Delayed Effect of Thorotrast Deposition in Humans: Carcinogenesis and Suppression of the Reticuloendothelial System

Hiroshi Irie*  
Department of Pathology, Teikyo University School of Medicine, 2–11–1 Kaga, Itabashi-ku, Tokyo 173–8605, Japan  
Received for publication, July 26, 2000

Thorotrast is a colloidal solution containing 20% thorium (232Th), and used to be used primarily in diagnostic radiology for visualization. Once thorotrast is infused into the body, it is not eliminated easily. Over 90% of thorotrast forms deposits. It emits α-rays, and thorotrast-injected patients are semipermanently irradiated from α-particles deposited in the body. Thorotrast gives rise to malignant tumors by delayed effect of deposition, and especially liver cancer is highly associated with thorotrastosis. Deposition of thorotrast as an exogenous substance inhibits phagocytosis by suppressing the reticuloendothelial system (RES) as well and bringing about a Shwartzman preparatory state (pre-shock state) in patients. A majority of thorotrast-injected patients died abruptly. This short natural history of thorotrastosis is thought to have some relation to RES suppression by long-term thorotrast deposition resulting in vulnerability to shock and infection.

Key words: thorotrast, α-ray emitter; carcinogenesis; reticuloendothelial system

INTRODUCTION

With the use of radioactive imaging materials, the potential for internal injury, (i.e., internal irradiation) is always of concern. A radioactive substance known as thorotrast was used widely in Japan, Portugal, Germany, France and the United States from 1930 to 1955 as a contrast medium for X-ray photography, but was discontinued when it was discovered that it is difficult to excrete and that it forms long-term deposits in the body. There are two primary concerns for patients having undergone X-ray studies with thorotrast: one is the long-term deposition of exogenous substance and the other is chronic internal irradiation by an α-emitter. Malignant tumors, especially liver cancer, are highly associated with thorotrastosis. Most patients given thorotrast in the past, have died, and its effects have been virtually forgotten. It is important, however, to remember cases wherein accepted medical treatments have long-term adverse affects. This paper treats the character and process of thorotrastosis and its implications with regard to exogenous substances.

HISTORY OF THOROTRAST

Oka and Radt first used colloidal thorium dioxide (ThO2) as a radiologic contrast medium for hepatoliography in 1928 (43, 47). In 1930, another thorium dioxide preparation, thorotrast, was developed by the German firm Heyden and exported and used widely at military hospitals and other medical institutions, mainly for angi- and hepatoliography. Thorotrast provided good contrast with relatively low risk of allergic reaction and was widely utilized in western countries until 1955. This contrast agent remained in certain body tissues indefinitely and continued to emit α-rays.

Use of thorotrast continued in Japan until about 1954, and the number of persons injected with it is estimated at somewhere between 20,000 and 33,000. Thorotrast was used mainly in angiographies for wounded Japanese soldiers, most of whom were not suffering from disease (35, 38, 39).

DISTRIBUTION OF THOROTRAST IN THE BODY AFTER INFUSION

Once thorotrast is infused into the blood, it is not eliminated easily from the body; over 90% of thorotrast forms deposits (6, 15, 30, 32, 55). After injection as a contrast material into the blood, it circulates to the systemic organs, soon accumulating in the liver, spleen and lymph nodes (8, 18, 29, 32, 56, 57). Figure 1 is a plain abdominal X-ray photo showing numerous spots of a radiopaque substance on the liver, spleen and abdominal lymph nodes. This substance is thorotrast that distributed in the body 24 years after injection.

Autoradiography (Fig. 2) is the only method traditionally used to detect thorium dioxide in conventional histological specimens, but more recently it has been detected by X-ray analysis, which is quicker and more easily performed. We examined distribution of...
Thorotrast deposit in liver tissue is demonstrated by $\alpha$-tracks (HE staining × 400).

We found that 70Å thorotrast particles that enter the sinusoid of the liver are taken up by endocytosis of the hepatocyte and macrophage phagocytosis (Fig. 4). Particles ingested by hepatocytes are excreted into bile canaliculi through the phagosomes. Circulating particles aggregate, increasing in size to 150–200Å or more, which is too large for endocytosis and subsequent elimination via hepatocytes (13, 14, 19, 52). Though very little excretion is found in urine and the air passage (12, 51), more than 90% of $^{232}$Th introduced into the body is deposited in the reticuloendothelial system (RES), thus accumulating in the liver (71 to 73%), spleen (7–17%), and bone marrow (6–10%) (16). Although the term RES has been replaced by the term mononuclear phagocytic system (MPS), the former will be used here since it is the most familiar and has been used in previous thorotrast papers. Both refer to cells in the bone marrow, peripheral blood, and tissues that are highly specialized for endocytosis (pinicytosis, phagocytosis and intracellular digestion).

Thorotrast particles are phagocytosed by macro-
Fig. 3. Thorotrast particles in the human liver 25 years after injection. Numerous and aggregated thorotrast particles are present mainly in the portal triad (a) (U-Pb staining × 3,000); energy dispersion X-ray spectrum of thorium and X-ray mapping of thorium (a window to X-ray of 2.996 peak of thorium) in the same visual field (b); and energy disperse X-ray spectrum (lower).

Fig. 6. Thorotrast particles in a human spleen 20 years after injection (HE staining × 20). Inset is soft X-ray photograph. The spleen weighs 4 g.

Fig. 7. Thorotrast liver with cholangiocellular carcinoma (a) and its soft X-ray photograph (b). Arrowheads on Fig. 7a indicate the border of cholangiocellular carcinoma.

Fig. 8. Thorotrast liver with angiosarcoma (a), and its soft X-ray photograph (b).
phages or Kupffer cells and deposited in the portal area, sinusoid and subcapsular region of the liver several decades after injection. Hepatocytes have been generally thought to be free from thorotrast particles in the late period. However, small thorium particles have been found in the hepatocytes. Fibrosis of the liver is manifested several decades after the ingestion of thorotrast (Fig. 5). In the spleen, it deposits around the lymphatic duct, resulting in fibrosis and eventual shrinkage of the spleen due to repeated infarction or hemorrhage induced by circulatory disturbance. Marked atrophy of the spleen is one of the characteristics of thorotrastosis (24, 32, 53); a spleen weighing even less than 5 g was observed in an extreme case (Fig. 6) (45).

Thorotrast also deposits in the lymph nodes of the paraaortic region and mesenterium, causing the lymphofollicles to shrink. Thorotrast deposits in the bone marrow as well, but the extent of deposition is less severe than in other organs. After macrophages phagocytose thorotrast particles, their function, especially phagocytic activity, is thought to be suppressed. It may be that a compensatory mechanism such as increase of NK cells plays a role in immunity in place of compromised macrophage (1), and that aging and cancer development accelerate RES suppression over time. Patients are then more vulnerable to infection and develop malignant tumors more easily than do normal persons.

CARCINOGENESIS CAUSED BY THOROTRAST INJECTION

Thorium-232 belongs to the thorium chloric series, so if the α nuclear species is released, there is no influence unless it is taken into the body because the range of the α track is less than 100 μm. Once it is infused into the body, however, very little is excreted (its biological half-life is 200–400 years) (17). Thorotrast-injected patients are semipermanently irradiated from α-particles deposited in the body (total doses were 10–100 ml, 0.2–2 μCi, with a physiological half-life of 1.4 × 10^10 years) (27).

The relation between internal radiation exposure from α-particles and carcinogenesis came into question very early, especially in the U.S., when it was found that watchmakers exposed to the radium in luminous dials developed osteogenic sarcoma at a high rate.

Twice, in 1932 and 1937, a warning was issued by the American Medical Association to restrict the use of thorotrast (8, 9). A link between thorotrast and carcinogenesis was established in a rate study by Oberling and Guerin (42), followed by many reports of carcinogenesis in mice, hamsters, rabbits, guinea pigs and other animals (44, 49, 58). Bauer estimated the term required for carcinogenesis by thorotrast to be 12 to 18 years after infusion, based on research into human 226Ra exposure (3).

In 1947, MacMahon reported the first case of liver hemangiosarcoma 12 years after the ingestion of thorotrast (33). Subsequently, reports of hepatic carcinoma, leukemia, and liver cirrhosis related to cases of thorotrastosis appeared all over the world.

The author examined 300 autopsied cases, carried out during the 43 years from 1945 to 1987, listed by the Ministry of Education, Science and Culture. The autopsy cases consisted of 260 males and 40 females. The population study included civilians, though the majority were wounded soldiers. Thorotrast was injected intravascularly for diagnosis and the amount of thorotrast injected ranged from 5 to 182 ml (0.1–3.6 Ci). Table 1 shows the carcinomas that occurred in association with the 300 cases of thorotrastosis. The period between ingestion of thorotrast and death was a mean of 35 years. The total incidence of malignant hepatic tumors was approximately 87% (Figs. 7–9). Liver cancer, leukemia and lung cancer were observed quite frequently, but only a few cases of gastric cancer and colon cancer were found. Cholangiocellular carcinoma accounted for the greatest number (n = 102) of malignant tumors, but hepatic angiosarcoma (n = 48) was considered the most specific to thorotrastosis since angiosarcomas are very rare in the normal population (1 in 4,000,000 population) (5, 25, 46). Hematologic disorders such as leukemia and idiopathic acquired sideroblastic anemia were seen, but many were atypical (2, 26). It is thought that fibrosis of microhematogenic circumstance in addition to damage to multipotent stem cells by thorotrast deposition and α-ray emission complicated the pattern of disease. The development of cancer accelerated over about 20 years after thorotrast injection as shown in Fig. 10, and the incidence of multiple cancers (double cancers were 28 cases and triple cancers were 2 cases) increased with time (Table 1) (36–38).

Now cancer is known to result from DNA mutated in the course of DNA repair (54). When α-ray emission injures the chromosome, the cell dies if the damage is severe and cancer develops if the damage is not so severe and the cell lives on. Thorotrast has been clarified as carcinogenic to humans. This has become clear pathologically as well as epidemiologically. The total irradiation dose of thorotrast is a mean of 800 to 1,000 rads, much less than the two to several thousand
Table 1. Carcinomas associated with thorotrastosis.

<table>
<thead>
<tr>
<th>1st Cancer</th>
<th>CCC</th>
<th>HCC</th>
<th>AS</th>
<th>Gastric</th>
<th>Leukemia</th>
<th>Lung</th>
<th>MM</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCC</td>
<td>17</td>
<td>9</td>
<td>3*</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>260</td>
</tr>
<tr>
<td>HCC</td>
<td>14</td>
<td>8</td>
<td>4**</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>7</td>
<td>3*</td>
<td>4**</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gastric</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers shown are number of cancers out of 300 cases examined.
* Included one case of triple cancers (CCC+AS+HCC).
** Included one case of triple cancers (AS+HCC+Renal).

Abbreviations: CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma; AS, angiosarcoma; MH, multiple myeloma; Gastric, gastric cancer; Lung, lung cancer; Colon, colon cancer; Renal, renal cancer.

rads that the workers exposed to luminous paint experienced, which caused the development of osteogenic sarcoma. Though the irradiation dose is considered small, thorotrast injected as it has been in the past has proven to be very harmful. Decreased immune response with age may also influence the long-term potential for carcinogenesis in cases of thorotrastosis.

Although the carcinogenic effects of thorotrast appear to be due to chronic internal irradiation, we must not neglect the potential effect of long-term deposition of thorotrast particles as an exogenous substance. There is the example of zirconium hydroxide sol, a non-radioactive contrast media, causing cancer in animal experiments (4).

THOROTRAST LIVER FIBROSIS AND PORTAL HYPERTENSION

Some human diseases involve metals deposits in organs such as the liver, spleen and bone marrow; hemosiderosis (excessive iron deposition) and Wilson’s disease (excessive copper deposition) for example. These diseases lead to liver cirrhosis and, less commonly, are associated with liver cancer. The clinical symptoms and laboratory data for thorotrastosis several decades after injection of thorotrast look like those of post-necrotic liver cirrhosis. Pathohistologically, the liver shows fibrosis around the thorotrast particles in the portal triad, sinusoid and subcapsular region. The convex surface of the liver recalls post-necrotic liver cirrhosis by subcapsular fibrosis, but nodular regeneration on the cut surface is not conspicuous, thus, the condition is considered thorotrast fibrosis (Fig. 11) (23). The macrophages laden with thorotrast form thrombi in the sinusoid that disturb the intrahepatic circulation and accelerate liver damage by ischemia (Fig. 12) (20). Splenic atrophy is one of the characteristics of thorotrastosis, but there are a few cases of splenomegaly. Histologically,
the liver change resembles that in Banti’s disease (idiopathic portal hypertension) in which liver fibrosis, splenomegaly and esophageal varices are seen, except that esophageal varices of thorotrastosis are relatively few in comparison.

Portal blood flow is disturbed in the liver by post-necrotic liver cirrhosis or liver fibrosis, as in Banti’s disease, and the overflowing blood that cannot flow into the liver bypasses via the esophageal venous plexus to the superior caval vein. Thus, esophageal varices are
formed as collateral circulation (Fig. 13). The severity of esophageal varices is relatively mild compared with the degree of fibrosis in the thorotrastosis of the 300 autopsy cases. We examined 211 thorotrast-injected autopsy cases whose spleen weights were recorded and divided into four groups (A, B, C, D) according to spleen weight (Table 2). Group A (below 20 g of spleen weight) was frequently accompanied by marked liver atrophy (severe fibrosis). Approximately 10% showed splenomegaly. Particularly, splenomegaly exceeding over 200 g (Group D) was seen in 13 cases, 9 of which had marked esophageal varices. It may be that portal blood pressure was accelerated in cases of splenomegaly exceeding 200 g. Though the specific mechanism by which portal blood pressure is increased is unclear, it may result in part from increased flow from the splenic artery as well as increased intrahepatic vascular resistance in the portal vein.

Rupture of esophageal varices associated with portal hypertension is lethal in most cases. Most thorotrast-injected patients with splenomegaly died by rupture of the esophageal varices; in the cases studied, the time until death was 10 years shorter than in cases without splenomegaly (Table 2) (24).

Splenectomy decreases the portal pressure, which implies decreased blood flow into the esophageal varices. Splenic atrophy is a pathologic change associated with thorotrastosis, but it is possible that such atrophy works to prevent increased variceal blood flow as does splenectomy (24). On the other hand, hepatic failure was conspicuous in Group A.

**THOROTRASTOSIS AND DISSEMINATED INTRAVASCULAR COAGULATION**

Disseminated intravascular coagulation (DIC) is an acquired thrombohemorrhagic disorder that occurs as a complication in a variety of diseases. It is characterized by activation of a coagulation sequence that leads to the formation of microthrombi. Fibrinolytic mechanisms are activated as a result of thrombotic diathesis, and hemorrhagic diathesis results.

The Shwartzman reaction that develops in animals has its counterpart in human DIC (48). When an animal is sensitized by a subcutaneous injection of endotoxin, intravenous injection of the same antigen 24 hr later will induce minute thromboses at the site of the prior sensitization. When both sensitizing and challenge doses are given intravenously, a systemic Shwartzman reaction develops and generalized microthromboses appear, which cause various infarctions, particularly renal cortical necrosis (48, 50). The Shwartzman reaction follows a two-step process, first sensitization and then provocation. The first step is thought to cause suppression of the RES, and thorotrast injection has been shown in animals to suppress the RES and bring about the preparatory sensitization needed for the Shwartzman reaction (7, 10, 11, 28). Thorotrast-injected patients are thought to be in a kind of Shwartzman preparatory state due to chronic suppression of the RES following thorotrast administration.

Pseudomembranous colitis, fulminant hepatitis, and renal cortical necrosis have been attributed to the DIC or Shwartzman reaction (22, 34, 40, 41, 48). Phagocytes make a major contribution to the first line of host defense, but RES suppression disturbs the phagocyte function, making it difficult to engulf and destroy organisms. In thorotrast autopsy cases, petechiae, massive hemorrhage, microthrombosis, and necrosis of organs are frequently observed, and in the liver of thorotrast-injected patients, disruption of the sinusoidal lining is less frequently observed, as shown in Fig. 14. These findings, which were not observed in the liver biopsy specimens of living thorotrast-injected patients, suggest that severe circulatory disturbance like DIC or shock is provoked at the end stage of thorotrastosis (21, 59).

We observed 9 cases of clinically diagnosed fulminant hepatitis out of the 300 thorotrast autopsy cases (Table 3). Eight cases were associated with malignancy,
Table 3. Massive hepatic necrosis in thorotrast-injected autopsied cases.

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>SN-27</th>
<th>SN-51</th>
<th>SN-72</th>
<th>SN-82</th>
<th>SN-137</th>
<th>SN-152</th>
<th>SN-163</th>
<th>SN-178</th>
<th>SN-265</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (yr)*</td>
<td>65/M</td>
<td>49/M</td>
<td>65/M</td>
<td>52/M</td>
<td>60/M</td>
<td>70/M</td>
<td>67/M</td>
<td>62/M</td>
<td>74/M</td>
</tr>
<tr>
<td>NH (days)</td>
<td>10</td>
<td>40</td>
<td>9</td>
<td>?</td>
<td>50</td>
<td>120</td>
<td>150</td>
<td>270</td>
<td>75</td>
</tr>
<tr>
<td>Clin diag</td>
<td>FH</td>
<td>FH</td>
<td>A HF</td>
<td>?</td>
<td>A HF</td>
<td>FH</td>
<td>A HF</td>
<td>A HF</td>
<td>A HF</td>
</tr>
<tr>
<td>Path diag</td>
<td>MHN</td>
<td>MHN</td>
<td>MHN</td>
<td>MHN</td>
<td>MHN</td>
<td>MHN</td>
<td>MHN</td>
<td>MHN</td>
<td>MHN</td>
</tr>
<tr>
<td>Malig</td>
<td>-</td>
<td>CCC</td>
<td>HCC</td>
<td>AS</td>
<td>AS</td>
<td>AS</td>
<td>AS</td>
<td>AS</td>
<td>TCC</td>
</tr>
<tr>
<td>Ascites</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hepatic coma</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Bil (mg/dl)</td>
<td>15</td>
<td>12.6</td>
<td>25.6</td>
<td>49.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>GOT</td>
<td>785</td>
<td>849</td>
<td>469</td>
<td>65</td>
<td>1,936</td>
<td>1,936</td>
<td>1,936</td>
<td>1,936</td>
<td>1,936</td>
</tr>
<tr>
<td>GPT</td>
<td>425</td>
<td>572</td>
<td>1,220</td>
<td>2,780</td>
<td>1,600</td>
<td>1,325</td>
<td>1,730</td>
<td>2,210</td>
<td>1,750</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>5</td>
<td>145</td>
<td>50</td>
<td>20</td>
<td>40</td>
<td>25</td>
<td>13</td>
<td>90</td>
<td>22</td>
</tr>
<tr>
<td>Spleen (g)</td>
<td>1,220</td>
<td>2,780</td>
<td>1,600</td>
<td>1,325</td>
<td>1,730</td>
<td>2,210</td>
<td>1,750</td>
<td>2,600</td>
<td>2,200</td>
</tr>
</tbody>
</table>

* Period: Duration of life between injection of thorotrast and death.
NH: Natural history (period between onset of symptoms and death).
Abbreviations: Clin diag, clinical diagnosis; Path diag, pathological diagnosis; FH, fulminant hepatitis; A HF, acute hepatic failure; DIC, disseminated intravascular coagulation; MHN, massive hepatic necrosis; Malig, malignancy; CCC, cholangiocellular carcinoma; HCC, hepatocellular carcinoma; AS, angiosarcoma; TCC, transitional cell carcinoma.

Fig. 14. Rupture of the sinusoidal lining (arrows) of the liver of a thorotrast-injected patient (HE staining ×400).

especially angiosarcoma (5 cases). In pathohistological findings, the liver was swollen and centrilobular parenchymal necrosis was observed. It is thought that massive hepatic necrosis is provoked by endotoxin at the end stage of thorotrastosis due to RES suppression after long-term thorotrast deposition (22). The possibility exists that these hepatic necroses were produced by severe circulatory disturbance related to Shwartzman reaction in addition to chronic liver disease, so-called "thorotrast liver."

NATURAL HISTORY OF THOROTRASTOSIS

To study the effect of RES suppression by deposition of exogenous substance, we observed the natural history of the disease (period between the onset of clinical symptoms and death). The initial symptoms were nonspecific, such as general malaise, abdominal fullness and appetite loss. The main direct causes of death in cases of thorotrastosis were renal failure, hepatic failure, and gastrointestinal bleeding or DIC. Figure 15 shows the natural history. One-hundred-and-fifty of the 300 autopsy cases were selected and the time to death was calculated and compared with that of 100 persons who died from causes other than thorotrastosis; serial autopsy cases. Mean (± SD) time to death was significantly shorter in the thorotrast cases than in the serial autopsy cases (252 ± 407 days, 1,439 ± 2,415 days, re-
spectives). A majority of the thorotrast-injected patients died abruptly. The short natural history of thorotrastosis is thought have some relation to RES suppression by long-term thorotrast deposition (31) resulting in vulnerability to shock and infection.

**CONCLUSION**

The author worked in a certain hospital of Ibaraki Prefecture between 1977 and 1979, and encountered four thorotrast-injected patients (one woman and three men) from the same village. These patients were originally from Nagano Prefecture and emigrated in a group to the same settlement in Manchuria before World War II, where they were injected with thorotrast. The detailed history of the thorotrast injection in these cases was unclear. After the war, they returned to Japan and immigrated to the same village in Ibaraki Prefecture. Unfortunately, it is unclear how many persons injected with thorotrast were there in that village, because we have had no chance to check the total number of thorotrast-injected patients. If it was done, we would have some knowledge of thorotrast usage in Manchuria. Now, 20 years later, most of the thorotrast-injected patients have died. While their deaths are unfortunate, much is to be learned of the harm of internal irradiation and the effects that exogenous substances can have upon the RES over the long term. It is important, however, to remember cases wherein accepted medical treatments have long-term adverse affects.

**Acknowledgements.** The author gratefully acknowledges the effective and conscientious assistance of all the persons concerned.

**REFERENCES**


