# Quantitative and Morphological Studies on the Influence of Zinc Deficiency on the Liver of Pregnant Rats

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ABSTRACT. The effect of zinc deficiency on trace metals in the liver, spleen, kidney, pancreas and duodenum was investigated in the control and zinc-deficient rats at 17 days and 20 days of pregnancy. Zinc-deficient rats fell into limosis after 5 days of pregnancy. The contents of zinc, iron, copper and manganese in the maternal tissues were measured by colorimetry with 5NPPF. The morphological changes of the liver were observed by light and electron microscopy. The contents of zinc in the pancreas and duodenum were less in the zinc-deficient group than in the control at both 17 days and 20 days of pregnancy. The contents of the copper and manganese in the liver, kidney, pancreas, duodenum and spleen, however, were not significantly different from the control. The contents of iron in the liver, spleen and kidney in the deficient group increased greatly at 17 days and 20 days of pregnancy compared with the control group. The combination rate of transferrin with iron also increased significantly in the deficient group. Staining with Berlin blue or Turnbull's blue showed intense reaction to iron around the interlobular connective tissue of the liver in the zinc deficient group at 20 days of pregnancy. Ultrastructurally, the liver of the zinc-deficient rats showed the decrease of glycogen granules and increase of lipid-like granules and lysosomes with various sizes and electron densities. These findings suggest that zinc deficiency causes the increase of iron contents in the various organs during pregnancy, and that there is an intimate interrelationship between zinc and iron in the metabolism of iron during pregnancy.—KEY WORDS: iron content, pregnancy, rat liver, zinc deficiency.

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There have been so far published many reports on the various influence of the zinc deficiency on the fetuses during intrauterine life [5, 6, 15, 20, 24–26]. It is still unknown, however, how the zinc deficiency effects the pregnant animals to cause a congenital malformation in the fetuses. Since various trace metals interact within the body [4, 9, 17], it is necessary to gain the exact knowledge of quantity of each trace metal in each tissue to understand the effects of zinc deficiency in the interaction of trace metals in the pregnant animals. Therefore, the quantitative analysis was made in the present study on the trace metals in the liver, kidney, spleen, pancreas and duodenum of the pregnant rats under intact (control) or zinc-deficient condition to reveal the effects of zinc deficiency on the pregnant animals. The liver was further examined morphologically since it plays a very important role in the metabolism of trace metals [28].

## MATERIALS AND METHODS

Adult female rats of the Wistar strain weighing approximately 250 g were used in the present study. They were placed overnight with male rats of the same strain and were examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was detected was counted as 0 day of pregnancy. The pregnant females were then divided into 2 groups, each consisting of 10 rats; the control group was fed a commercial diet (Orientl Solid Stock including 50 ppm of zinc), and the deficient group was fed a

zinc-deficient diet (Oriental Pulverized Stock including 1.5 ppm of zinc), respectively, during pregnancy. Food and water were given *ad libitum*. Animals were sacrificed at 17 days or 20 days of pregnancy to obtain the liver, kidney, spleen, pancreas and duodenum for the assay of metalic elements. A part of the liver was processed for light and electron microscopy.

The contents of zinc, iron, copper and manganese were measured in the tissues above mentioned in both groups. Each tissue was pretreated with an acid mixture (sulfic acid: perchloric acid: nitric acid=1: 3: 3), and then processed for colorimetry with 1-(5-nitro-2-pyridy)-3, 5-diphenyl formazan (5NPPF) [27].

The combination rate of serum transferrin with iron was measured by the method of International Committee for Standardization in Human Hematology (ICSH) [16] in both groups.

For light microscopy, the liver was fixed in buffered formalin and embedded in paraffin by the routine procedure. Paraffin sections were stained with Berlin blue or Turnbull's blue to demonstrate iron and counterstained with carmin. For electron microscopy, a part of the liver was fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C for 1 hr, postfixed with 1% osmium tetroxide in the same buffer for 1 hr at 4°C and embedded in Polybed 812. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined with a JEM-100B electron microscope. Semithin sections were stained with Mallory's azur II-methylene blue [23] for light microscopy.

## RESULTS

*General findings:* In the present study, zinc-deficient rats completely lacked appetite and fell into limosis after 5 days of pregnancy. The body weight of zinc-deficient group decreased 14% and 40% at 10 days and 20 days of pregnancy, respectively, compared with the control group.

*Quantitative analysis:* The contents of zinc and iron in each tissue of both control and deficient groups were shown in Table 1. The content of zinc was significantly low in the pancreas and doudenum of the deficient group at both 17 days and 20 days of pregnancy in comparison with that in the control group, but showed no signifi cant difference in the other tissues of both groups. On the other hand, the content of iron was remarkably high in all the tissues except pancreas and duodenum of the deficient group at both 17 days and 20 days of pregnancy; especially, it amounted in the liver of the deficient group more than 3 times as much as that of the control group at 20 days of pregnancy. The contents of copper and manganese showed no significant difference in every tissue examined between control and deficient group at both 17 days and 20 days of pregnancy.

The combination rate of transferrin with iron was 53% and 53% in the control group, 93% and 95% in the deficient group, respectively, at 17 days and 20 days of pregnancy.

*Light microscopy:* The liver of the deficient group at 20 days of pregnancy showed a more intense reaction to Berlin blue or Turnbull's blue than that at 17 days of pregnancy to suggest the extensive accumulation of iron in the liver (Figs. 1, 2), while the liver of the control group showed only a slight reaction to these dyes at both 17 days and 20 days of pregnancy. In the semithin epon sections, the liver cells of the deficient group underwent metachromasia around the interlobular connective tissue and contained numerous sky-blue granules (Fig. 3). In addition, the space of Disse was rather expanded in this group. These histological findings tended to be more evident at 20 days of pregnancy than at 17 days.

*Electron microscopy:* The liver cells of the

Table 1. Zinc and iron contents in organs of rats fed control and zinc-deficient diets at 17 days and 20 days of pregnancy

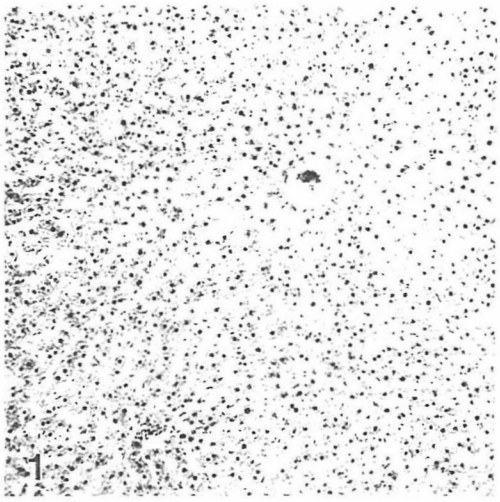
| Organs | Groups | Zinc | | Iron | |
|---|---|---|---|---|---|
| | | 17 | 20 | 17 | 20 (days) |
| | | | | | ($\mu$g/g wet) |
| Liver | Control | 31.0±2.0 | 30.0±1.2 | 131.0± 42.0 | 110.0± 40.0 |
| | Deficient | 30.0±3.0 | 32.0±1.4 | 350.0± 30.0* | 380.0± 60.0* |
| Kidney | Control | 29.0±1.2 | 29.0±4.0 | 63.0± 1.0 | 66.0± 9.4 |
| | Deficient | 24.0±2.0 | 22.0±1.0 | 71.0± 1.5* | 75.0± 6.0* |
| Spleen | Control | 20.0±0.8 | 20.0±0.4 | 490.0±180.0 | 600.0±130.0 |
| | Deficient | 20.4±0.5 | 21.2±1.7 | 750.0±210.0* | 970.0±260.0* |
| Pancreas | Control | 34.0±2.3 | 29.0±4.0 | 16.1± 7.0 | 19.0± 2.3 |
| | Deficient | 17.0±2.0 | 17.0±2.0* | 22.0± 2.0 | 20.0± 3.0 |
| Duodenum | Control | 26.0±6.0 | 25.0±2.0 | 76.0± 31.0 | 93.0± 20.0 |
| | Deficient | 19.3±1.0* | 19.0±2.0* | 59.0± 9.0 | 40.0± 14.0 |

Zinc contens in control and deficient diets were 50 ppm and 1.5 ppm, respectively. Each value represents M±SE for 10 rats.
* Significantly different from control group, $p<0.01$.

deficient group lost almost all of the glycogen granules at both 17 days and 20 days of pregnancy in striking contrast to control group (Figs. 4, 5). These liver cells contained extremely large lipid-like granules 0.6–2.8 $\mu$m in diameter (Fig. 6) and numerous lipid-like granules (Fig. 7) approximately 0.5 $\mu$m in diameter. The liver cells of the deficient group were further characterized by well-developed rough-(rER) and smooth-surfaced endoplasmic reticulum (sER), an increasing number of microbodies (Fig. 8) and free ribosomes (Figs. 6, 8) and the appearance of large vacuoles. The liver cells were divided into light and dark cells according to the composition of cell organelles. The Kupffer's stellate cells of the deficient group often contained electron-dense lysosomes (Fig. 9). The light liver cells tended to contain electron-dense lysosomes about 0.6 $\mu$m in diameter, while the dark liver cells often contained lipid-like granules 4 times larger in diameter than former ones (Fig. 10).

## DISCUSSION

It is generally accepted that zinc is essential for the synthesis of nucleic acids and protein [13]. In addition, with the recent progress of the quantitative analysis of zinc, it has been revealed that zinc is contained in or required by numerous enzymes that play important roles in the metabolism of hormones and other substances [8, 10, 22]. Therefore, zinc deficiency causes for one thing a marked depression of appetite and digestive disturbances in various species of mammals [1, 14, 19]. In the present study, zinc-deficient rats completely lacked appetite and fell into limosis. This seems to explain the decrease of glycogen granules in the liver cells of zinc-deficient rats, because glycogen granules are used as an energy source in case of malnutrition [11]. The development of rER and sER in the liver cells of the deficient group is similar to the findings of the liver cells observed in the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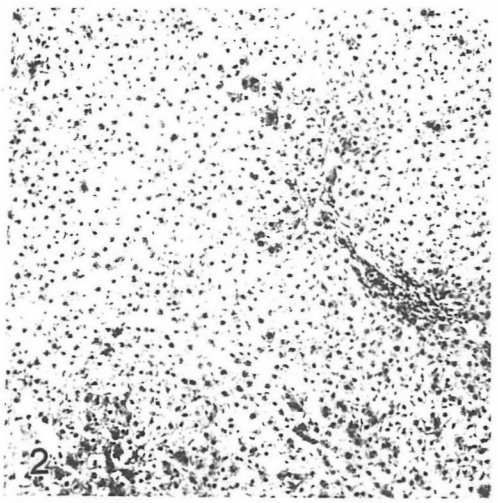
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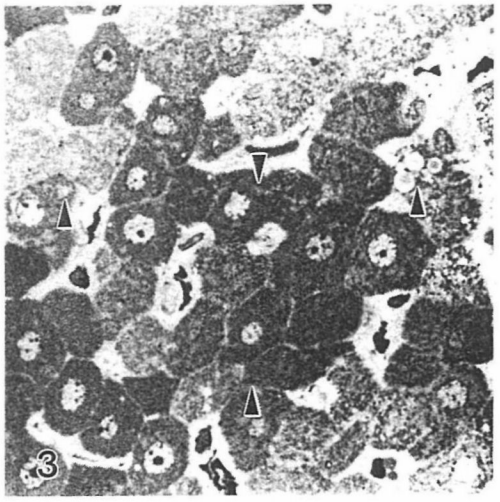
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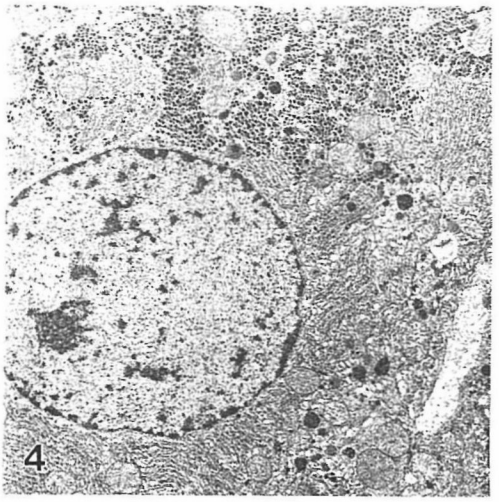
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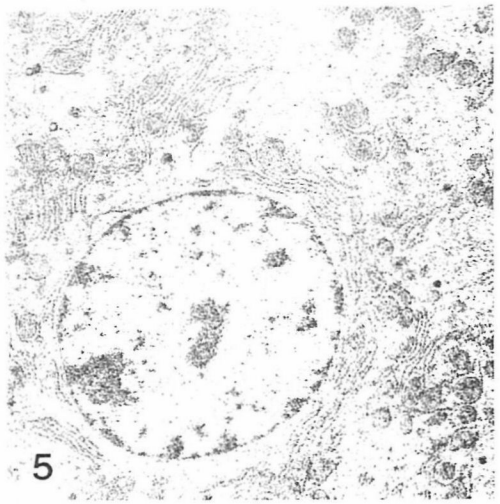
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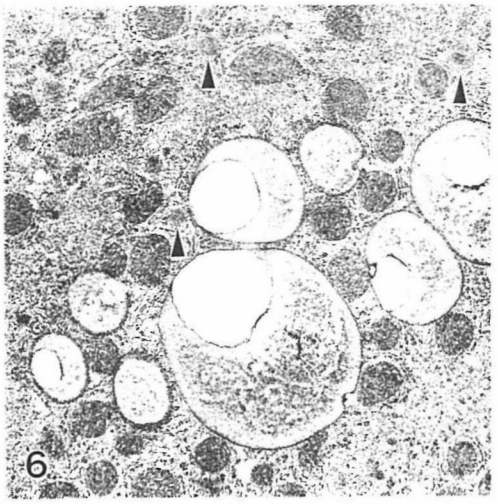
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intoxication of noxious substances such as carbon tetrachloride [21, 29], where the protein synthesis is suppressed [21]. Therefore, the present findings may also suggest the suppression of protein synthesis in the zinc-deficient group. The significance of the other ultrastructural changes such as appearance of electron-dense lysosomes and lipid-like granules is further to be investigated.

The influence of zinc deficiency on the interaction of trace metals was manifested in the present study as the alteration of iron contents in several tissues and of the combination rate of transferrin with iron. In the zinc-deficient group, the content of iron increased remarkably in the liver, kidney and spleen. On the contrary, the content of iron decreased in the duodenum where iron is absorbed [7]. While, the combination rate of transferrin with iron was 53% and 53% in the control group, 93% and 95% in the deficient group, respectively, at 17 days and 20 days of pregnancy. Although iron is present in relatively large quantities in the organism and of great significance as a component of hemoglobin, myoglobin and various enzymes [12], it is usually combined with transferrin in the serum [7, 18]. Then, it is supplied to the cell by the action of transferrin [28]. Since only one third of transferrin is combined with iron in the normal men [28], the remaining transferrin can combine with iron if the excess iron is added to the serum [2]. On the other hand, zinc is reported to protect the red blood cell membrane from the detrimental action of calcium [3]. In the zinc deficiency, therefore, iron seems to be unable to transfer to hemoglobin because of the detrimental action of calcium to the red blood cell membrane and left combined with transferrin in the serum. This may lead to the saturation of transferrin with iron. The remaining iron in the serum seems to be accumulated in the several tissues. The remarkably high combination rate of transferrin with iron in the deficient group of the present study suggests that transferrin is saturated with iron owing to zinc deficiency. The increase of iron contents in the liver, kidney and spleen of the present study also suggests that the excess iron is stored in these tissues because transferrin is already saturated with iron and cannot afford to combine with the excess iron. Since transferrin supply iron to the cell

Fig. 1. Light micrograph of hepatic lobules stained with Berlin blue at 20 days of pregnancy in control group. ×263.

Fig. 2. Light micrograph showing the intense reaction to Berlin blue around the interlobular connective tissue of hepatic lobules at 20 days of pregnancy in zinc-deficient group. ×263.

Fig. 3. Light micrograph of epon section showing numerous metachromatic sky-blue granules (arrow) in the liver cells around the interlobular connective tissue at 20 days of pregnancy in zinc-deficient group. Mallory's azur II-methylene blue ×1,800.

Fig. 4. Electron micrograph showing the formation of conspicuous rosettes by numerous glycogen granules in the liver cells at 20 days of pregnancy in control group. ×4,000.

Fig. 5. Electron micrograph showing the disappearance of glycogen granules in the liver cells at 17 days of pregnancy in zinc-dificient group. ×4,550.

Fig. 6. Electron micrograph showing the appearance of large lipid-like granules 0.6 to 2.8 $\mu$m in diameter, and increase of free ribosomes and microbodies (arrow) in the liver cells at 20 days of pregnancy in zinc-deficient group. ×9,200.

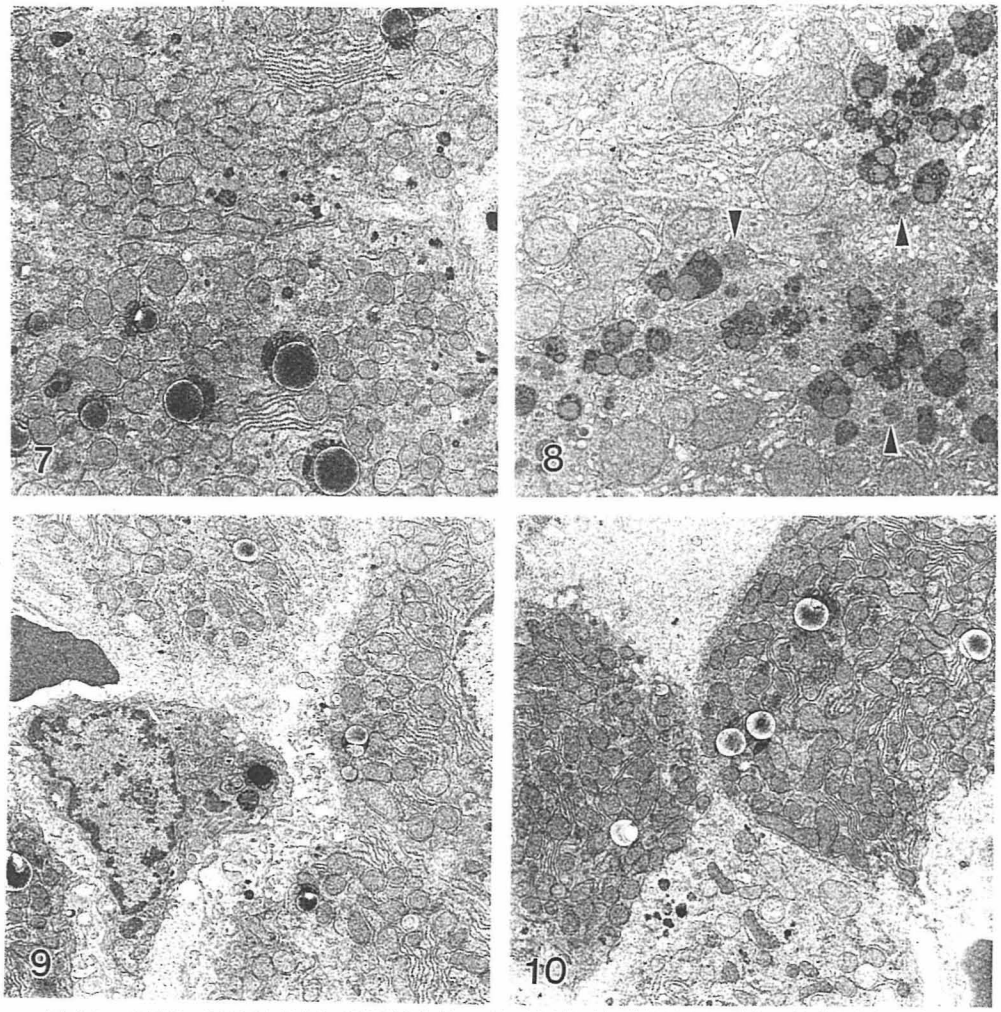


Fig. 7. Electron micrograph showing numerous lipid-like granules 1.4 $\mu$m in diameter in the liver cells at 20 days of pregnancy in zinc-deficient group. ×4,900.

Fig. 8. Electron micrograph showing well-developed rER and sER, lysosomes of various size and numerous microbodies (arrow) in the liver cells at 20 days of pregnancy in zinc-deficient group. ×8,500.

Fig. 9. Electron micrograph showing dense lysosomes in the Kupffer's stellate cells of the liver at 20 days of pregnancy in zinc-deficient group. ×5,000.

Fig. 10. Electron micrograph showing electron-dense lysosomes 0.6 $\mu$m in diameter in light cells and lipid-like granules 2.4 $\mu$m in diameter in dark cells of the liver at 20 days of pregnancy in zinc-deficient group. ×3,300.

[28], the accumulation of excess iron around the interlobular connective tissue, which is caused by zinc deficiency, may inhibit transferrin to supply iron to the liver cells and may also cause the saturation of transferr with iron.

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要　約

亜鉛欠乏の妊娠ラット肝臓における定量的および形態学的研究：萬場光一・谷口和之[1]・利部　聰・牧田登之（山口大学農学部家畜解剖学教室，[1]岩手大学農学部家畜解剖学教室）——亜鉛欠乏飼料を妊娠ラットに与えて，その肝臓，腎臓，脾臓，膵臓，および十二指腸の微量金属（亜鉛，鉄，銅，マンガン）の定量をおこない，代謝の中心的存在である肝臓の形態学的変化を検討した．その結果，亜鉛欠乏飼料飼育妊娠17および20日目のラットの各臓器内金属含有量の変化は，亜鉛は膵臓と十二指腸では低値を示したが，他の臓器では変化が認められなかった．銅，マンガンは各種臓器に有意差を示さなかった．しかし，亜鉛欠乏が鉄代謝に及ぼす影響は大で，欠乏飼料飼育の妊娠ラットのほとんどの臓器に高い鉄含有量がみられた．特に肝臓に鉄の異常増加が見られた．光顕的に肝臓ではベルリン青やターンブル青染色により，小葉間結合織の周囲の肝細胞に鉄の強い陽性反応が観察された．また，肝臓の電顕像では，グリコーゲンの減少や大小さまざまな，電子密度の異なる多数のライソゾームや脂肪様顆粒が観察された．これらのことから妊娠のラットの亜鉛欠乏はラットの飢餓状態をまねき，ヘモグロビンと鉄の結合を阻害することにより血清鉄を増加させ，肝臓と各種臓器における鉄の異常増加をもたらし，それらが肝臓の超微構造に変化を与えたものと考えられた．以上の所見より，亜鉛欠乏は各種臓器に鉄の増加を招来し，この亜鉛と鉄の間に強い関係のあることが示唆された．