Changes in some hormones by low doses of di (2-ethyl hexyl) phthalate (DEHP), a commonly used plasticizer in PVC blood storage bags & medical tubing

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**Background & objectives:** Di (2-ethyl hexyl) phthalate (DEHP), a plasticizer commonly used in PVC blood storage bags leaches out in significant amounts into blood during storage. In view of many reports on the toxicity of this compound, it was considered necessary to investigate the effect of DEHP at the low level solubilized in blood on some important hormones in rats and in human blood stored in DEHP plasticized blood bags.

**Methods:** Rats were administered DEHP at a low level of 750 µg/100 g body weight on alternate days for 14 days. Changes in the serum insulin, blood glucose, liver glycogen level and $T_3$, $T_4$ and thyroid stimulating hormone (TSH) as well as cortisol in the serum were studied. Changes in the hormones were also studied in blood stored in DEHP plasticized PVC bags.

**Results:** The results indicated decrease in serum insulin, cortisol and liver glycogen, and increase in blood glucose, serum $T_3$ and $T_4$ in rats receiving DEHP. These changes were reversed when administration of DEHP was stopped. Similar changes in hormones were also observed in the blood stored in DEHP plasticized blood bags.

**Interpretation & conclusion:** The results indicated that administration of DEHP at low levels to rats caused symptoms of diabetes, thyroid and adrenocortical dysfunction. Though the results obtained in rats cannot be extrapolated to human, the fact that similar hormonal changes seen in human blood stored in DEHP plasticized blood bags may suggest possibility of DEHP causing similar changes in human. The fact that these changes were reversed in rats when DEHP administration was stopped, indicates that transfusion of a few units of blood to a recipient may not be harmful, but it may pose a problem during repeated transfusions such as in thalassaemia patients.

**Key words** Blood transfusion - cortisol - DEHP - thyroid hormones

Di (2-ethyl hexyl) phthalate (DEHP), a plasticizer commonly used in PVC blood storage bags, leaches out in significant amounts into blood and blood products resulting in exposure of patients to this compound during transfusion$^{1-3}$. A number of reports are available on the toxicity of DEHP, particularly in the liver and reproductive organs in experimental animals$^{4-9}$. Almost all the studies on the toxicity have been carried out with large doses of DEHP ($³2g/kg$ body weight) administered by oral route, which has no relevance to blood transfusion where the amount of DEHP leaching out into blood during transfusion is several times smaller (approximately about 10 mg/100 ml blood after 21 days of storage) and the administration is intravenous. In an earlier study from our laboratory$^{10}$ it was shown that the administration of DEHP in rats in doses similar to those solubilized in blood...
during storage produced no serious toxic effects as evidenced by lack of any histopathological changes in the liver and testes or significant alterations in many biochemical parameters. However possible changes in some important hormones like insulin, thyroid hormones and cortisol were not investigated in that study.

Only a few reports are available which show alterations in the thyroid by histochemical studies\(^\text{11-14}\), and a fall in liver glycogen and decrease in glucose tolerance in rats on administration of DEHP\(^\text{15-17}\). All these studies were performed using high doses of DEHP given orally. Very little information is available on the effect of DEHP at a level as it is solubilized in blood on systemic administration in experimental animals or on the changes in the various hormones in the blood stored in DEHP plasticized bags. The present study was undertaken to observe the changes in insulin, thyroid hormones and cortisol in rats administered low levels of DEHP corresponding to that solubilized in blood during storage and also in human blood stored in DEHP plasticized blood bags.

### Material & Methods

**Animal experiments:** Female albino rats (Wistar strain, average body weight 150 g) were grouped randomly into two groups (control and experimental) with 16 rats in each group.

The experimental group rats were administered DEHP at a dose of 750 µg/100 g body weight. Taking the average body weight of a human recipient of blood transfusion as 60 kg and the amount of DEHP solubilized as 10 mg/100 ml blood after 21 days of storage, this dose corresponds to the transfusion of ten units of blood in a human recipient\(^\text{10}\). Emulsion of DEHP was prepared in 2.2 per cent glycerol containing 1.2 per cent egg yolk lecithin by sonication under sterile conditions and was administered intraperitonially as described earlier\(^\text{10}\). The control rats received the same volume of vehicle. The animals were caged individually in polypropylene cages and maintained on normal laboratory feed (M/s. Sai Durga Feeds and Foods, Bangalore) in rooms maintained at temperature 28±1°C. Food and water were available to the rats *ad libitum*.

Administration of DEHP was made on alternate days and a total of 7 injections were given. At the end of 14 days, 8 rats in each group were deprived of food overnight and blood was collected from ocular vein. These rats were sacrificed and liver was collected into ice-cold containers for glycogen estimation. Thyroid and pancreas were also collected in 10 per cent buffered formalin for histopathological examination. For biochemical analysis, blood and liver of six rats in each group were used, and for histopathological studies in thyroid and pancreas the remaining two rats were also used.

The injection of DEHP was stopped in the remaining 8 rats in the experimental group and these rats, along with the control rats, continued on their diet for another 7 days. They were then deprived of food overnight, sacrificed and blood and organs were collected for various estimations. This was done to ascertain whether the effect produced by DEHP was reversible or not, when DEHP administration was stopped.

The study protocol was approved by the Institutional Animals Ethics Committee.

**Studies using human blood:**

Blood stored in glass bottles in the presence of DEHP-Blood (350 ml) was collected under sterile conditions from young healthy volunteers by following standard procedures\(^\text{18}\) into 49 ml citrate-phosphate-dextrose-adenine (CPDA)\(^\text{19}\) solution in glass bottles and in CPDA solution containing DEHP. The concentration of DEHP was 20 mg/100ml blood, which corresponded to approximately twice the amount of DEHP solubilized when blood is kept in DEHP-plasticized bags (about 10 mg/100 ml blood after 21 days of storage). An emulsion of DEHP in the CPDA solution was prepared by sonication and solution sterilized by autoclaving and this emulsion was added to CPDA before blood collection.

Blood stored in DEHP plasticized PVC bag-Blood (350 ml) was collected into 49 ml of CPDA in Terumo-Penpol blood bag (DEHP plasticized PVC blood bags manufactured by Terumo Penpol Limited\(^\text{TM}\), Thiruvananthapuram) from young healthy volunteers as per standard procedures\(^\text{18}\).

In both cases, blood immediately after collection from donors was transferred to 4±1°C. Aliquots were withdrawn immediately after collection and after 7, 14 and 21 days of storage for various analysis.
Analytical procedures: Glycogen in the liver was estimated by the method of Carrol et al. Serum cortisol was estimated by the method described by Mattingly. Commercial kits were used for the estimation of serum insulin (Mercodia Insulin ELISA, Mercodia AB, Sweden), T₃, T₄, and TSH (Monobind Inc. CA, Sweden) and blood glucose (Randox, UK).

Statistical analysis was carried out using Students t test for animal experiments and ANOVA for blood bag experiments.

Results

Animal experiments: There was significant (P<0.01) increase in blood glucose and decrease (P<0.01) in liver glycogen in rats administrated DEHP when compared to control rats. These changes were however reversed to very near control values after 7 days of stopping DEHP administration. Concentration of serum insulin showed significant (P<0.01) decrease in the rats administered DEHP. This decrease was reversed to near normal levels 7 days after stopping administration of DEHP (Table I).

Concentration of serum T₃ and T₄ showed significant (P<0.01) increase in the rats administered DEHP, while that of TSH was not very significantly altered. The level of T₃ and T₄ returned to near normal levels 7 days after stopping administration of DEHP. Serum cortisol showed significant (P<0.01) decrease in the rats administered DEHP when compared to control rats. This decrease was also reversed 7 days after stopping administration of DEHP (Table II).

No histopathological changes were observed in the β-cells of pancreas in the rats administered DEHP. But thyroid gland showed initial reactional hyperplasia when compared to that in control rats.

| Table I. Effect of administration of DEHP on the levels of glucose, glycogen and insulin in the experimental animals and the effect of its withdrawal |
|---|---|---|---|
| Group | Blood glucose (mg/dl) | Liver glycogen (mg/100 g wet tissue) | Serum insulin (mU/l) |
| Control | 64.5±2.902 | 139.83±4.89 | 1.937±0.089 |
| Experimental | 72.54±3.34* | 121.36±4.24* | 1.677±0.077* |
| Effect of withdrawing of DEHP after 7 days |
| Control | 64.3±2.89 | 139.8±4.86 | 1.91±0.085 |
| Experimental | 61.79±2.84 | 140.2±4.9 | 1.89±0.088 |

Values given are the mean ±SD of 6 rats
*P<0.01 compared to controls

| Table II. Effect of administration of DEHP on the levels of thyroid hormones and cortisol in the experimental animals and the effect of its withdrawal |
|---|---|---|---|---|
| Group | T₃ (ng/dl) | T₄ (µg/dl) | TSH (µlU/ml) | Cortisol (µg/dl) |
| Control | 55.75±2.45 | 3.9±0.198 | 0.34±0.017 | 10.02±0.45 |
| Experimental | 88.5±4.4* | 4.68±0.239* | 0.32±0.016 | 8.98±0.404* |
| Effect of withdrawing of DEHP after 7 days |
| Control | 55.65±2.75 | 3.93±0.196 | 0.34±0.017 | 10.08±0.423 |
| Experimental | 50.15±2.95 | 4.4±0.22 | 0.347±0.0172 | 10.09±0.428 |

Values given are the mean ±SD of 6 rats
*P<0.01 compared to controls
TSH, thyroid stimulating hormone
Changes in the blood stored in glass bottles with and without DEHP and in DEHP plasticized PVC blood bags: Level of insulin decreased progressively in the blood stored in glass bottle with increase in storage time. This decrease was more in the blood in the presence of added DEHP. Blood stored in blood bag also showed progressive decrease in insulin level with storage time. This decrease was significantly ($P<0.01$) more when compared to that in blood stored in glass bottle without DEHP. A similar pattern of change was also observed in the level of cortisol (Table III).

$T_3$, $T_4$ and TSH also showed decrease in the blood stored in glass bottle during storage. Presence of DEHP (glass bottle + DEHP and DEHP plasticized bag) reduced the extent of this decrease in $T_3$ and $T_4$ significantly. The concentration of $T_3$ and $T_4$ was higher in the presence of DEHP. In the case of TSH the decrease in the presence of DEHP was more (Table III).

**Discussion**

The dose of DEHP administered to the rats was similar to the concentration of DEHP received by a thalassaemia patient during a period of six months through blood transfusion. The present results showed significant alteration in many important hormones. Serum insulin was decreased significantly and there was an increase in blood glucose and decrease in liver glycogen. The decrease in liver glycogen was similar to the findings reported by Mann et al\textsuperscript{16} in rats administered larger dose (50 to 2000 mg DEHP/kg body weight orally). Mushtaq et al\textsuperscript{15} also reported similar findings in rats given high dose of DEHP. The decrease in insulin may be the cause of decrease in liver glycogen and increase in blood glucose. Van Dooren\textsuperscript{23} reported a decrease in the level of various drugs including insulin by interaction with the plasticizer on storage.

The increase in $T_3$ and $T_4$ in the DEHP treated rats may indicate hyperactivity of this gland. Persistent hyperactivity of the thyroid was reported by Price et al\textsuperscript{13} in rats administered DEHP by electron microscopy, similar to the present results obtained biochemically. Histochemically also the DEHP treated rats showed initial reactional hyperplasia in the present study. Hinton et al\textsuperscript{14} reported marked hyperactivity in the thyroid in rats administered DEHP orally. The fact that TSH is not significantly altered in rats administered DEH may indicate that the anterior pituitary, which secretes this hormone, may not be significantly affected by DEHP.

The decrease in cortisol may either be due to the toxic effect of DEHP on the adrenal gland or may be a consequence of suppression of pituitary gland. But the results with TSH indicate that pituitary gland may not be affected by DEHP. Therefore the decrease in cortisol may be due to the direct effect of DEHP on the adrenal gland.

The administration of DEHP at low levels caused symptoms of diabetes (lower serum insulin and liver glycogen and increase in blood glucose), hyperthyroidism (increase in $T_3$ and $T_4$ as well as hyperplasia of thyroid gland on histopathological examination) and adrenocortical suppression (decrease in cortisol). The results on the effect of DEHP in rats have to be considered in the light of observation that rats and mice appear to be particularly sensitive to some effects of DEHP and the findings may not be similar in humans. Thus, the results obtained with rats cannot be extrapolated to human but the fact that similar changes in $T_3$, $T_4$ and cortisol were observed in human blood stored in glass bottles to which DEHP was added and in blood stored in DEHP plasticized bags suggested that similar changes might possibly take place in recipients of repeated transfusion of DEHP containing blood. If so, these observations may suggest the possibility of similar changes in the recipients of transfusion of blood containing DEHP.

The fact that the effect of DEHP was reversible in rats when the administration of DEHP was stopped indicates that transfusion of a few units of blood to a recipient may not be harmful. A reversal of the effect of DEHP on withdrawal in mice has also been reported by David et al\textsuperscript{24}. The present observation on lowering of insulin level by DEHP suggests that the repeated transfusion of DEHP containing blood to thalassaemia patients may lead to diabetes in these patients. A detailed study on this aspect in the thalassaemia patients is warranted in the light of the present observation.

In conclusion, the results of the present study indicate the necessity to reduce the level of DEHP leaching into the blood by modifying the preservative solution or the bag surface or using a non-DEHP plasticized bag.
Table III. Changes observed in hormonal levels in the plasma of blood when stored in glass bottles, glass bottle containing DEHP and blood bags

<table>
<thead>
<tr>
<th>Hormone (units)</th>
<th>Glass bottle</th>
<th>Glass bottle + DEHP</th>
<th>Blood bag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days after storage</td>
<td>Days after storage</td>
<td>Day after storage</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>6.1±0.22</td>
<td>5.0±0.15**</td>
<td>4.6±0.138**</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>383.42±13.8</td>
<td>375.29±12.35</td>
<td>351.9±11.9**</td>
</tr>
<tr>
<td>T₃ (ng/dl)</td>
<td>77.5±2.61</td>
<td>60±1.98**</td>
<td>53±1.89**</td>
</tr>
<tr>
<td>T₄ (µg/dl)</td>
<td>8.1±0.23</td>
<td>6.6±0.16**</td>
<td>5.3±0.158**</td>
</tr>
<tr>
<td>TSH (µlU/ml)</td>
<td>1.69±0.052</td>
<td>1.33±0.039**</td>
<td>1.23±0.055**</td>
</tr>
</tbody>
</table>

Values given are the mean ±SD of 6 experiments

*P<0.05;   **<0.01 compared to day 1
† P<0.01 compared to corresponding value in glass bottle

TSH, thyroid stimulating hormone
References


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