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Histochemical and Ultrastructural Studies of Hepatic Fibrogenesis; Its Initiation and the Effect of Dexamethasone in Rats

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Intralobular fibrogenesis of the liver following hepatocellular damage has long been a controversial subject in regard to its initiation and prevention. To investigate the site of hepatic fibrogenesis and the effect of glucocorticoids during the early stage of hepatic fibrogenesis, dexamethasone was administered in a daily dose of 8 mg/rat following a single dose of CCl_4 to induce hepatic necrosis with or without combination of vitamin A to stimulate lipocytes. Light and electron microscopic examinations of the liver at 2, 4, 6, 8 and 10 days after CCl_4, vitamin A and dexamethasone treatment demonstrated that hepatocellular damage stimulated perisinusoidal lipocytes which in turn actively produced collagens. Concomitant administration of vitamin A enhanced stimulation of lipocytic activity and consequently increased collagen formation, while administration of dexamethasone suppressed lipocytic activity leading to an inhibition of collagen formation.

Key Words : Liver, Hepatic fibrosis, Hepatic fibrogenesis

The most undesirable result of hepatic injury is fibrosis, which is usually irreversible and progressive by replacing normal hepatic parenchyme until finally resulting in hepatic failure.

The genesis of intralobular hepatic fibrosis has long been controversial and two theories have been advanced. Hartroft(1950), and Hartroft and Ridout(1951) suggested that intralobular fibrosis results from condensation of preexisting reticulin fibers due to collapse of hepatic parenchyme. Meader(1963), on the other hand, suggested that fibroblasts are newly generated within the lobule and actively produce collagens leading to hepatic fibrosis.

Since there is not only condensation of reticulin but also active collagen production in hepatic fibrosis, Meader's (1963) suggestion has been considered more plausible. However, for many years, fibroblasts were not recognized within the hepatic lobules until the recent discovery of perisinusoidal lipocytes in the space of Disse by histochemical and electron microsco-

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pic observations (Ito and Nemoto, 1952; Bronfenmajor *et al*, 1966; Ito, 1973).

Popper and Udenfriend(1970) and Kobayashi *et. al* (1973) reported that excessive vitamin A administration activates lipocytes and transforms them into fibroblast-like cells resulting in collagen formation. Thus, they considered perisinusoidal lipocytes as the precursors of fibroblasts.

Although several drugs and chemicals; such as histamine (Dabrowski and Maslinski, 1970), amino-acetonitrile (Fiume, 1962) and penicillamine(Nimni *et al*, 1967; Klein and Nowacek, 1969) have been used to try and suppress hepatic fibrogenesis, the best drug known to have actual merit is corticosteroid(Hutterer *et al*, 1962). Hepatic injury leading to diffuse hepatic fibrosis can easily and regularly be induced experimentally by various chemicals, among which CCl_4 is most widely used(Aterman, 1954; Reddy *et al*, 1962; Rubin, 1963; Reuber, 1968).

Despite the accumulating evidence that lipocytes play the key role in hepatic fibrogenesis, clear cut documentation is still insufficient. Therefore, the present investigation is undertaken to elucidate the site of intralobular hepatic fibrogenesis following hepatic injury, and the effect of glucocorticoid during the early phase of fibrogenesis to explore the possibility of preventing hepatic fibrosis.

MATERIALS AND METHODS

A total of 90 male rats, weighing about 200 grams, were divided into two major groups: the control and the experimental. The control group (group I) was subdivided into salad oil only (I-A), vitamin A only (I-B), and dexamethasone only (I-C) treated groups. The experimental group(group II) was also divided into CCl_4 alone(II-A), CCl_4 with dexamethasone (II-B) and CCl_4 with vitamin A and dexamethasone(III-C) treated groups. Each subgroup consisted of 15 animals. All animals had been conditioned for 10 days prior to initiation of the experiment with balanced laboratory chew and water ad libitum. Salad oil was administered in a dose of 2 ml/rat per os, vitamin A 100,000 IU/rat orally, and dexamethasone 8 mg/rat intramuscularly once every day, and CCl_4 was administered in a single dose of 2 ml/rat intramuscularly. Three animals of each group were sacrificed at the 2nd, 4th, 6th, 8th and the 10th day of treatment. Under ether anesthesia, a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.15 mol phosphate buffer (pH 7.4) was infused via the portal vein, at a rate of 10 ml per minute for three minutes. For the light microscopic examination, pieces of liver were fixed in 10% neutral formalin and embedded in paraffin. For the evaluation of routine morphologic alterations, hematoxylin-eosin staining was applied to all sections. For the evaluation of fibrogenesis, Masson's trichrome method to demonstrate young collagen and Gomori's method to demonstrate reticulin were applied to each section. For the identification and evaluation of lipocytic activity, oil red-O staining was performed on frozen sections from formalin fixed tissue and gold impregnation by the method of Wake(1971). For the electron microscopic examination, perfusion fixed liver tissue was minced into 1 mm^3 size and fixed in 1% OsO_4 in phosphate buffer(pH 7.4) for two hours, followed by dehydration through graded alcohol and embedding in Epon 812. Ultra-thin sections were made with a glass knife and stained with uranyl acetate and lead citrate, and periodic acid methenamine silver (PAM) stain for the demonstration of collagen fibrils. Observations were made with a Hitachi H-500 model electron microscope, especially on the ultrastructural features of lipocytic activity.

RESULTS

1) Light microscopic findings:

The liver of animals treated with salad oil only showed scant numbers of lipocytes in the perisinusoidal space of Disse, particularly at the angle of adjoining hepatic parenchymal cells without apparent perisinusoidal collagen formation. There was no necrosis or inflammatory reaction.

In animals treated with vitamin A alone, neither liver cell necrosis nor inflammatory cell infiltration were noted. However lipocytes began to increase in numbers from the 2nd day of treatment, which became more marked on the 4th and 6th days with the appearance of small amounts of perisinusoidal collagen. These lipocytes reacted positively to oil red-O, PAS and gold impregnation.

In animals treated with dexamethasone only, no increase in the number of lipocytes or evidence of perisinusoidal collagen formation was noted.

The animals treated with CCl_4 alone showed apparent liver cell necrosis on the 2nd and 4th days accompanied by inflammatory infiltration. The liver cells began to regenerate after the 6th day of treatment and to restore the normal architecture without notable necrosis on the 10th day. Lipocytes began to increase in number after the 2nd day along with necrosis and infiltration of inflammatory cells. This was followed by a decrease as the regeneration of the liver cells began. Perisinusoidal collagen formation was accompanied by lipocytic proliferation at the areas of parenchymal damage.

The animals treated with CCl_4 together with vitamin A showed more pronounced lipocytic activity than in animals treated with CCl_4 alone.

The animals treated with dexamethasone following CCl_4 and vitamin A administration showed also some parenchymal necrosis, lipocytic proliferation and perisinusoidal collagen formation but much less than those seen in animals treated with CCl_4 and vitamin A only.

These light microscopic findings are summarized in table 1.

2) Electron microscopic findings:

The lipocytes were located in the space of Disse at the angle of adjoining hepatocytes as relatively small and scantily vacuolated cells with moderate amounts of RER. The lipocytes in the liver of animals treated with salad oil only showed inactive forms as evidenced by a small amount of cytoplasm, only a few lipid droplets, small amounts of RER, and poorly developed cytoplasmic processes. PAM staining revealed no demonstrable intracytoplasmic collagen formation. A minimal amount of perisinusoidal collagen fibers were observed, but they were located apart from lipocytes without direct connection. No subendothelial basement membrance formation was observed.

In the animals treated with vitamin A only, lipocytes were activated as evidenced by increase of cytoplasm accompanied by increase and prolongation of cytoplasmic processes, increase in lipid droplets, and increase and activation of RER. These morphologically transformed cells mimicked fibroblasts. PAM staining demonstrated the presence of collagen substance within the cytoplasm and collagen fibers in the sinusoidal space attached or connected to the surface membrane of activated lipocytes. No subendothelial basement membrane formation, however, was noted.

The animals treated with dexamethasone alone did not show evidence of lipocytic activation.

The animals treated with CCl_4 alone demonstrated prominent proliferation and activation of lipocytes, more pronounced than those seen in animals treated with vitamin A only. Fibroblas-

Table 1. Light microscopic findings

Groups		Days	Findings				
			Liver cell necrosis	Infl. cell infiltration	Lipocyte proliferation	Perisinus collagen formation	Cental vein sclerosis
Group I (Control)	I-A (Salad oil)		−	−	+	−	−
	I-B (Vit. A)	2	−	−	++	+	−
		4	−	−	+++	++	+
		6	−	−	+++	++	+
		8	−	−	++	+	+
		10	−	−	++	+	+
	I-C (Dexamethasone)	2	−	−	+	−	−
		4	−	−	+	−	−
		6	−	−	+	−	−
		8	−	−	+	−	−
		10	−	−	+	−	−
Group II (Experimental)	II-A (CCl_4)	2	+++	++	++	+	+
		4	+++	++	+++	+++	++
		6	+	+	++	++	+
		8	−	−	++	++	+
		10	−	−	+	+	−
	II-B (CCl_4+vit. A)	2	+++	+++	+++	+	+
		4	+++	+++	+++	+++	+
		6	++	+	++	++	+
		8	+	+	++	++	+
		10	−	−	++	++	+
	II-C (CCl_4+vit. A+ dexamethasone)	2	++	+	++	−	+
		4	+	+	++	+	+
		6	−	−	++	−	−
		8	−	−	+	−	−
		10	−	−	+	−	−

−:negative, +: mild, ++: moderate, +++: severe.

tic transformation was more pronounced, and there was a large amount of intracytoplasmic and perisinusoidal collagen formation. A newly formed basement membrane was observed subendothelially from the 2nd day after CCl_4 treatment.

The animals treated additionally with vitamin A after CCl_4 administration showed evidence of enhanced lipocyte activation.

The animals treated with dexamethasone together with vitamin A after CCl_4 administration showed evidence of suppression of lipocytic

Table 2. Electron microscopic findings

Groups		Days	Findings				
			Lipocytes			Others	
			Activation	Fat vacuoles	RER	Collagen	BM
Group I (Control)	I-B (Salad oil)		−	+	+	±	−
	I-B (Vit. A)	2	+	++	++	+	−
		4	+++	+++	+++	+++	−
		6	+++	+++	++	++	−
		8	++	++	++	++	−
	I-C (Dexamethasone)	2	−	±	+	−	−
		4	+	++	+	±	−
		6	+	+	+	±	−
		8	±	+	+	−	−
		10	±	+	+	−	−
Group II (Experimental)	II-A (CCl_4)	2	++	++	++	+	+
		4	+++	+++	+++	+++	+
		6	++	++	++	++	±
		8	++	++	++	+	−
		10	+	+	++	+	−
	II-B (CC_4+vit. A)	2	++	++	++	++	+
		4	+++	+++	+++	+++	+
		6	+++	+++	+++	+++	+
		8	++	++	++	++	−
		10	††	++	++	+	−
	II-C (CCl_4+vit. A+ dexamethasone)	2	++	++	++	±	−
		4	++	++	+	±	−
		6	+	++	+	±	−
		8	+	++	±	+	−
		10	±	+	±	±	−

−: negative, ±: minimal, +: mild, ++: moderate, +++: severe.
BM: basement membrane.

activation in comparison to animals treated with CCl_4 alone or CCl_4 and vitamin A. Lipocytes in this group were smaller in size, less vacuolated, with lesser cytoplasmic processes, and especially markedly suppressed collagen formation both within and outside of lipocytes. The electron microscopic findings are summarized in table 2.

DISCUSSION

The theory that collagen fiber in the hepatic lobule is made by condensation of reticulin

fiber at the site of hepatic cell necrosis resulted from and ignorance about the presence of fibroblasts in the hepatic lobule in the past, because most studies had been carried out only with the light microscope (Popper and Udenfriend, 1970). However, electron microscopic examination and autoradiographic studies have disclosed presence of fibroblast-like cells in the hepatic lobule (Popper *et al*, 1961; Patrick and Kennedy, 1964; Stenger, 1965).

Ito (1951) first described new cells containing numerous cytoplasmic fat droplets in the perisinusoidal space, the space of Disse, and called them fat storing cells. Since then many studies were carried out on these cells and various names such as lipocytes (Bronfenmajor *et al*, 1966), adventitious connective tissue cells (Schnack *et al*, 1967), Sternzellen (Wake, 1971), Ito cells (Yamamoto, 1975), and perisinusoidal cells (Wisse, 1970) were given. These cells are indigenous to the hepatic lobule. Popper and Udenfriend (1970) considered them to be precursors of intralobular fibroblasts, containing not only well developed rough endoplasmic reticulum and Golgi complex in the cytoplasm as those in the connective tissue, but also numerous fat droplets. These lipocytes store vitamin A and are different from Kupffer cells or endothelial cells in that they are in the space of Disse or between the liver parenchymal cells, and have neither phagocytic activity (Bronfenmajor *et al*, 1966; Kobayashi, 1973), nor alkaline phosphatase, acid phosphatase, and elastase activities (Tanaka *et al*, 1980). When containing excessive vitamin A in the cytoplasm, lipocytes have been proven to be activated and form collagen fiber, resulting in liver cirrhosis (Popper and Udenfriend, 1970; Yamamoto, 1975; Kent *et al*, 1976; Yamamoto *et al*, 1978). On electron microscopic examinations, these cells contain well developed RER in which flocculent material exists. The flocculent material exhibits the same stainability with periodic acid methenamine silver as extracellular flocculent material between the collagen fibrils which is considered to be the precursor of collagen fibrils, protocollagen (Kawanami, 1973).

Vitamin A is known to have unstabilizing effects on lysosomal membrane and can induce liver cirrhosis by destroying lysosome and mitochondria when treated excessively in experimental animals (Nieman and Klein, 1954; Dingle *et al*, 1962; Muenter *et al*, 1971). Ehrlich *et al* (1973) observed dermal fibrosis by excessive treatment with vitamin A. In patients with chronic vitamin A intoxication, Russel *et al* (1974) observed lipocytic proliferation in the perisinusoidal space, resulting in perisinusoidal fibrosis and hepatic parenchymal atrophy due to occlusion of the sinusoid by collagen fibers, and confirmed the autofluorescent vitamin A in the lipocytes. The vitamin A in the lipocytes reduce gold chloride and make the lipocyte react positively to gold impregnation (Wake, 1971).

In the present study, animals receiving vitamin A only showed lipocytic proliferation with development of RER and fat droplets in the cytoplasm. These lipocytes transformed into fibroblast-like cells and reacted positively to gold impregnation, suggesting that the fat droplets in the cytoplasm were vitamin A complexes. On PAM staining many collagen bundles were observed, especially in contact with or near the cytoplasmic membrane of the lipocytes. This finding strongly suggests lipocytic involvement in perisinusoidal collagen fiber formation.

For a long time, only the fibroblasts in connective tissue had been considered to produce collagen fibers and involvement of hepatic parenchymal cells in the production of perisinusoidal collagen had never been thought of. But Ohuchi and Tsurufuji (1972) recognized protocollagen proline hydroxylase in rat liver cells, which was confirmed by Ooshima (1977) with a

immunohistochemical method. Sakakibara and Sato (1976) found collagen formation by liver parenchymal cell in tissue culture. Henceforth, it should be taken into account that the liver cell participates in collagen fiber formation. Previously, Popper (1977) reported that lipocytes and endothelial cells were the cells capable of collagen formation. In the present study evidence of participation of endothelial cells or hepatocytes in fibrogenesis was lacking. It was evident, however, that lipocytic proliferation with activation is closely related to the collagen formation.

The factors activating fibroblasts were not definitely recognized although lysosomal enzymes in the inflammatory cells infiltrated at the area of necrosis were considered to stimulate the fibroblasts, bring about collagen fiber formation. In groups treated with CCl_4 alone or with vitamin A, hepatic cell necrosis, leukocytic infiltration, and lipocytic proliferation associated with collagen formation were found. It is not certain whether only lysosomal enzymes from the inflammatory cells stimulate the fibrogenesis, since the authors could not find any hepatic cell necrosis or inflammatory reaction in the vitamin A only treated group, where perisinusoidal collagen formation was obvious. Popper and Udenfriend (1970) reported that low oxygen concentration and high sinusoidal pressure had a stimulating role on the lipocytes. Tanaka *et al* (1980) supported this view by showing an increase in r-glutamyl transpeptidase, an enzyme necessary for connective tissue protein synthesis in those conditions.

In chronic hepatitis or cirrhosis, formation of a subendothelial basement membrane like structure is noted in addition to collagenization at the site of lipocytic proliferation (Tanikawa, 1975), leading to a decrease in the number of pores of sinusoidal endothelium (Schaffner and Popper, 1963) and aggravation of hepatic cell necrosis by the interference of blood supply. The present experiment suggests that the basement membrane is also produced by activated lipocytes.

In the inhibition of collagenization, two possible mechanisms have been proposed; one is an interference of the formation process and the other is a dissolution of pre-formed collagen (Riley and Peacock, 1967; Lazarus *et al*, 1968; Jefrey and Gross, 1970; Perez-Tamayo, 1970). Schwartz and Kutzsche (1966) observed that steroid depressed collagen formation in tissue culture of fibroblasts. Corticosteroid is known to have no effect on pre-formed fibrosis (Spain *et al*, 1950), but have profound inhibitory effect on collagen formation (Seol *et al*, 1979).

In the present study, CCl_4 alone or in combination with vitamin A induced hepatic cell necrosis and activated lipocytes, producing perisinusoidal collagenous fibrosis. Additional treatment with dexamethasone suppressed activation of lipocytes and thereby collagen formation.

Therefore, it is concluded that the perisinusoidal fibrosis seen in association with hepatic necrosis is initiated by activation of lipocytes, and dexamethasone inhibits the lipocyte activation, resulting in suppression of fibrogenesis.

REFERENCES

Aterman K: *Studies in fibrosis of the liver induced by carbon tetrachloride. I. Relation between hepatocellular injury and new formation of fibrous tissue. Arch Pathol 57:1, 1954*

Bronfenmajor S, Schaffner F, Popper H: *Fat-storing cells (lipocytes) in human liver. Arch Pathol 82:447, 1966*

Dabrowski R, Maslinski C: *The effect of histamine on collagen formation and collagen polymerization in the skin wound healing of*

guinea pigs. Life Sci 9:189, 1970

Dingle JT, Glauert AM, Daniel M: *Vitamin A and membrane synthesis. I. The actions of the vitamin on the membranes of cells and intracellular particles. Biochem J 84:76, 1962*

Ehrlich HP, Tarver H, Hunt TK: *Effects of vitamin A and glucocorticoids upon inflammation and collagen synthesis. Ann Surg 177:222, 1973*

Fiume L: *Inhibition by aminoacetonitrile of early lesions induced in the liver of rats by carbon tetrachloride. J Pathol Bacteriol 83:291, 1962*

Hartroft WS: *Accumulation of fat in liver cells and lipodiastaemata proceeding experimental dietary cirrhosis. Anat Rec 106:61, 1950*

Hartroft WS, Ridout JH: *Pathogenesis of the cirrhosis produced by choline deficiency. Escape of lipid from fatty hepatic cysts into the biliary and vascular systems. Am J Pathol 27:951, 1951*

Hutterer F, Rubin E, Gall E, Popper H: *Cortisone effect in stages of liver injury. A cytochemical, autoradiographic and histologic study. Exp Mol Pathol 1:85, 1962*

Ito T: *Cytological studies on stellate cells of Kupffer and fat storing cells in the capillary wall of the human liver. Acta Anat (Nippon) 26:2, 1951*

Ito T: *Recent advances in the study on the fine structure of the hepatic sinusoidal wall: A series. Gunma Rep Med Sci 6:119, 1973*

Ito T, Nemoto M: *Die Kupfferschen Sternzellen und die "Fettspeicherungszellen" (fat storing cell) in der Blutcapillarenwand in der menschlichen Leber. Okajima Folia Anat Jap 24: 243, 1952*

Jefrey JJ, Gross J: *Collagenase from rat uterus. Isolation and partial characterization. Biochemistry 9:298, 1970*

Kawanami O: *Electron microscopic study on mammalian liver with periodic acid methenamine silver stain. Basement membrane and fibrogenesis in space of Desse. Acta Pathol Jpn 23:717, 1973*

Kent G, Gay S, Popper H: *Vitamin A-containing lipocytes and formation of type III collagen in liver injury. Proc Natl Acad Sci 73:3719, 1976*

Klein L, Nowacek C: *Effect of penicillamine of new and pre-existing collagen in vivo. Acta Biochim Biophys 194:504, 1969*

Kobayashi K, Takahashi Y, Shibasaki S: *Cytologic studies of fat-storing cells in the liver of rats given large doses of vitamin A. Nature 243:186, 1973*

Lazarus GS, Daniels JR, Brown RS, Bladen HA, Fullmer HM: *Degradation of collagen by a human granulocyte collagenolytic system. J Clin Invest 47:2622, 1968*

Meader RD: *Treatment of nutritional cirrhosis in rats with choline and methionine; with special reference to fibrogenesis and fibroclasia. Anat Rec 145:123, 1963*

Muenter MD, Perry HO, Ludwig J: *Chronic vitamin A intoxication in adults; hepatic, neurologic and dermatologic complications. Am J Med 50:129, 1971*

Nieman C, Klein O: *The histochemistry and pathology of hypervitaminosis A. Vit Horm 12:69, 1954*

Nimni ME, Gerth N, Bavetta LA: *Reversibility of a penicillamine induced defect in collagen aggregation. Nature (London) 213:921, 1967*

Ohuchi K, Tsurufuji S: *Protocollagen proline hydroxylase in isolated rat liver cells. Acta Biochim Biophys 258:731, 1972*

Ooshima A: *Immunohistochemical localization of proline hydroxylase in rat tissue. J Histochem Cytochem 25:1297, 1977*

Patrick RS, Kennedy JS: *S-35-labelled amino acids in experimental liver disease. J Pathol*

Bacteriol 88:107, 1964

Perez-Tamayo R: *Collagen resorption in carrageenin granulomas. II. Ultrastructure of collagen resorption. Lab Invest 22:142, 1970*

Popper H: *Summary, in: (ed by) Wisse E and Knook DL, Kupffer cells and other sinusoidal cells. Elsevier/Horth-Holland Biochemical Press, Amsterdam, p. 509-514, 1977*

Popper H, Paronetto F, Schaffner F, Ferez V: *Studies on hepatic fibrosis. Lab Invest 10: 265, 1961*

Popper H, Udenfriend S: *Hepatic fibrosis; correlation of biochemical and morphologic investigations. Am J Med 49:707, 1970*

Reddy DG, Krishnamurthy KR, Bhaskar GR: *Carbon tetrachloride cirrhosis in rats. Arch Pathol 74:73, 1962*

Reuber MD: *Carbon tetrachloride induced cirrhosis. Arch Pathol 85:275, 1968*

Riley WB, Peacock E: *Identification, distribution and significance of a collagenolytic enzyme in human tissues. Proc Soc Exp Biol Med 124:207, 1967*

Rubin E: *Cell proliferation and fiber formation in carbon tetrachloride intoxication. Am J Pathol 42:715, 1963*

Russell RM, Boyer JL, Bagheri SA, Hruban Z: *Hepatic injury from chronic hypervitaminosis resulting in portal hypertension and ascites. N Engl J Med 291:435, 1974*

Sakakibara K, Sato M: *Native collagen formation by liver parenchymal cells in culture. Nature 262:316, 1976*

Schaffner F, Popper H: *Capillarization of hepatic sinusoids in man. Gastroenterology 44:239, 1963*

Schnack H, Stockinger L, Wewalka F: *Adventitious connective tissue cells in the space of Disse and their relation to fiber formation. Rev Int Hepatol 17:855, 1967*

Schwarz W, Kutzsche A: *Elektronenmikroskopische Untersuchungen ueber die anabole und katabole Wirkung von Dexamethason und Methenolonester auf Fibroblasten in vitro. Klin Wschr 44:979, 1966*

Seol JH, Park CI, Kim CS, Lee YB: *The effects of penicillamine and corticosteroid on the collagen formation during the repair of skin wound in rats. Yonsei J Med Sci 12:95, 1979*

Spain DM, Molonut N, Haber A: *Biological studies on cortisone in mice. Science 112: 335, 1950*

Stenger RJ: *Hepatic sinusoids in carbon tetrachloride-induced cirrhosis. An electron microscopic study. Exp Mol Pathol 4:359, 1965*

Tanaka M, Ekunaga M, Watanabe K, Kaneko Y, Takahashi T, Ishikawa E: *Fat storing cells (Ito's cell) of human liver; biological characteristics and changes occurring in circulatory disturbance. Acta Histol Jpn 43:241, 1980*

Tanikawa K: *Collagen metabolism in the liver. Popper H and Becker K ed. Stratton International Medical Book Corp., New York, p. 93-99, 1975*

Wake K: *"Sternzellen" in the liver perisinusoidal cells with special reference to storage of vitamin A. Am J Anat 132:429, 1971*

Wisse E: *An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoid. J Ultrastruct Res 31:125, 1970*

Yamamoto M: *Ultrastructure and function of Ito cell (fat-storing cell) in the liver. Med J Hiroshima Univ 23:245, 1975*

Yamamoto M, Enzan H, Hara H, Iijima S: *Fluorescence and electron microscopic studies on the perivascular mesenchymal cells and fibroblasts after vitamin A administration. Acta Pathol Jpn 28:513, 1978*

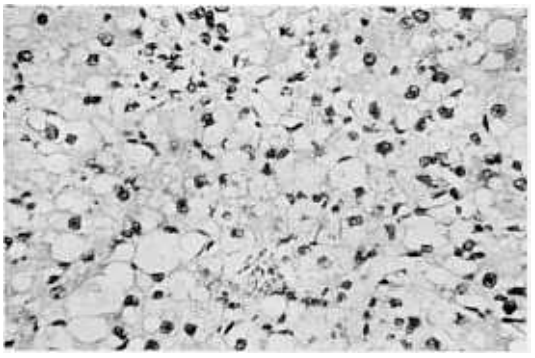

Fig. 1. Liver 4 days after CCl_4 only; showing a prominent proliferation of sinusoidal cells associated with inflammatory infiltration and liver cell necrosis. H-E stain, 100 x.

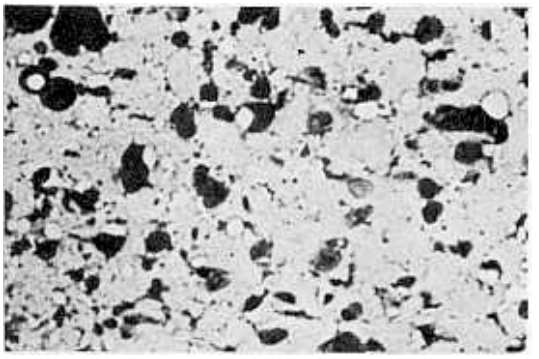

Fig. 2. Liver 4 days after CCl_4 only; showing oil red-O positive lipocytes at sinusoidal wall at the angle between the parenchymal cells. Oil red-O stain, 400 x.

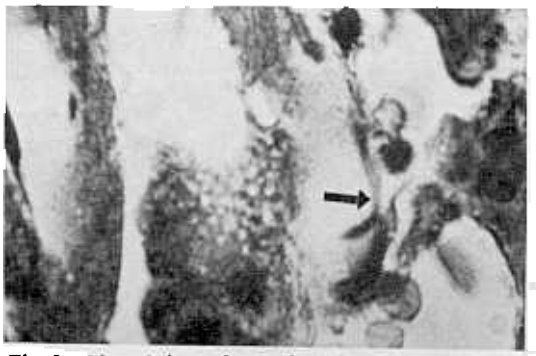

Fig. 3. Liver 4 days after CCl_4 only; showing collagen fibers (arrow) extending along cytoplasmic membrane of perisinusoidal cell. Masson's trichrome stain, 1,000 x.

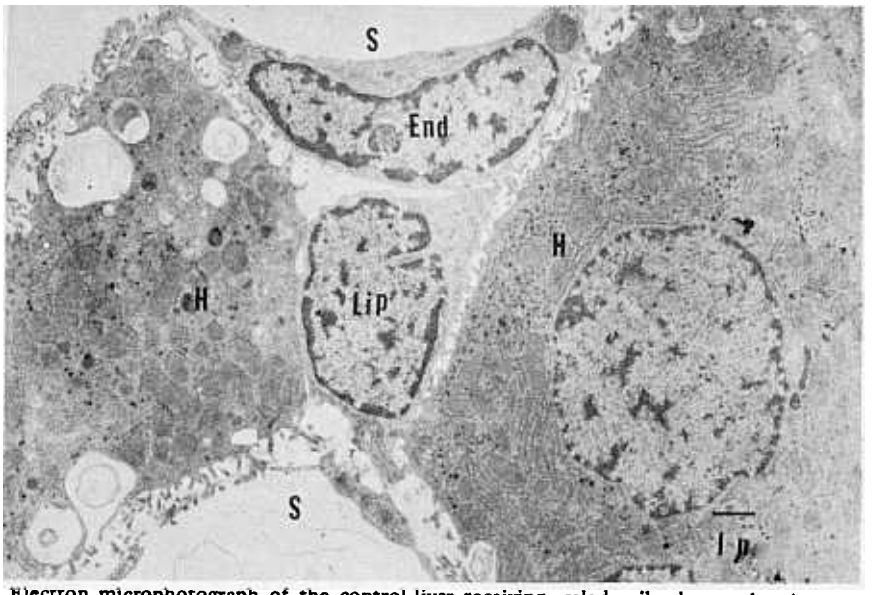

Fig. 4. Electron microphotograph of the control liver receiving salad oil alone; showing a small inactive form of lipocyte (Lip) between a endothelial cell and two hepatic cells. 7,500 x.

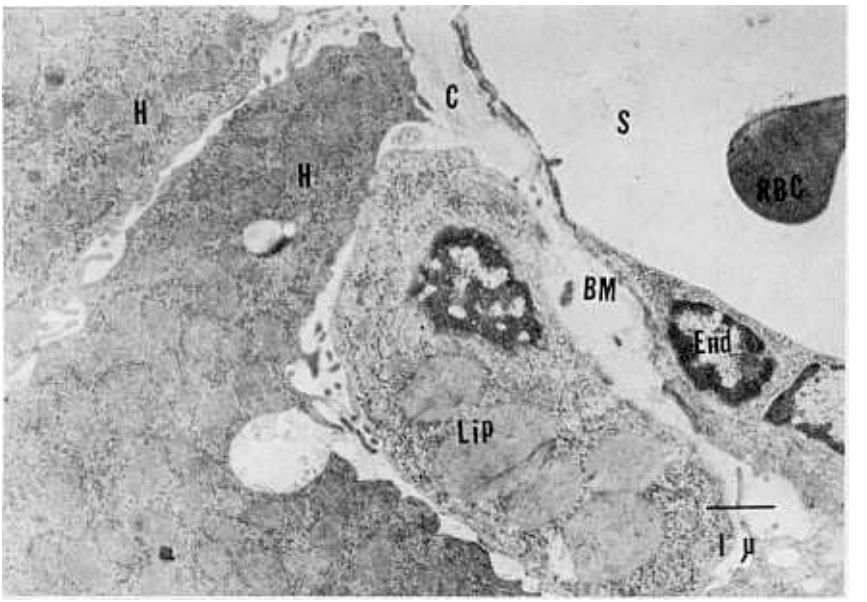

Fig. 5. Electron microphotograph of the liver 4 days after CCl_4 only; showing lipocyte containing fat droplets in perisinusoidal space, collagen fiber (C) attached to lipocyte and subendotherlial basement membrane formation (BM). 12,000X.

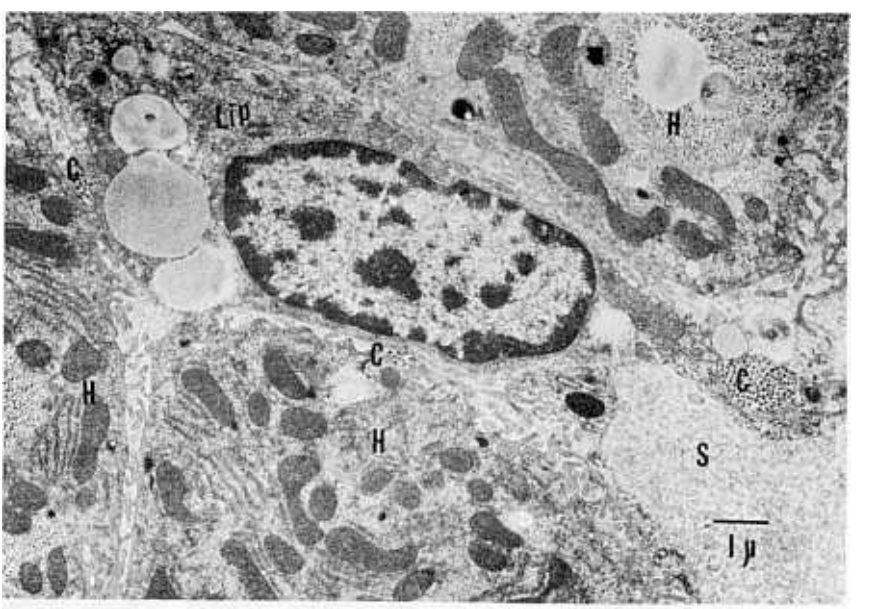

Fig. 6. Electron microphotograph of the liver 6 days after vitamin A alone; showing numerous collagen fibers (C) adjacent to lipocyte in the space of Disse. PAM stain, 10,000X.

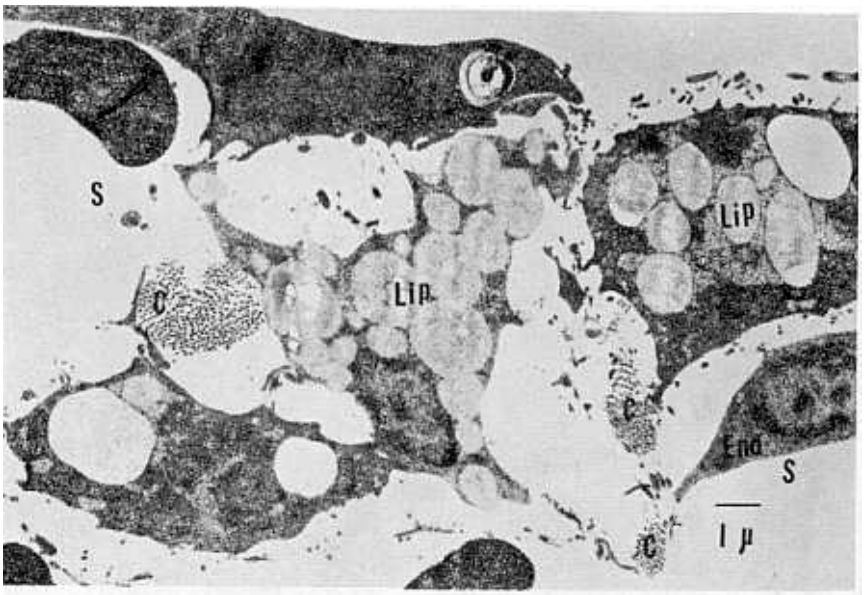

Fig. 7. Electron microphotograph of the liver 4 days after CCl_4 and vitamin A; showing a large activated lipocyte (Lip) with abundant cytoplasm and processes, and collagen fibers adjacent to the cell membrane. PAM stain, 8,700X.

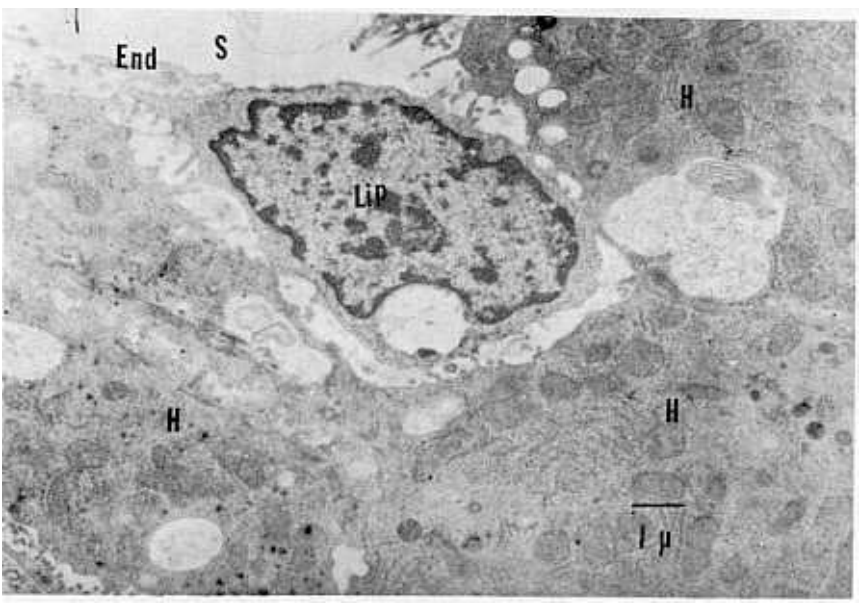

Fig. 8. Electron microphotograph of the liver on the 4th day after combined administration of CCl_4, vitamin A and dexamethasone; showing relatively inactive form of lipocyte (Lip) as evidenced by a small size with poor development of RER and lack of cytoplasmic processes small amount of lipid droplets and no evidence of collagen formation. 10,000X.