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肝臓のクッパー細胞に対するビタミンCの影響

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The Effect of vitamin C on Kupffer cells in liver tissue

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Summary

This study examined the effect of vitamin C deficiency on guinea pig Kupffer cells, which are in the liver sinusoid and are members of the mononuclear phagocytic system. The experimentally deficient group was fed a vitamin C-deficient diet (vitamin C 12 mg per 100 g diet) and the control group the laboratory chow (vitamin C 60 mg per 100 g diet). To measure the peroxidase activity in Kupffer cells, the frozen liver sections were incubated in a solution containing DAB (3,3'-diaminobenzidine) and H_2O_2. Kupffer cells showing a DAB positive reaction were counted under a light microscope. The number of Kupffer cells decreased markedly in vitamin C-deficient liver compared with normal controls in a histochemical study. Decreased ability to cope with foreign materials and poor immune activity due to vitamin C deficiency are discussed.

KEY WORDS Kupffer cell, liver, vitamin C, peroxidase

Vitamin C, a water-soluble vitamin, has various important biological functions, such as playing a role in collagen formation [1-4], and deoxidation associated with glutathione or cytochrome c. Man, monkey and guinea pig can not synthesize vitamin C *in vivo* because they lack l-gulonolactone oxidase which catalyzes the terminal reaction in the biosynthesis of vitamin C[5]. Deficiency of this vitamin causes scurvy but clinical report of this disease are now rare in Japan.

Recent interest in vitamin C has developed due to its pharmacological use in mega doses. When vitamin C is given to animals deficient in the vitamin, its level in the leukocytes [6, 7, 15] rises and increased immunity of the organisms[8] has been found, e.g., chemotaxis of leukocytes [9, 10, 11], blast-formation of lymphocytes[12, 13], and manifestation of IgM and IgG receptors on T-lymphocytes[14, 16] increased.

Lymphocytes, leukocytes and macrophages belong to the mononuclear phagocytic system and display active phagocytosis for foreign materials. Kupffer cells in the liver sinusoid are also members of the mononuclear phagocytic system[17]. They can remove foreign materials and the wasted erythrocytes in the portal blood flow.

Kuppfer cells adhere to the sinusoidal wall[18]. *In vivo*, they can change their form under various conditions, and many pseudopodia on their surface catch foreign materials or the wasted erythrocytes.

This paper describes the effect of vitamin C deficiency on Kupffer cells. The difference in the number of Kupffer cells between the control and the vitamin C deficient guinea pig liver was studied histochemically.

METHODS

Animals Male guinea pigs weighing 200 to 250 g were divided into experimental and control groups. The experimental group was fed vitamin C deficient diet which contained only 12 mg of vitamin C per 100 g of the diet. The control group fed the laboratory chow containing vitamin C 60 mg per 100 g diet. Each group was consisted with six guinea pigs. The degree of vitamin C deficiency was determined by measuring the plasma concentration of vitamin C of the above groups

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after feeding on the diet for 2, 3 and 4 weeks. The level of vitamin C in the liver, spleen, adrenal gland and kidney were determined in the organs removed after the guinea pigs had been anesthetized with nembutal.

Histochemical detection of Kupffer cells The peroxidase activity in hepatic tissue was determined according to the method of Fahimi[19]. A guinea pig was anesthetized with nembutal, its liver was perfused with 0.9% NaCl solution through the portal vein for 3 minutes, then removed, cut into small blocks and frozen for 20 minutes at −40°C. The frozen tissue was cut with cryostat into 8 μm thin sections at −20°C. To measure the peroxidase activity in Kupffer cells, the frozen sections were incubated in a solution containing 2.5 mg DAB (3,3'-diaminobenzidine, Sigma Chemical Co.) in 100 ml of 50 mM Tris-HCl buffer with 0.1 ml of 5 % H_2O_2. The incubation was performed at 25°C for 30 minutes. Next, the sections were fixed with 2% OsO_4 solution and stained with hematoxylin. Kupffer cells showing the DAB positive reaction were countered under a light microscope in 6 sections selected at random and 8 fields of each section were observed. The number of Kupffer cells per mm^2 was then calculated.

RESULTS

When guinea pigs were fed on the vitamin C deficient diet for more than 2 weeks, their body weights decreased markedly compared with the control. After 3 weeks, some guinea pigs showed abnormal walking syndrome and after 4 weeks, some died due to vitamin C deficiency.

Wet weight of organs The weight of liver and spleen of the vitamin C deficient guinea pigs were about half that of the respective control organs. The relative weights per 100 g body weight of the organs of the deficient animals were significantly lower than those of the control. However, no significant difference was found in weights of the kidney and adrenal glands between the two groups, and the relative weights per 100 g body weight of the organs were even heavier than the controls (Table 1).

Vitamin C concentration of liver and plasma Table 2 shows the vitamin C concentration of the liver and plasma. In the control guinea pigs, the vitamin C concentration of the liver was about 30 mg%, while the level in the vitamin C deficient guinea pigs was too low to be detected. In the plasma, the vitamin C concentration of the control group was 0.7 mg% and that in the vitamin C deficient guinea pig was too low to determine. Thus, vitamin C deficiency could be produced within 3 weeks from the beginning of feeding on a vitamin C deficient diet.

Table 1

Body weights and weights of organs (g)

	Control	Vitamin C deficient
Body weight	316.3 ± 1.79	258.9 ± 21.3*
Liver weight	17.95 ± 1.44	9.34 ± 1.43**
Spleen weight	0.46 ± 0.03	0.27 ± 0.04**
Adrenal weight	0.18 ± 0.03	0.18 ± 0.03
Kidney weight	2.84 ± 0.08	2.74 ± 0.19
	g/100 g body weight	
Liver	5.50 ± 0.50	3.59 ± 0.44*
Spleen	0.14 ± 0.01	0.10 ± 0.02
Adrenal	0.05 ± 0.01	0.07 ± 0.01
Kidney	0.85 ± 0.07	1.07 ± 0.10

The values are expressed in mean ± SEM. of six guinea pigs. Significantly different from the control (*$p < 0.05$, **$p < 0.01$).

Table 2

Concentrations of vitamin C in plasma and liver (mg%)

	Plasma	Liver
Control	0.68 ± 0.05	27.79 ± 1.22
Vitamin C deficient	0	0

The values are expressed in mean ± SEM. of six guinea pigs. The guinea pigs were fed on either the vitamin C deficient diet or the laboratory chows (control) for three weeks.

Peroxidase acittivity in Kupffer cells Frozen sections of the guinea pig liver were stained with hematoxylin (Fig. 1) and Kupffer cells were identified. To find sinusoidal cells, the DAB reaction was examined. Kupffer cells were stained brown by the method, in the frozen sections (Fig. 2). Fig. 3 shows a frozen section which had been treated with DAB solution and stained with hematoxylin to clarify the border between cells. There were a number of Kupffer cells in the sinusoids. Fig. 4 shows a frozen section of the liver from the vitamin C deficient guinea pigs treated with the DAB reaction. The pictures on the vitamin C deficient liver were compared with those on the control liver (Fig. 3, 5, 7: control group, Fig. 4, 6, 8: the vitamin C deficient group). Six specimens were selected

at random from both the control and vitamin C deficient livers. The number of Kupffer cells in eight visual fields were counted under a light microscope. The area of each field was measured to estimate the cell number per mm^2 in the section (Table 3). The average number

Table 3
Numbers of Kupffer cells

	No. Area (A)	Number (N)	N/A
Control	1. 7.8 mm^2	815	103.3/mm^2
	2. 7.6	854	112.4
	3. 7.8	905	116
	4. 8.2	947	115.4
	5. 8.3	952	115.2
	6. 8.7	819	94.3
	Mean ± SEM (n=48)		109.5 ± 3.6
Vitamin C deficient	1. 9.2	374	40.5
	2. 9.9	464	46.7
	3. 9.7	369	38.2
	4. 9.7	357	37.0
	5. 9.4	393	41.6
	6. 9.6	309	32.1
	Mean ± SEM (n=48)		39.3 ± 2.2**

Significantly different from the control (**$p<0.01$).

of Kupffer cells was calculated to be 110 per mm^2 in the section of the control guinea pigs. The average number of Kupffer cells was 40 per mm^2 in vitamin C deficient guinea pig liver.

DISCUSSION

Vitamin C is known to be associated with synthesis of collagen *in vitro*, but its physiological effect is not clear. Much recent work has concerned the role of vitamin C *in vivo*, espacially when mega doses has been shown to promote the synthesis of interferon[20, 21] and to affect the serum cholesterol level[22]. These studies suggest that vitamin C has some relation to the immune system because it is distributed in leukocytes where its consumption is increased in infected patients. In culture medium leukocytes locomotion[9, 11] and blast-formation of lymphocytes[12, 13] are increased by the addition of vitamin C.

Polymorphonucleic leukocytes are members of the mononuclear phagocytic system, which includes marcophages, and display energetic phagocytosis. Kupffer cells adhering to the liver sinusoidal wall, which destroy foreign materials from the portal blood by phagocytosis are considered to be members of the monocuclear phagocytic system[17]. Kupffer cells seem to not only display phagocytosis but also to affect the systematic immune response in the whole body by inducing synthesis of complement and antibody[23] and blast-formation of lymphocytes. Foreign materials absorbed from the small intestine via portal blood into the liver may be trapped by Kupffer cells in the liver sinusoid. Interestingly, Kupffer cells are the first line of defense decrease markedly in the vitamin C deficient guinea pig liver. Only 40 per mm^2 of the liver tissue was present, while the control guinea pig tissue contained 100 per mm^2. This was approximately 36% of the control. As the liver weight of vitamin C deficient guinea pig decreased to half of the contorl value, the decrease in Kupffer cell number is estimated to be approximately 19% for the vitamin C deficient liver. Vitamin C deficient guinea pigs have been reported to have ability to cope with foreign materials and poor immune system activity. Today scurvy is almost nil in Japan, but resistance to various disease may be attenuated vitamin C shortage or deficiency.

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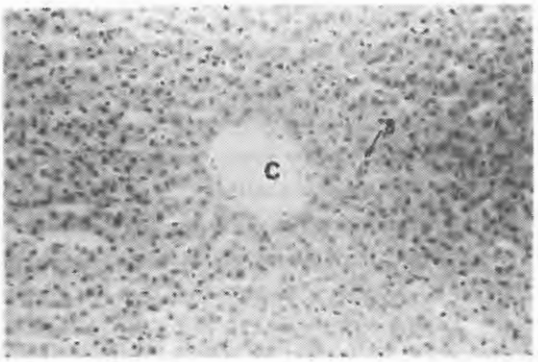


Fig. 1

Fig. 2

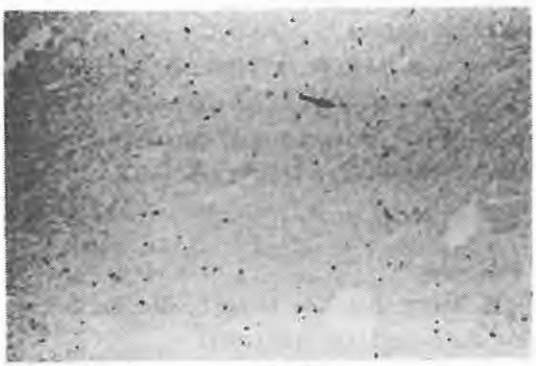
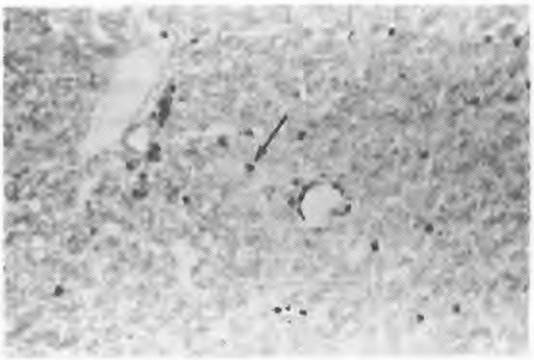


Fig. 3

Fig. 4



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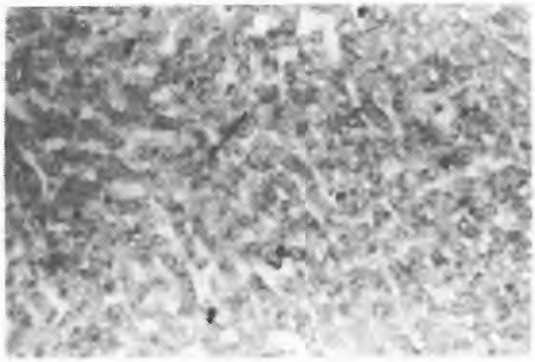

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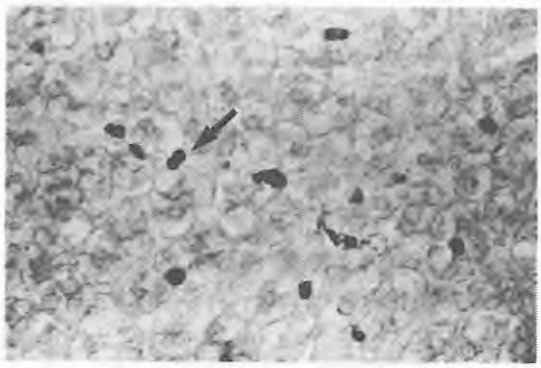

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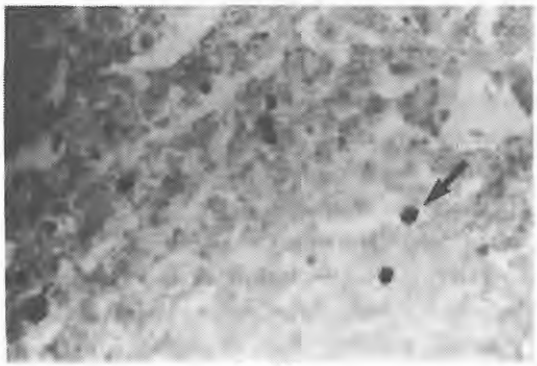

Fig. 8

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和 文 要 約

本研究は，ビタミンC投与量とKupffer細胞（肝臓の類洞に存在するmononuclear phagocytic systemのメンバー）の数との相関関係をしらべ，ビタミンCの効用

Figures legend

Fig. 1 Liver tissue stained with hematoxylin.
S: Sinusoid, C: Central vein
Purple points are the nuclei of hepatocytes or other cells (Kupffer cells, endothelial cells and other sinusoidal cells).
Magnification is × 200.

Fig. 2 Liver tissue treated with DAB (3,3′-diaminobenzidine) reaction.
Brown points (arrow) are Kupffer cells.
Magnification is × 300.

Fig. 3 DAB reaction and hematoxylin stain (control guinea pigs.).
Brown points (arrow) scattered in the liver tissue along the sinusoid.
Magnification is × 100.

Fig. 4 DAB reaction and hematoxylin stain (vitamin C-dificient guinea pigs).
Brown point (arrow) show Kupffer cells. Fewer Kupffer cells were found in the vitamin C-deficient guinea pigs than in the control guinea pigs (Fig. 3).
Magnification is × 100.

Fig. 5 DAB reaction and hematoxylin stain (control guinea pigs).
Brown points (arrow) in sinusoids are Kupffer cells.
Magnification is × 200.

Fig. 6 DAB reaction and hematoxylin stain (vitamin C-deficient guinea pigs).
Brown points (arrow) are Kupffer cells. Fewer Kupffer cells are present than in the control sample (Fig. 5).
Magnification is × 300.

Fig. 7 DAB reaction and hematoxylin stain (control guinea pigs). Kupffer cells are clearly stained brown (arrow).
Magnification is × 400.

Fig. 8 DAB reaction and hematoxylin stain (vitamin C-dificient guinea pigs).
Brown points (arrow) are Kupffer cells.
Magnification is × 400.

を生体の免疫系との関係で観察した。

250g前後のモルモットを (1)実験群；ビタミンC欠乏飼料（12㎎ビタミンC／100g）で飼育と (2)対照群；固型飼料（60㎎ビタミンC／100g）で飼育に分け，それぞれ3週間飼育したのちに肝臓の凍結切片を作製した。Kupffer細胞のperoxidase活性を検出するために，肝臓の凍結切片をDAB（3，3'－Diaminobenzidine）とH_2O_2を含有した溶液中で反応させ，DAB反応陽性を示すKupffer細胞を光学顕微鏡下において計測した。

ビタミンC欠乏飼料で飼育したモルモット肝臓のKupffer細胞の数は，対照のモルモットにくらべて，顕著に減少した。

生体のビタミンCが欠乏すると，生体外異物を補足する能力が減少し，免疫能が低下することを考察した。