of the liver from a control rat shows uniform hepatic parenchyma with round nuclei and centrally placed nucleolus. H/E 400 × .

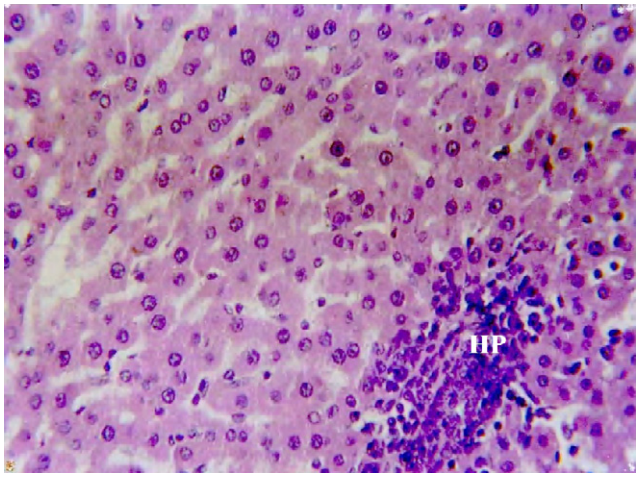


**Fig. 3.** Representative section of the liver of an arsenic fed rat shows hyperplasia (HP) and vascular lesions. H/E 400 × .

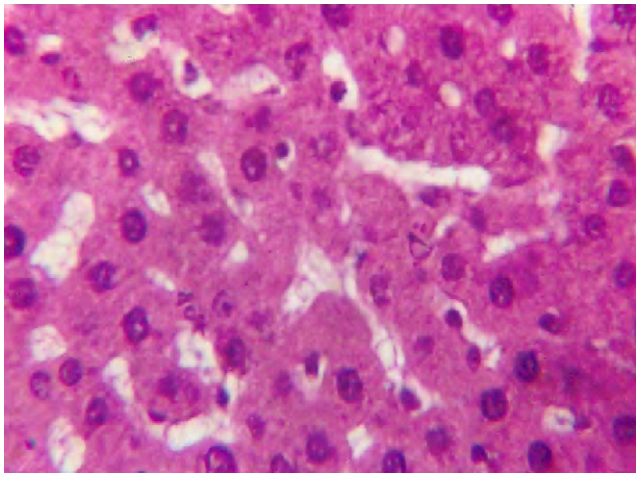

**Fig. 4.** Representative section of the liver shows inflammed hepatocytes after administration of arsenic to PTU pretreated rats. H/E 400 × .

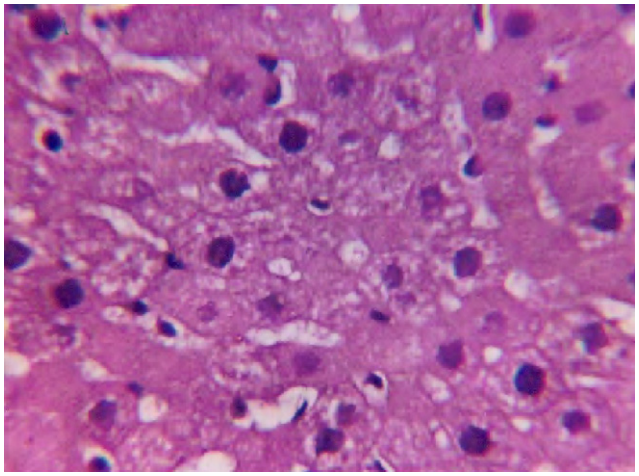

**Fig. 5.** Representative section of the liver of a $T_4$ pretreated and arsenic-administered rat showed necrosis at few locations. Several binucleated cells were also observed. H/E 400 × .

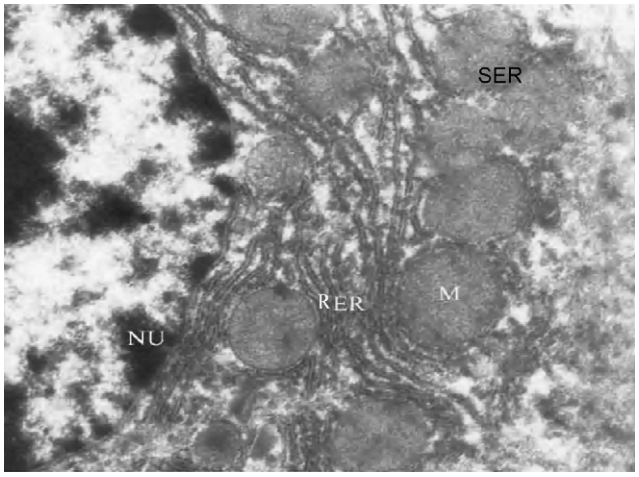


**Fig. 6.** Representative transmission electron micrograph of the liver of a control rat shows round mitochondria (M) and intact endoplasmic reticulum (RER, SER). 5400 × .

## Ultrastructural observations on liver

Ultrathin section of liver from a control rat examined under transmission electron microscope showed no particular lesion. Normal structure of organelles was observed (Fig. 6). However, in the liver of arsenic-treated rats, enlarged intercellular spaces, vacuolization of cytoplasm, a change in nuclear shape and size, inflammation of mitochondria and desquamated endoplasmic reticulum (ER) were observed. Fragmented and marginalized chromatin indicated apoptosis (Fig. 7). Administration of arsenic to PTU pretreated rats, induced peroxisomal proliferation. The change in shape and size of mitochondria were also observed. However, chromatin was not marginalized. It suggested absence of apoptosis (Fig. 8). The liver of $T_4$ pretreated and

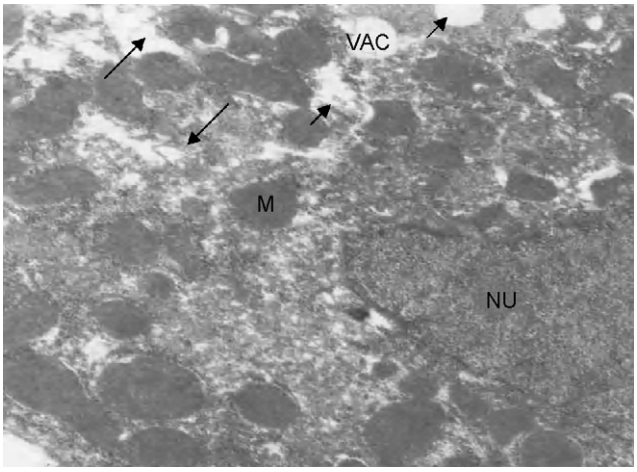


**Fig. 7.** Representative transmission electron micrograph of the liver of an arsenic-treated rat shows several prominent vacuoles (VAC) in the cytoplasm. 4200 × .

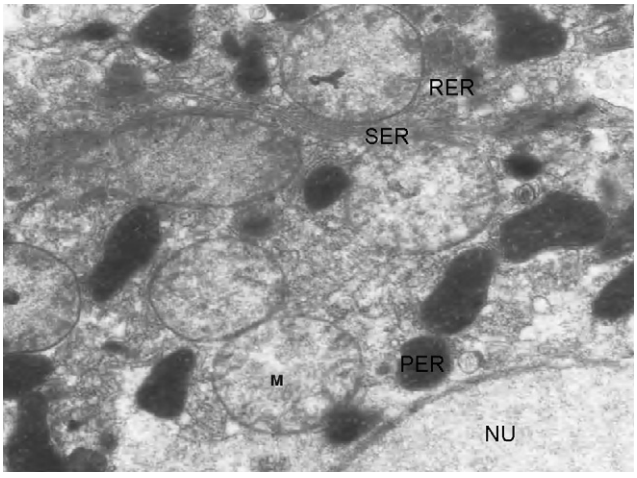


**Fig. 8.** Representative transmission electron micrograph of the liver of PTU pretreated and arsenic-fed rat shows peroxisomal (PER) proliferation and round mitochondria (M). 5400 × .

arsenic-administered rats showed proliferation of granular ER. Mitochondria were irregular in shape and size. Nucleus was round. Nucleolus was marginally placed. Marginalization of chromatin was observed (Figs. 9 and 10).

## Histopathological observations on thyroid

In the thyroid of $As^{III}$-treated rats, several discharged follicles were observed. The colloid deposition was greatly reduced (Fig. 11). However, in PTU and $As^{III}$-treated rats, collapsed follicles with severe epithelial cell damage were recorded (Fig. 12). In $T_4$ and $As^{III}$-treated rats, follicles were observed but the epithelial cells were found to be intact. A significant reduction in colloid was observed (Figs. 13 and 14).

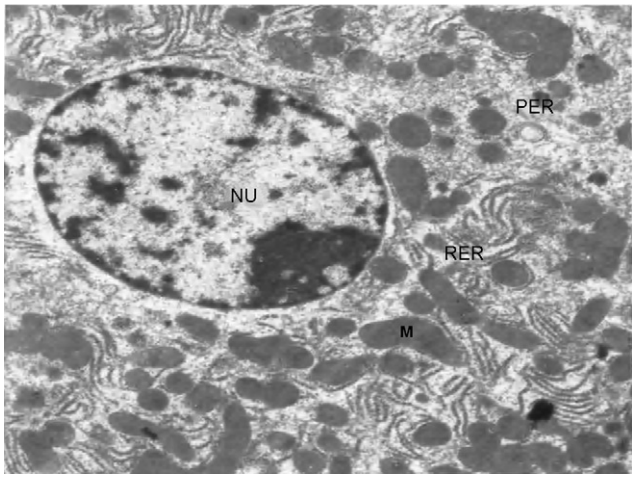


**Fig. 9.** Representative transmission electron micrograph of the liver of a $T_4$ pretreated and arsenic-fed rat shows increased number of mitochondria (M) of irregular shape and size. 2050 × .

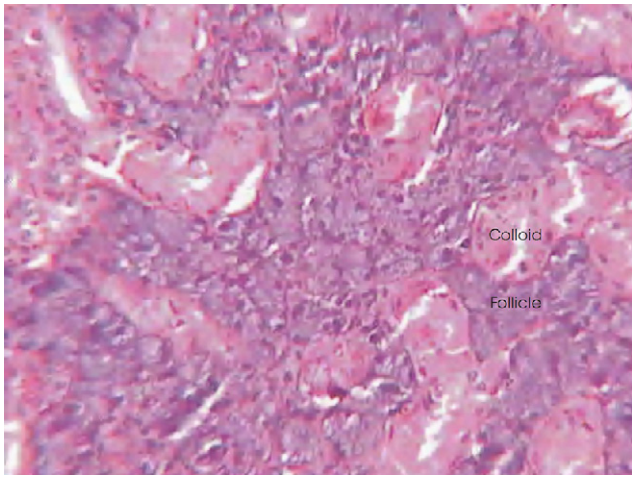


**Fig. 10.** T.S. of the thyroid of a control rat shows intact follicles. Parafollicular space/cells show no lesions. H/E 400 × .

## Ultrastructural observations on thyroid

Ultrastructural studies support the light microscopical observations. On $As^{III}$ treatment, epithelial lining of follicles got highly vacuolated. Large phagosomes were observed. At several places phagocytic vesicles fared with lysosomes. Mitochondrial movement towards apical surface was quite conspicuous (Fig. 15). Noteworthy observations in the thyroid of PTU and $As^{III}$-treated rats included shrinkage of epithelial cells, a reduced number of phagosomes, mitochondrial changes and appearance of secretary vesicles (Fig. 16). In $T_4$ and $As^{III}$-treated rats, reduced lumen of the follicles was observed. Polarity of the follicles was also changed. Cisternae of rough ER appeared as thread-like

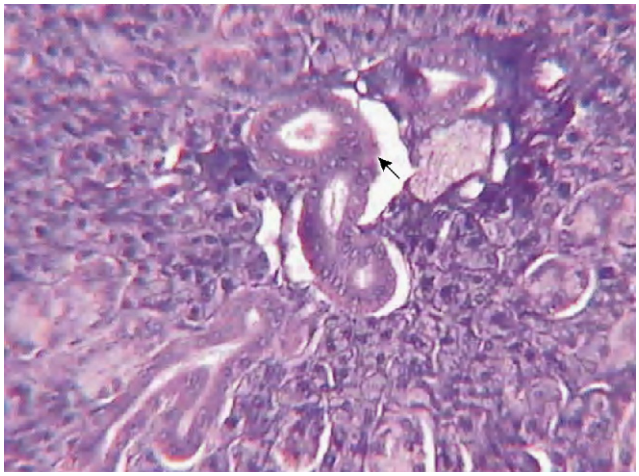

**Fig. 11.** Several discharged and shrunken follicles were observed in the thyroid of arsenic-treated rats. Colloid deposition greatly reduced. H/E 400 × .

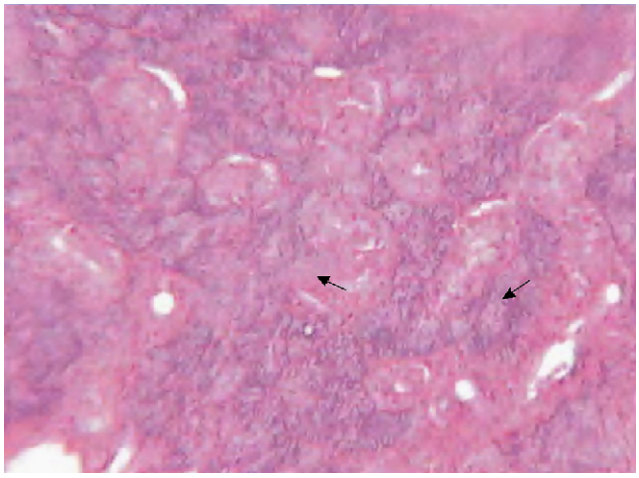

**Fig. 12.** Thyroid of hypothyroidic rat after arsenic administration showed collapsed and constricted follicles. Loss of colloid was noticed. H/E 400 × .

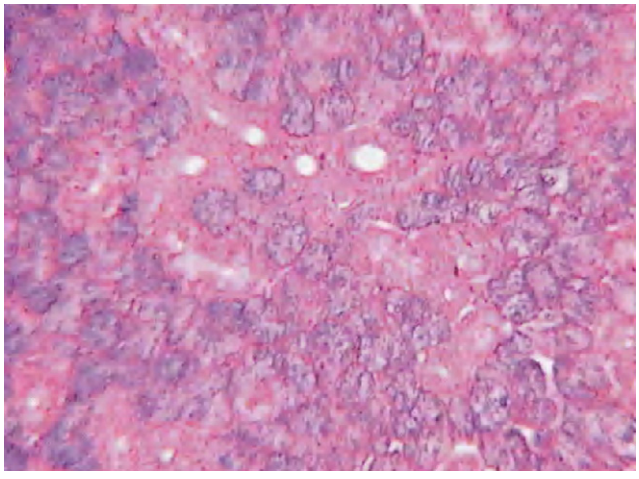

**Fig. 13.** Follicles of different sizes were observed in hyperthyroidic rats after arsenic administration. Epithelium was columnar. Intact follicles contained colloid. H/E 400 × .

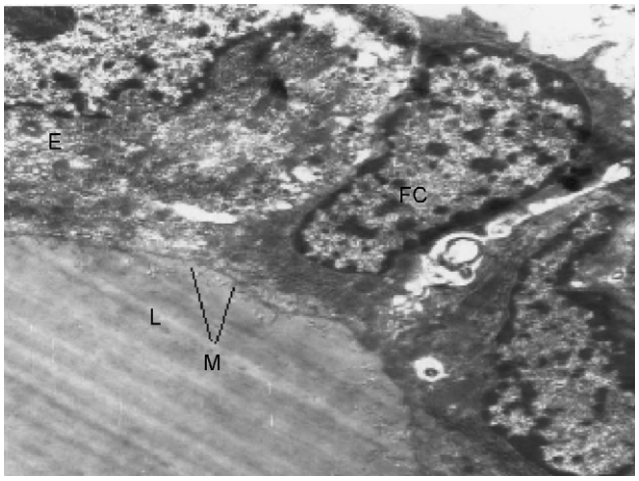


**Fig. 14.** TEM study of a normal thyroid shows in fact follicular cells and lumen of the follicle 2650 × .

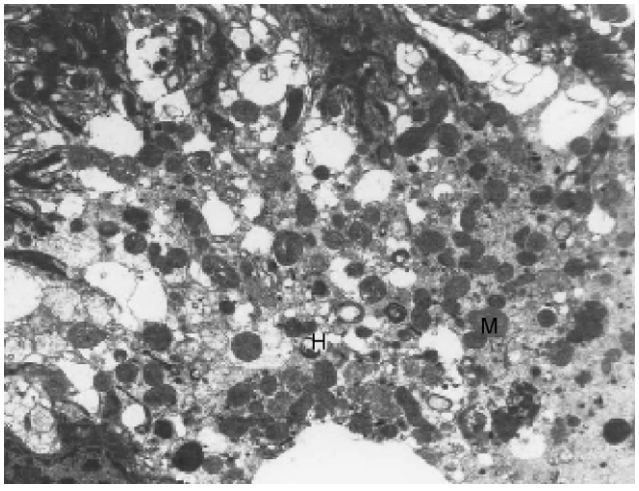


**Fig. 15.** TEM study of a thyroid of an arsenic fed rats shows highly vacuolated epithlium and apical movement of mitochondria (M). 4550 × .

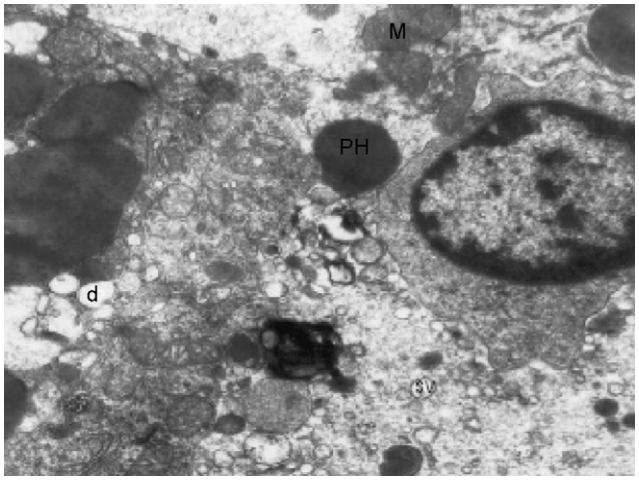


**Fig. 16.** Several desmosomes and secretory vesicles appeared in PTU and arsenic-treated thyroid of rat. 4600 × .

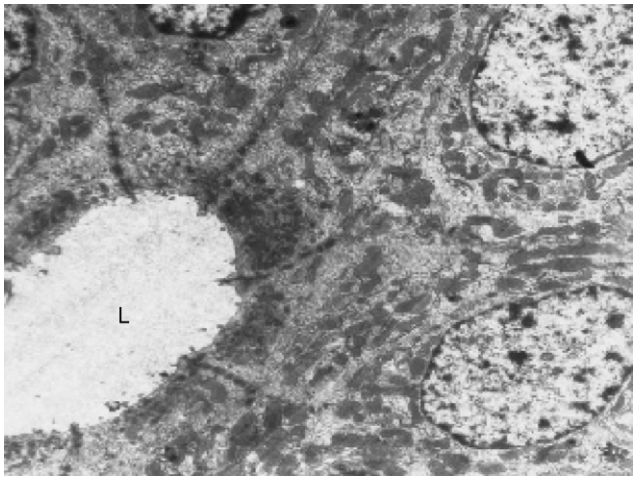


**Fig. 17.** TEM study of thyroid of $T_4$ and arsenic-fed rats shows reduced lumen and stimulated follicular epithelium 2950 × .

structures. Several secretory vesicles were observed on the trans surface of Golgi bodies (Fig. 17).

## Discussion and conclusion

Hepatotoxicity of arsenic trioxide was found to be profoundly altered in rats pretreated with thyrotoxic (PTU) and thyroactive ($T_4$) agents. Involvement of thyroid in the metabolism and toxicity of xenobiotics has been reported earlier as well [22–25]. $As^{III}$ disturbs the secretion of thyroid hormones. Values of both $T_3$ and $T_4$ were higher in the serum of $As^{III}$-treated rats. The mechanism responsible for the increased $T_3$ and $T_4$ values should apparently be an inhibition of 5-monodeiodination in pituitary thyrotrophs, which should lead to increased thyrotropin (TSH) secretion and thus higher $T_3$ and $T_4$ values. Alternatively, enhanced TSH secretion might also lead to increased synthesis of $T_3$ and $T_4$. Further metabolism of $T_4$ and PTU in liver may contribute in $As^{III}$ hepatotoxicity. $T_4$ generates ROS [26] whereas PTU offers protection against ethanol [27], carbon-tetrachloride [28], salicylate [29], and acetaminophen [30].

We could notice the effect of thyroidal activity on concentration of arsenic in liver. Both PTU and $T_4$ mobilized arsenic, perhaps by increasing its biliary or urinary excretion [31]. Antagonistic relationship between $T_4$ and arsenic as reported earlier [33] offers support to present observations [32].

Chronic oral exposure to $As^{III}$ is known to produce significant pathological changes in kidney and liver of experimental animals [33]. Influence of thyroid hormones on these lesions induced by $As^{III}$ was confirmed during present investigations. Pretreatments with PTU was protective whereas $T_4$ excerbated $As^{III}$ toxicity. Similar conclusions have been drawn in an earlier report on kidney [15]. We concluded that PTU when given in doses sufficient to induce hypothyroidism increased hepatic glutathione (GSH) that offered protection against hepatic injury.

Liu et al. [32] have reported that $As^{III}$ treatment increased ALT values in the serum. Increased release of lactate dehydrogenase was also observed. Pretreatment with $T_4$ further increased the values of ALT, AST and alkaline phosphatase in the serum. Contrarily, PTU pretreatment inhibited the release of alkaline phosphalase. No effect of $T_4$ or PTU was observed on bilirubin in $As^{III}$-treated rats. Available literature shows that thyroid hormones and testosterone decrease hepatic bilirubin conjugation whereas BUGT activity increases by a combination of progestational and estrogenic steroids [34].

It was interesting to note that administration of $As^{III}$ increased calcium concentration in the liver. However, $T_4$ and PTU pretreatments made significant effects on $Ca^{2+}$ concentration in the liver. Although $Ca^{2+}$ plays an important role in hepatocellular injury, its role in $As^{III}$ hepatotoxicity needs further conformation [35]. $Ca^{2+}$ concentration in hepatic parenchymal cell is a highly controlled mechanism. It depends on the exchange with extracellular compartments, on the one hand and on the other hand, with the release or segregation of $Ca^{2+}$ from intracellular elements like mitochondria. Since mitochondria are involved in biotransformation of arsenic, release of $Ca^{2+}$ from mitochondria during $As^{III}$ toxicity was expected [36]. Hormones can rapidly elevate the cytoplasmic concentration of $Ca^{2+}$ by changing the properties of plasma membrane and thereby accelerating passive uptake. Further, hormones can specially bring about the opening of calcium channels and/or facilitate the $Ca^{2+}$ transport mechanisms.

Studies made earlier have indicated a tissue-specific regulatory role of thyroid hormones with respect of energy metabolism in mitochondria and turnover of their protein components [37]. It is also known that the activity of thyroid gland is predominantly regulated by pituitary TSH. Thyroid hormone exerts a negative feedback on pituitary secretion of TSH. When $T_4$ concentration drops, TSH concentration increases thus keeping $T_3$ levels stable. Thyroid hormones exert their influence at a nuclear level by regulating the transcription of thyroid hormone responsive genes. This process is initiated when $T_3$ binds to thyroid hormone receptors.

Amelioration of arsenite hepatotoxicity by thyroidal suppression was first reported by our laboratory [12]. Whether these effects of PTU were independent or caused by virtue of hypothyroidism had been a subject of debate. A few workers attribute this effect of PTU to reduced GSH. PTU administered in large doses leads to increase in hepatic GSH [31]. Further, it may act as a

nucleophile analogous to that of GSH and scavenge ROS. Similar hypothesis has been suggested in fish [38]. Whereas, pretreatments with $T_4$ reduced concentration of $As^{III}$ in liver, this might have consequently diminished the toxic attributes of $As^{III}$. Present results, however, suggest that a hypothyroid state produced by repeated treatments of PTU offers protection against $As^{III}$ toxicity.

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