EFFECT OF IRON LOADING UPON THE FORMATION OF COLLAGEN IN THE HEPATIC INJURY INDUCED BY CARBON TETRACHLORIDE

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No uniform agreement presently exists as to the cause of the tissue damage in hemochromatosis and the role of iron in the production of these changes. Certain observations, however, have favored the view that large deposits of iron in parenchymal organs have deleterious effects upon these organs. Foremost among these are the findings that the iron deposits in hemochromatosis are related to[1] and, in fact, precede tissue damage[2] and, furthermore, that the clinical course in hemochromatosis is favorably influenced by repeated phlebotomy.[3,4] Other observers have remained unimpressed with these arguments and consider the harmful effects of iron unproven. Thus, it is argued, the amount of iron and the degree of tissue damage do not run parallel, there being many instances in which massive amounts of iron are not accompanied by any degree of tissue damage.[5] Moreover, it is contended that all attempts to reproduce the tissue changes of hemochromatosis in experimental animals have been uniformly unsuccessful.

Golberg and Smith[6] have recently reported that cirrhosis could be more rapidly induced in iron-loaded animals by the administration of ethionine or a low protein diet, suggesting that the siderotic liver was vulnerable to certain adverse circumstances. Since similar nutritional or metabolic hazards may play a role in human siderosis, an inquiry into the mechanism leading to the tissue changes appeared in order. This study accordingly presents observations on the effects of iron loading upon the carbon tetrachloride injury in the rat.

MATERIAL AND METHODS

Male Sprague-Dawley rats, with an average weight of 150 gm., were treated as follows: (1) Thirty animals received subcutaneous injections of carbon tetrachloride, 0.1 ml. per 100 gm. of body weight twice weekly. (2) Thirty animals received carbon tetrachloride, as in group 1, and in addition were given intramuscular injections of iron dextran (supplied through the courtesy of Lakeside Laboratories, Milwaukee, Wisconsin) in doses of 10 mg. of iron per 100 gm. of body weight. The injections of

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carbon tetrachloride and iron dextran were started on the first day of the experiment, the carbon tetrachloride then being continued twice weekly while the iron dextran was given every third day for one month, then once a week until sacrificed. (3) Another 30 rats received carbon tetrachloride, as in group 1, and in addition were given intramuscular injections of dextran (Lakeside Laboratories). The dextran was the same as that employed in the preparation of iron dextran and was given in equivalent amounts and at the same intervals as iron dextran. (4) Iron dextran was administered to 30 rats according to the schedule used for group 2. (5) There were 30 normal controls. All animals were maintained on Purina Checkers and tap water *ad libitum.* Blood was obtained from the tail vein at 2, 4 and 6 weeks for serum transaminase determinations by the method of Reitman and Frankel.[7] Ten animals in each group were sacrificed 1, 3 and 5 months respectively after the beginning of the experiment.

Under Nembutal® anesthesia, a small piece of liver was excised and fixed for electron microscopy. The liver was weighed, and sections taken for histologic study were fixed in formalin or alcohol. Paraffin sections were stained with hematoxylin and eosin, and processed by Wilder's method for reticulum, Masson's trichrome method for connective tissue and by Perls's ferrocyanide reaction for iron. The amount of reticulum observed in histologic sections was graded from 0 to 4+, 0 indicating the normal amount in control rats and 4+ the maximum amount of fibrosis after 5 months. Tissue for electron microscopy was fixed in Palade's buffered osmium at 0 to 4° C. and embedded in Epon 812 as previously described.[8] Blocks were sectioned with a Cambridge ultramicrotome and stained with uranyl acetate for 2 hours, or lead citrate[9] or with phosphotungstic acid followed by uranyl acetate. Micrographs were taken with an RCA electron microscope (EMU3G) at original magnifications of 2,500 to 48,000 diameters. Portions of liver were homogenized for determination of hydroxyproline[10] and total and soluble iron[11] as in previous studies. The hydroxyproline content was expressed as mg. per 100 gm. of dry defatted liver and also as mg. per whole liver.

Results

Light Microscopy and Chemical Data

The early, intermediate and advanced stages of the chronic carbon tetrachloride injury were studied 1, 3 and 5 months after the beginning of the experiment.

Early Stage. One month after the beginning of the experiment, the animals receiving carbon tetrachloride alone exhibited the characteristic changes described by Cameron and Karunaratne.[12] These consisted essentially of centrilobular necrosis and hydropic degeneration of liver cells, as well as central and midzonal fatty changes. A few inflammatory cells were observed in the centrilobular zones, but ductular cells were not in evidence. Connective tissue stains revealed no increase in collagen or in argentophil fibers (Fig. 1) over those seen in control rats. Chemical values for collagen, determined as hydroxyproline (Table I), likewise were not increased above normal (123 mg. per cent). The body weight of these animals had increased in parallel with control animals, from an initial weight of about 150 gm. to approximately 310 gm. (Table II). The average liver weight was 14.1 gm. or 4.5 per cent of body weight and that of the control rats 13.9 gm. or 4.3 per cent of body weight. The

amount of iron in the liver was 16.2 mg. per cent, of which 11 mg. per cent was present in a soluble form. These values were of the same order as those obtained from control animals (Table III).

The rats receiving carbon tetrachloride and dextran were in all respects similar to those receiving carbon tetrachloride alone. The livers showed no increase in histologically visible fibers or in chemically estimated collagen, the hydroxyproline values being 116 mg. per cent or 3.1 mg. per whole liver.

The rats receiving iron dextran alone exhibited features as previously described.[11] Hepatic iron in the form of Prussian blue-positive granules was located almost exclusively in the Kupffer cells and amounted to

TABLE I

EFFECT OF IRON DEXTRAN (ID) ON HEPATIC RETICULUM AND HYDROXYPROLINE CONTENT IN NORMAL AND CARBON TETRACHLORIDE-TREATED ANIMALS

Treatment	No. of mo.	No. of animals	Reticulum *	Hydroxyproline			
				Mg. per 100 gm.		Mg. per whole liver	
				Mean	P	Mean	P
Control	1	10	0	113 ± 18	N.S. ‡	3.0 ± 0.55	N.S.
ID †		10	0	110 ± 10		3.4 ± 0.35	
CCl_4	1	9	0	123 ± 11	<0.001	2.7 ± 0.66	<0.005
CCl_4 + ID		10	1–2+	165 ± 19		3.8 ± 0.64	
CCl_4	3	9	1–2+	157 ± 29	<0.005	4.3 ± 0.78	<0.001
CCl_4 + ID		10	3+	216 ± 35		6.6 ± 1.1	
CCl_4	5	9	3+	335 ± 38	<0.001	10.5 ± 3.7	<0.005
CCl_4 + ID		3	4+	1040 ± 68		23.1 ± 5.1	

* Graded from 0 (normal amount) to 4+ (maximum observed at 5 months).
† Dextran and other controls are reported in text.
‡ Not significant.

TABLE II

BODY WEIGHT AND LIVER WEIGHT IN RATS GIVEN CARBON TETRACHLORIDE WITH AND WITHOUT IRON DEXTRAN (ID) *

Treatment	Body weight (gm.)			Liver weight †					
				Total (gm.)			% body weight		
	Months			Months			Months		
	1	3	5	1	3	5	1	3	5
None	320	445	495	13.9	16.0	17.7	4.3	3.6	3.6
ID	300	392	401	16.3	18.5	19.1	5.4	4.8	4.5
CCl_4	310	399	432	14.1	16.8	17.2	4.5	4.2	4.0
CCl_4 + ID	280	372	380	15.8	19.3	22.2	5.6	5.2	5.8

* Initial body weight of all groups averaged 150 gm.
† Calculated as mean of 10 animals for each group.

259.1 mg. per cent; of this, 181.6 mg. per cent was contained in the soluble fraction (Table III). The amount of hydroxyproline in the liver was not increased above normal (Table I). The body weights were slightly decreased as compared with the controls and averaged 300 gm. (Table II). The liver weights, by contrast, were increased in all animals

TABLE III

HEPATIC IRON IN RATS EXPOSED TO CARBON TETRACHLORIDE WITH AND WITHOUT IRON DEXTRAN (ID)

Treatment	No. of animals	Hepatic iron *			
		1 month		3 months	
		Total	Soluble	Total	Soluble
None	10	17.1 ± 1.2	11.8	21.3 ± 2.7	15.5
CCl_4	9	16.2 ± 1.8	11.0	18.0 ± 1.9	12.2
ID	10	259.1 ± 10.3	181.6	307.1 ± 31.6	214.2
CCl_4 + ID	10	269.7 ± 10.5	172.4	319.8 ± 23.2	221.4

* Expressed as mg. of iron per 100 gm. of wet liver.

receiving iron dextran, whether they received carbon tetrachloride or not. Thus, the livers in this group averaged 16.3 gm., representing 5.4 per cent of body weight.

The rats receiving carbon tetrachloride plus iron dextran exhibited regressive changes in the central zones much like those in the animals receiving carbon tetrachloride alone. The extent of hepatocellular damage did not appear greater as judged from the zonal involvement or type of injury. However, two additional features were evident: Fine fibrous septums, accompanied by delicate vascular channels were now observed in the central zones (Fig. 2) and formed connections between hepatic veins. Moreover, a strikingly close relationship was apparent between the septums and iron-laden macrophages (Fig. 3). The latter presumably were Kupffer cells which had migrated to the regions of the central veins and formed aggregates within and around the septums, more or less delineating these structures. Fiber formation was accentuated in areas where macrophages accumulated (Fig. 4). Prussian blue-positive material was also seen in the Kupffer cells, but no appreciable amount of iron was seen in the liver cells. Hydroxyproline values averaged 165 mg. per cent or 3.8 per whole liver (Table I) and were thus significantly higher than in the carbon tetrachloride controls. As in previous studies,[10] hydroxyproline values correlated remarkably well with histologically assessed fibrosis. The body weights of these animals averaged 280 gm. (Table II). The liver weights averaged 15.8 gm. or 5.6 per cent of body weight. The amount of iron in the liver was 269.7 mg. per cent with a soluble fraction of 172.4 mg. per cent (Table III). These values were

not significantly different from those in normal animals receiving corresponding amounts of iron dextran.

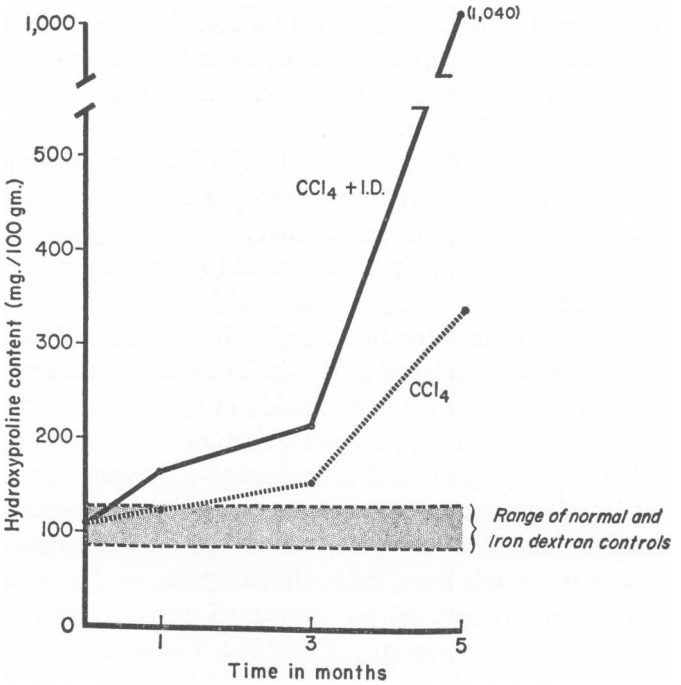
Intermediate Stage. Three months after the beginning of the experiment, the rats receiving carbon tetrachloride alone exhibited fine fibrous septums (Fig. 5) which were located in the centrilobular zones and connected hepatic veins. Ductular cells were seen only rarely. In general, the septums at this time were similar in extent and thickness to those seen after one month in animals receiving carbon tetrachloride plus iron dextran. This impression was borne out by the hydroxyproline values which averaged 157 mg. per cent or 4.3 mg. per whole liver (Table I). The body weight of these animals averaged 399 gm. (Table II). The liver weight was 16.8 gm. or 4.2 per cent of body weight. The amount of iron in the liver was 18 mg. per cent with a soluble fraction of 12.2 mg. per cent (Table III). Administration of dextran had no significant effect on the hydroxyproline content in the liver. It amounted to 161 mg. per cent or 4.1 mg. per whole liver.

The rats receiving iron dextran alone had hydroxyproline values very close to those of normal controls (110 mg. per cent). The body weight of these animals was 392 gm. and the liver weight 18.5 gm. or 4.8 per cent of body weight. Hepatic iron amounted to 307.1 mg. per cent with a soluble fraction of 214.2 mg. per cent (Table III).

Rats receiving carbon tetrachloride plus iron dextran for 3 months had developed a well-established cirrhosis. The central septums in many areas had now connected with portal fields (Fig. 6), and ductular cells were often conspicuous in the periportal septums. The latter were thinner than the central septums, thus reflecting a shorter duration. Aggregates of iron-laden macrophages were observed around both portal and central septums. Hydroxyproline values amounted to 216 mg. per cent or 6.6 mg. per whole liver, indicating a significant increase as compared to the rats not receiving iron dextran (Table I). The body weight of these animals averaged 372 gm. (Table II). The liver weight was 19.3 gm. or 5.2 per cent of body weight. The amount of iron in the liver was 319.8 mg. per cent, with a soluble fraction of 221.4 mg. per cent (Table III). This was not significantly greater than the hepatic iron content in animals given iron dextran alone, indicating that hepatic iron storage was not enhanced by the carbon tetrachloride injury.

Advanced Stage. After 5 months, all rats receiving carbon tetrachloride had developed cirrhotic changes, whether they had received iron dextran or not (Figs. 7 and 8). However, marked differences existed between these two groups: the rats receiving iron dextran contained considerably greater amounts of collagen in the liver (Table I) and revealed a tendency to develop large postnecrotic scars. Moreover, most of these

rats died spontaneously during the fifth month of the experiment. By contrast, the animals receiving carbon tetrachloride alone were all sacrificed at the end of the experiment. Their septums in general were delicate and, judging from connective tissue stains and chemical values, were hardly more extensive than in animals treated with carbon tetrachloride plus iron dextran for 3 months. The hydroxyproline values averaged 335 mg. per cent or 10.5 mg. per whole liver (Table I), while those of the iron-loaded animals were 1,040 mg. per cent or 23.1 mg. per whole liver. Although hydroxyproline values were only obtained in 3 animals (Table I), histologic evaluation, carried out in 10 animals, strongly supported the validity of these results. The comparative hydroxyproline values in



TEXT-FIG. 1. Effect of iron dextran on collagen formation in hepatic injury induced by carbon tetrachloride.

the two groups for the duration of the experiment are set out in Text-figure 1. Weight gain between the third and fifth months was only slight in the animals receiving carbon tetrachloride and negligible in those receiving also iron dextran (Table III). Hepatic iron was not determined in these animals.

Electron Microscopic Observations

Attention was focused on the composition of the centrilobular septums and adjacent cells in the iron-loaded animals. The liver cells about the

septums manifested varying degrees of fatty or hydropic change (Fig. 9). Some of these had lost most of their cytoplasmic content and had evidently undergone irreversible damage. Wedged between the degenerating liver cells and the collagenous septums and also between collagen fibrils were iron-laden macrophages (Fig. 9). These cells occurring singly or, more often, in clusters, were by far the most conspicuous elements in these areas. The septums themselves contained numerous delicate blood channels (Fig. 10) possessing basement membranes and occasionally also a narrow rim of collagen. The macrophages were packed with phagosomes of various sizes and shapes. Many of these were round or oval and had well-defined limiting membranes. Others had no recognizable membranes or had irregular contours, while still others contained large complex bodies (Fig. 11) devoid of structural detail, probably representing the partially digested remains of other cells.

Iron particles, identified as ferritin with surprising frequency, were observed in large concentrations throughout the cytoplasm of these cells. Where the macrophages bordered on collagen fibrils, the ferritin particles were often seen beyond the cellular boundaries (Figs. 12 and 13). In other areas, the extracellular location of ferritin was more difficult to establish with assurance. The collagen fibrils were oriented in various directions. The fibroblasts were evident mostly as slender cytoplasmic processes between the collagen fibrils. They were recognized mainly by a well-developed rough-surfaced and dilated endoplasmic reticulum (Fig. 14). The profiles of the cisternae formed anastomosing channels containing electron-lucid or slightly opaque material. Some of the fibroblasts had prominent Golgi vacuoles and fat droplets. A striking feature within the cytoplasm of the fibroblasts was the presence of membrane-bound bodies packed with iron particles. On closer inspection it became evident that the iron particles were also dispersed throughout the cytoplasm of the fibroblasts. In addition, they were regularly observed extracellularly in the ground substance between the collagen fibrils (Fig. 15). In most instances, characteristic profiles of ferritin could be made out in the fibroblasts and in the extracellular spaces. While the extracellular location of individual iron particles could be established with relative ease, this was more difficult with regard to larger iron-containing bodies. Although the latter were often surrounded by collagen and appeared to be extracellular in location (Figs. 16 and 17), their location within cells could not be excluded, in view of the vague cellular boundaries of fibroblast processes.

The large numbers of iron-containing bodies within fibroblasts often made it difficult to distinguish them from macrophages. Because of a prominent and dilated endoplasmic reticulum studded with ribosomes, these cells were interpreted as fibroblasts (Fig. 18), although their re-

semblance to macrophages posed the question of the relationship of these cells to one another. Electron microscopic study was also applied to the septums in the later stages of the experiment. The findings did not add significantly to the problem under discussion and are, therefore, not described here.

Serum Glutamic Pyruvic Transaminase Activity

Serum enzyme studies were undertaken to help assess the extent of hepatocellular damage in the various groups. As expected, the serum glutamic pyruvic transaminase activity was elevated at 2, 4 and 6 weeks after the beginning of the experiment in the rats exposed to carbon tetrachloride (Table IV). No greater degree of elevation was evident in the

TABLE IV

EFFECT OF IRON LOADING ON SERUM GLUTAMIC PYRUVIC TRANSAMINASE ACTIVITY (SGPT) IN LIVER INJURY INDUCED BY CARBON TETRACHLORIDE

No. of animals	Time (weeks)	SGPT activity *	
		CCl_4	CCl_4 + ID †
10	2	41 ± 21	35 ± 14
9	4	32 ± 12	23 ± 11
10	6	113 ± 33	52 ± 7

* Reported in Sigma-Frankel units; normal controls, 12 ± 3.
† Iron dextran.

iron-loaded rats. If anything, the serum enzyme levels were lower in the latter than in the animals receiving carbon tetrachloride alone.

DISCUSSION

It was evident in the present study that iron loading enhanced significantly the formation of collagen septums as well as the development of cirrhosis in rats exposed to carbon tetrachloride. The first septums appeared in the iron-loaded animals long before they were seen in the carbon tetrachloride controls. They were located in the centrilobular zones, in the areas of hepatocellular degeneration,[13] and were accompanied by large numbers of iron-laden macrophages. Later in the experiment, iron loading caused an earlier and accentuated development of cirrhosis with the formation of porto-portal and porto-central septums and the appearance of many ductular cells. In contrast to the fine septal cirrhosis finally evident in the animals given carbon tetrachloride alone, the iron-loaded animals also displayed extensive "post-necrotic" scarring and died prematurely.

The mechanism leading to the effects of iron loading is not clear. One

pathway to be considered is an aggravation of hepatocellular injury by the administered iron. This effect could be brought about by the adverse action of iron on liver cells [14] or by an impairment of the centrilobular blood supply following hypertrophy of iron-laden Kupffer cells.[15] The serum transaminase activities and histologic findings speak against these considerations. The enzyme activities in the iron-loaded animals, in fact, were relatively low and might even suggest a mild protective effect of iron dextran. In agreement with previous observations,[11] moreover, the carbon tetrachloride injury appeared to have no significant effect upon hepatocellular iron storage. Thus, parenterally administered iron was stored in the Kupffer cells and not in the liver cells and was unlikely to exert adverse effects upon the latter. The same does not apply to ethionine intoxication which, under similar circumstances, is known to cause a conspicuous parenchymal cell siderosis.[11,16] The effect of iron loading upon this injury [6] may well include a direct action of iron on liver cells.[17]

As an alternate explanation, a direct influence of iron loading on the connective tissue must be considered. While the carbon tetrachloride injury was associated with relatively few inflammatory cells, concomitant administration of iron dextran led to the appearance of many iron-laden macrophages in the centrilobular areas. A close topographic relationship became established between the macrophages and the developing septums, and a consistent accentuation of fiber formation was observed in areas where macrophages accumulated. The septums themselves displayed iron particles abundantly in fibroblasts and in the ground substance of the connective tissue, much of which could be identified as ferritin. Baker, Golberg, Martin and Smith [18] and Muir and Golberg [19] described similar features in the subcutaneous or intramuscular tissues of rats and other experimental animals following the injection of massive doses of iron dextran. These authors ascribed a fibrogenic effect to the injected material and attributed this to the presence of large masses of siderophages and of extracellular ferritin. They observed large iron-containing bodies with a complex internal structure, probably representing the ingested remains of other macrophages. In conjunction with the extracellular presence of cytoplasmic material, they considered these observations to reflect a local toxic action of ferritin in high concentrations. We have observed similar complex bodies within macrophages. Artifacts of fixation or embedding as the reason for the appearance of the extracellular iron were carefully considered throughout this study. These possibilities were disregarded because of the abundance of cellular debris within macrophages and the selective location of the extracellular iron in certain areas of the connective tissue as against others.

The iron-laden macrophages and extracellular ferritin then may be important factors in the enhanced fibrosis following iron loading. Singly or in combination, they may perhaps stimulate the production of excess collagen or interfere with its removal[20,21] and so exert detrimental effects in hepatic injury. The mechanism of this effect is open to speculation. The macrophages could influence fiber formation by serving as chemotactic agents,[22,23] by being transformed into fibroblasts,[18,19,22,24] or by merely serving as mechanical scaffolding for fibers, similar to the role postulated for ductules.[25,26] Ferritin in high concentrations, on the other hand, could have a lytic action on macrophages, similar to that proposed for quartz,[24,27] and could thus cause the release of irritating substances by these cells.

The observations we have presented may have a bearing on human hemochromatosis, since iron-laden macrophages feature prominently in the developing septums in both primary and secondary hemochromatosis.[1] The occurrence of Prussian blue-positive material, apparently outside of cells, has also been reported.[1,28] In primary hemochromatosis, the iron in the macrophages is derived from degenerating liver cells, and in secondary hemochromatosis it represents, in addition, one of the usual sites for iron storage.[1] While the basic defect underlying human hemochromatosis remains unknown, the carbon tetrachloride injury may well illustrate one of the pathways leading to the tissue alterations characteristic of this condition.

Summary

The effect of massive doses of iron dextran upon the formation of hepatic collagen was studied in rats exposed to carbon tetrachloride. The animals receiving iron dextran developed fibrous septums earlier and to a significantly greater extent than controls treated with carbon tetrachloride alone. The degree of cirrhosis, moreover, was considerably enhanced. Analogous doses of dextran had no such effect.

Serum transaminase activity and histologic evaluation suggested that the fibrogenic effect of iron loading was not due to an accentuation of the hepatocellular injury. On the other hand, a close topographic relationship existed between the developing septums and iron-laden macrophages, collagen formation being particularly pronounced in areas rich in these cells. The septums also contained much extracellular iron, often identifiable as ferritin.

The findings suggest that iron-laden macrophages or extracellular ferritin may be important factors in the fibrogenic effects of iron loading. They may thus also contribute to tissue alterations in human hemochromatosis.

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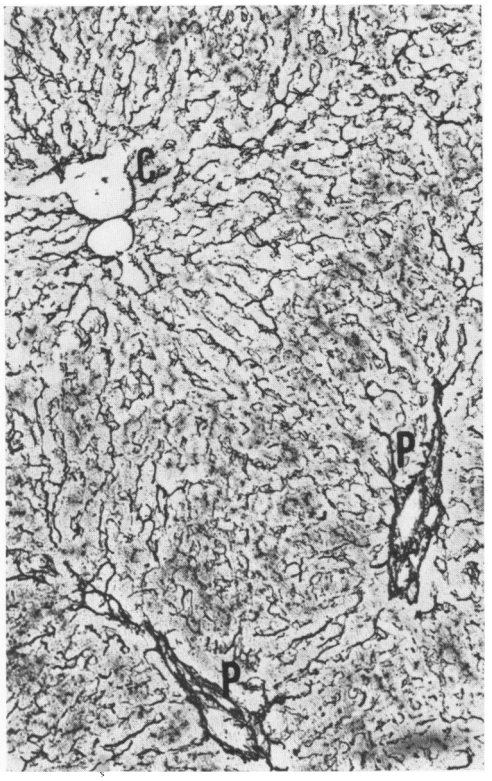
LEGENDS FOR FIGURES

FIG. 1. "Early" carbon tetrachloride injury, 1 month's duration. Two portal fields (p) are shown. The reticulum fibers about the central vein (c) are somewhat more distinct than in other zones. Wilder's reticulum stain. × 90.

FIG. 2. "Early" carbon tetrachloride injury in an "iron-loaded" rat, 1 month's duration. Clearly defined centrilobular septums connecting central veins (c) are seen at this stage. The portal fields (p) do not yet connect with centrilobular septums. Wilder's reticulum stain. × 80.

FIG. 3. "Early" carbon tetrachloride injury in an "iron-loaded" rat, 1 month's duration. Centrilobular areas of degeneration (arrows) contain conspicuous accumulations of iron-laden macrophages (black areas). Perls's Prussian blue reaction. × 80.

FIG. 4. Same as Figure 3. Reticulum fibers are increased in prominence in areas where macrophages (arrows)) have accumulated. Wilder's reticulum stain. × 400.



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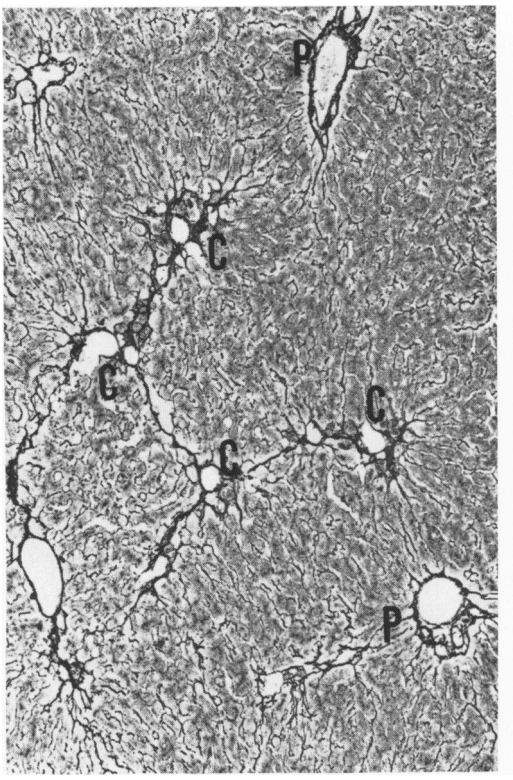

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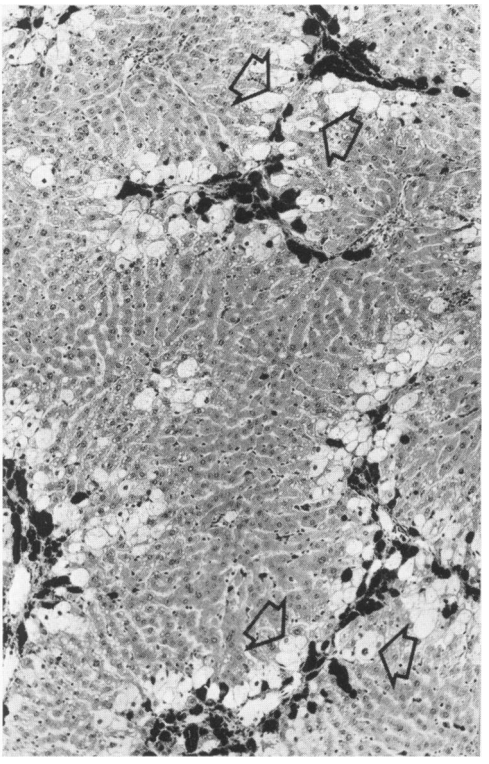

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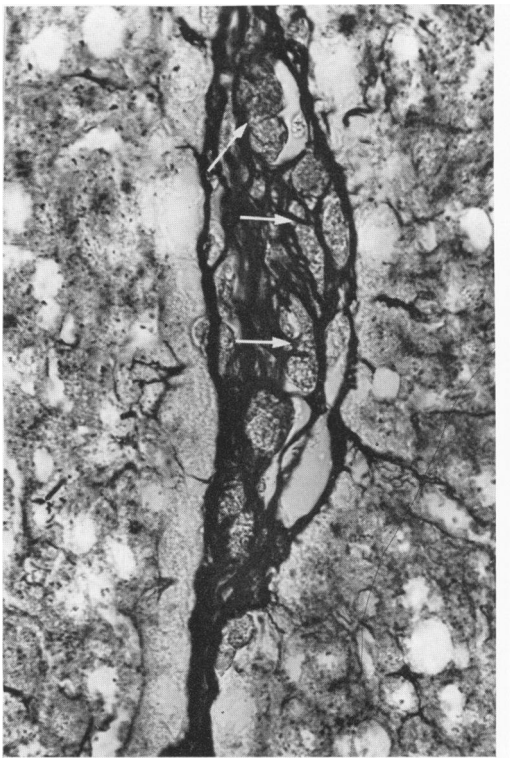

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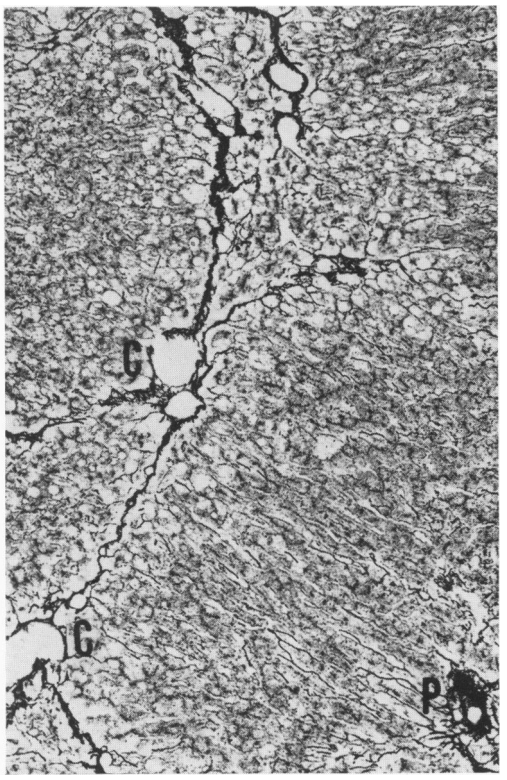
FIG. 5. "Intermediate" carbon tetrachloride injury, 3 months' duration. Centrilobular septums now connect central veins (c). The portal fields (p) do not yet connect with the fibrous septums. Comparison with Figure 2 shows that the carbon tetrachloride lesion at 3 months resembles in extent the 1 month lesion in the iron-loaded animals. Wilder's reticulum stain. × 90.

FIG. 6. "Intermediate" carbon tetrachloride lesion in an "iron-loaded" rat, 3 months' duration. In comparison with Figure 5, the fibrous septums are wider and more abundant. Moreover, cirrhosis is now apparent in the form of connections between portal (p) fields and centrilobular septums (c). Wilder's reticulum stain. × 80.

FIG. 7. "Advanced" carbon tetrachloride lesion, 5 months' duration. A fine septal cirrhosis is evident. The lesion is of about the same extent as that in "iron-loaded" rats after 3 months. Wilder's reticulum stain. × 80.

FIG. 8. "Advanced" lesion in an "iron-loaded" rat, 5 months' duration. Cirrhosis is advanced, and large "postnecrotic" scars surround regenerative nodules (R). Wilder's reticulum stain. × 80.



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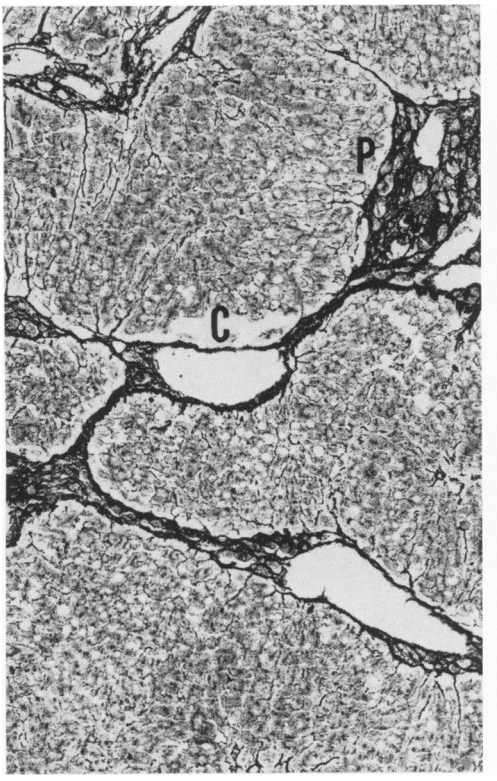

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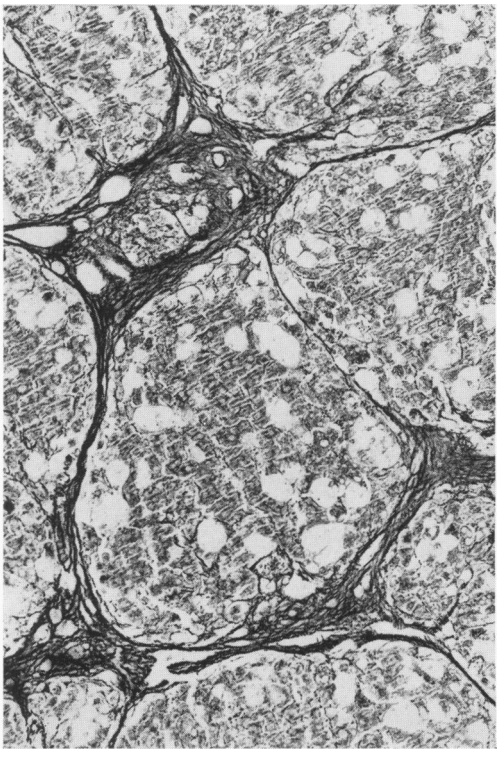

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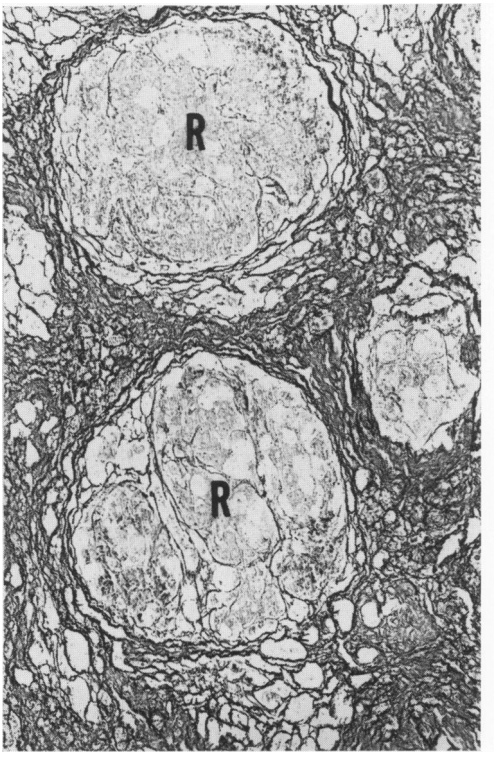

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FIG. 9. A portion of a septum is adjacent to an area of centrilobular degeneration in a rat receiving carbon tetrachloride and iron dextran. All subsequent micrographs are from similar areas. Iron-laden macrophages (m) are prominent, as is the relationship of these cells to collagen (c) on the one hand and to hepatic parenchymal cells (LC) on the other. The latter display hydropic and fatty changes. Stained with uranyl acetate. × 4,900.

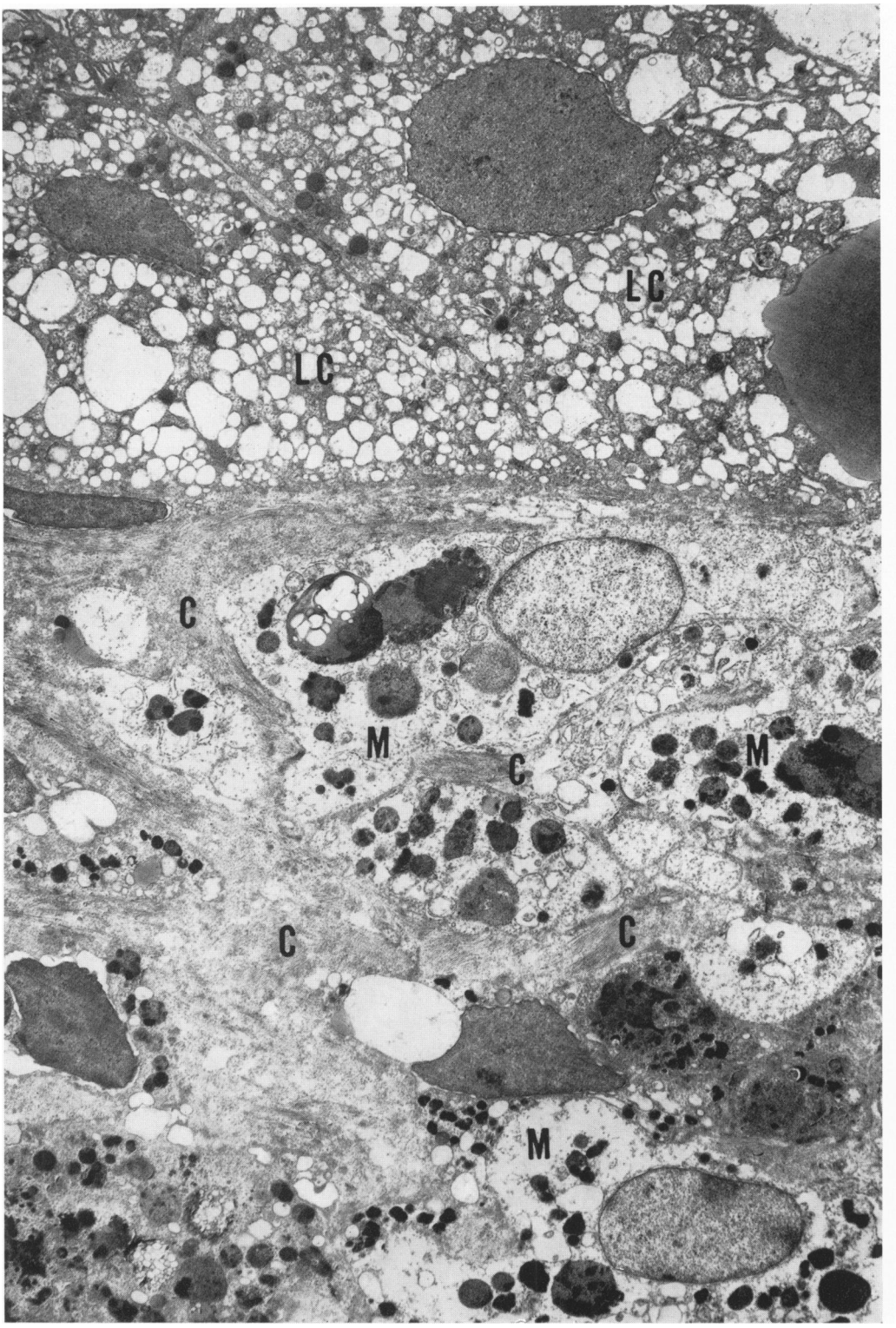

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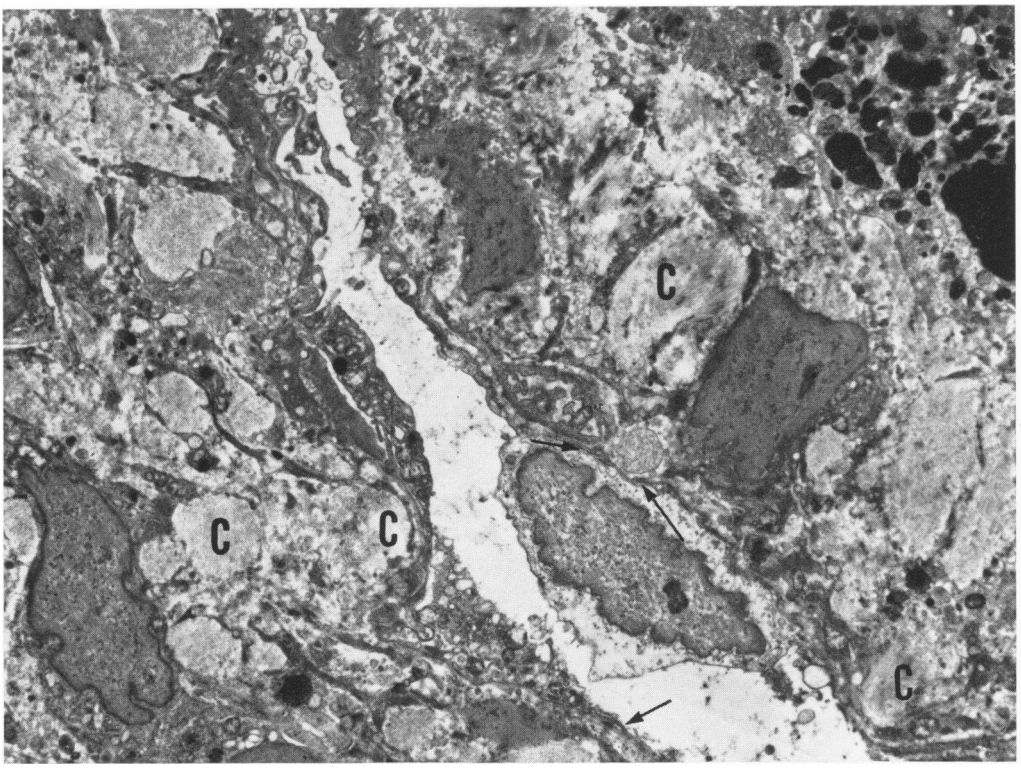
FIG. 10. A blood vessel is delineated from the surrounding collagen (c) by a basement membrane (arrows). A macrophage may be noted in the upper right corner of the micrograph. Lead citrate stain. × 4,800.

FIG. 11. A portion of huge macrophage contains phagosomes (arrows) and complex bodies (CB). The latter exhibit densely packed iron particles and some lipid material, but otherwise lack structural detail. They are thought to be partially digested remains of other cells. Uranyl acetate stain. × 9,100.



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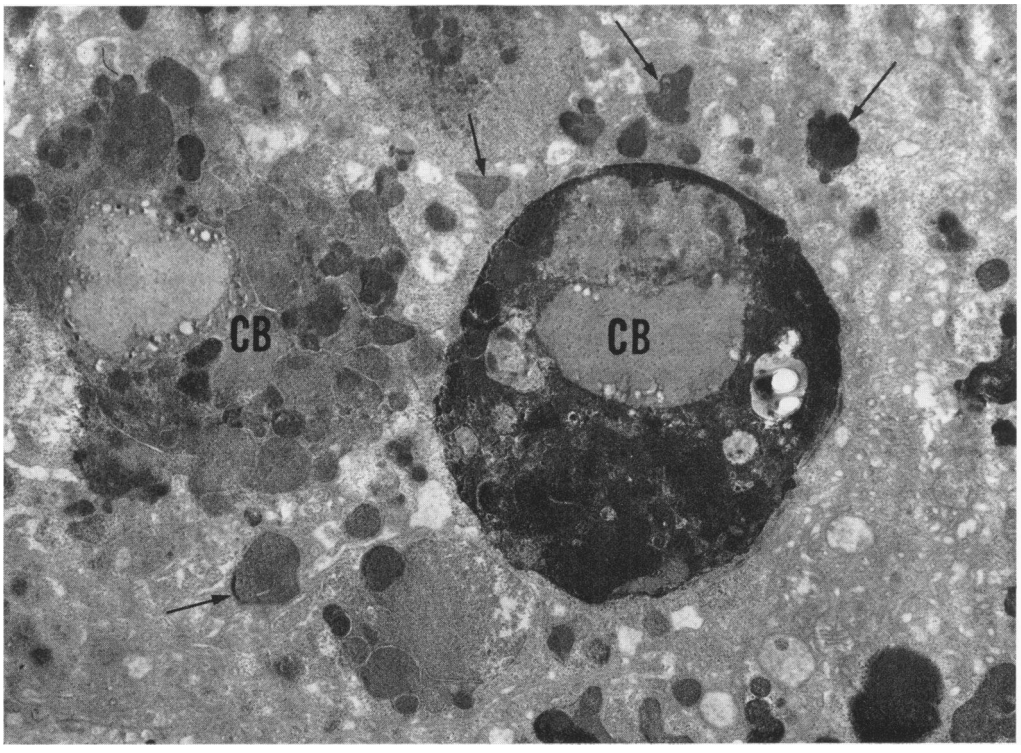

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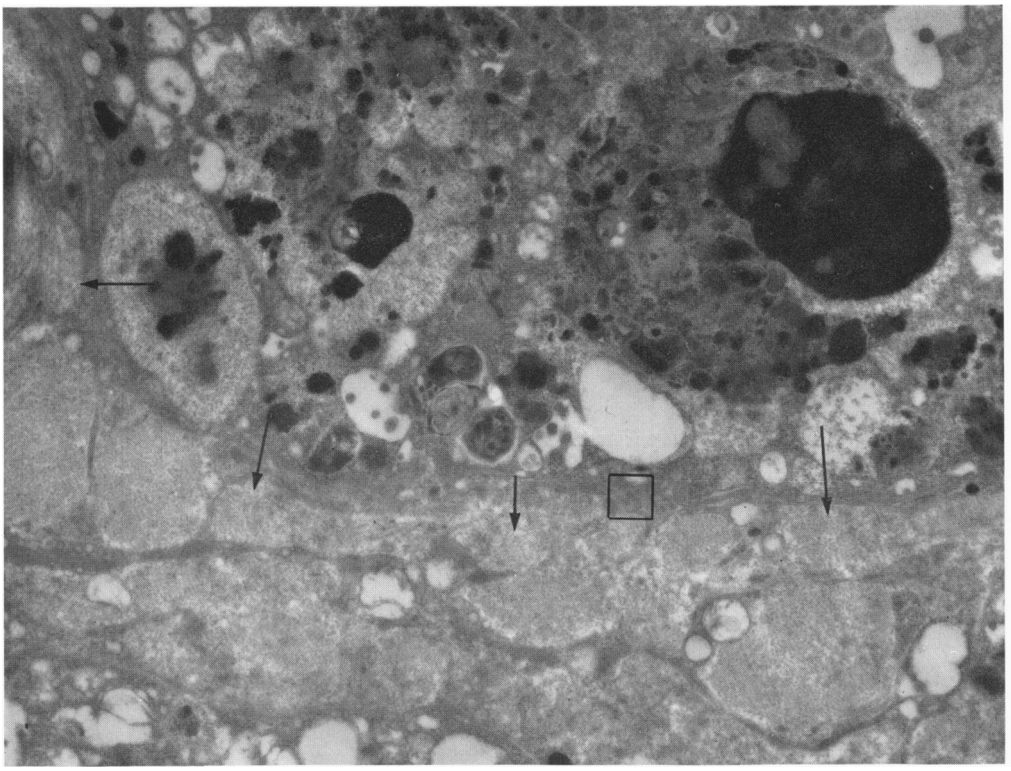
FIG. 12. A macrophage adjacent to collagen (arrows). The small square denotes an area shown at higher magnification in Figure 13. Uranyl acetate stain. × 8,000.

FIG. 13. Extracellular ferritin particles (circles) appear along the microvilli (M) on the outer surface of macrophages. Uranyl acetate stain. × 235,000.



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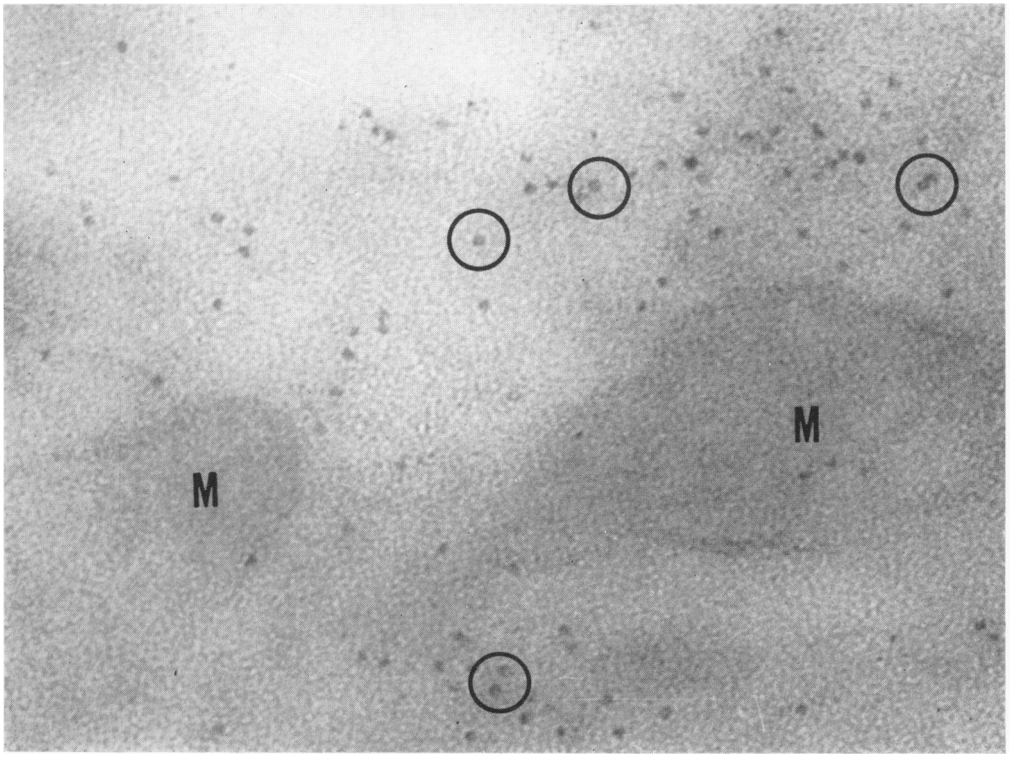

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FIG. 14. Cytoplasmic processes of a fibroblast (arrows)) separate collagen fibrils. The dilated endoplasmic reticulum (E) is studded with ribosomes, and numerous membrane-enclosed bodies are packed with iron. By light microscopy, these bodies might be interpreted as extracellular hemosiderin. Uranyl acetate stain. × 20,000.

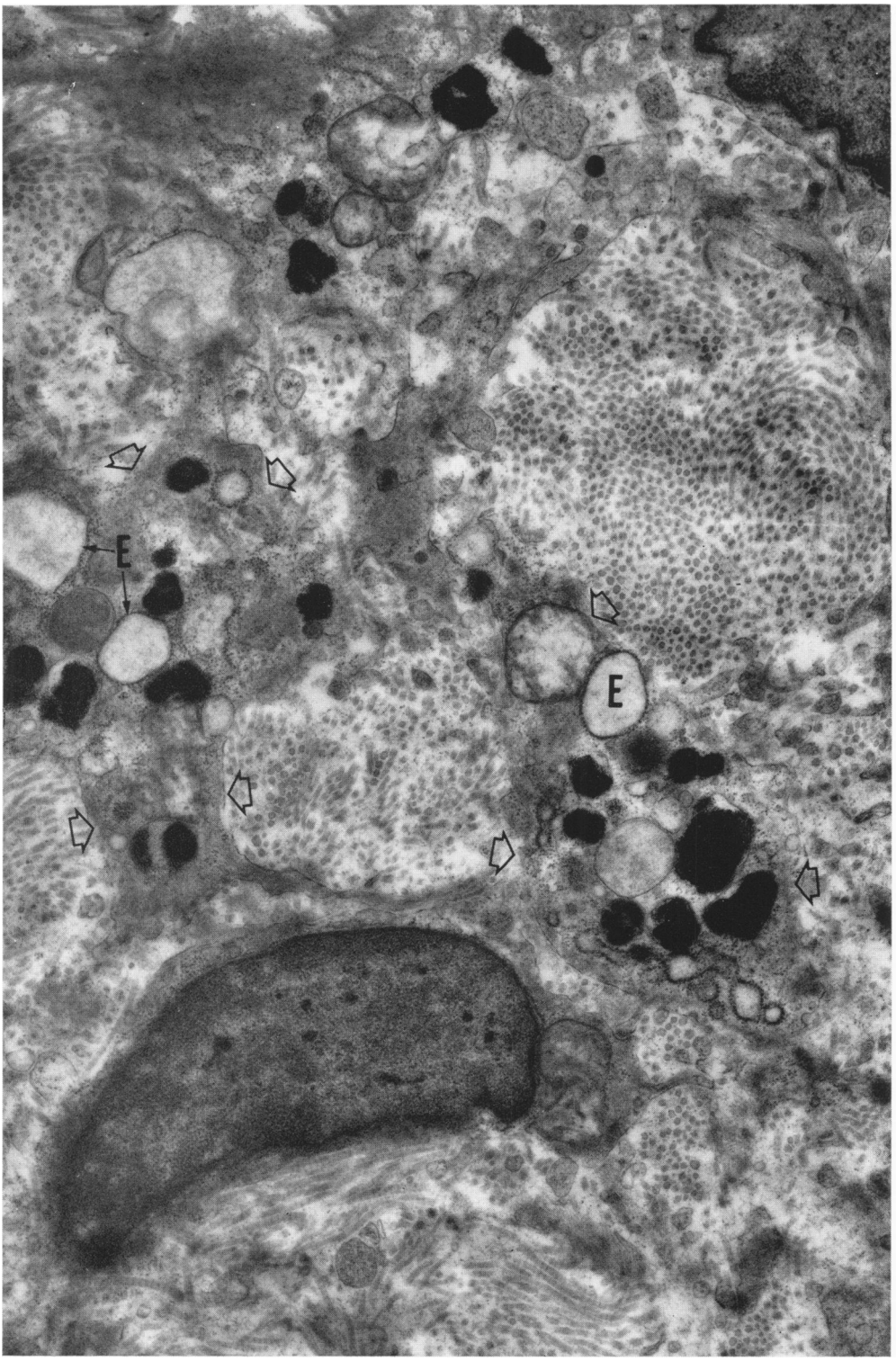

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FIG. 15. Extracellular ferritin particles (circles), mostly located between collagen fibrils (c), appear in the ground substance of the connective tissue. Uranyl acetate stain. × 245,000.

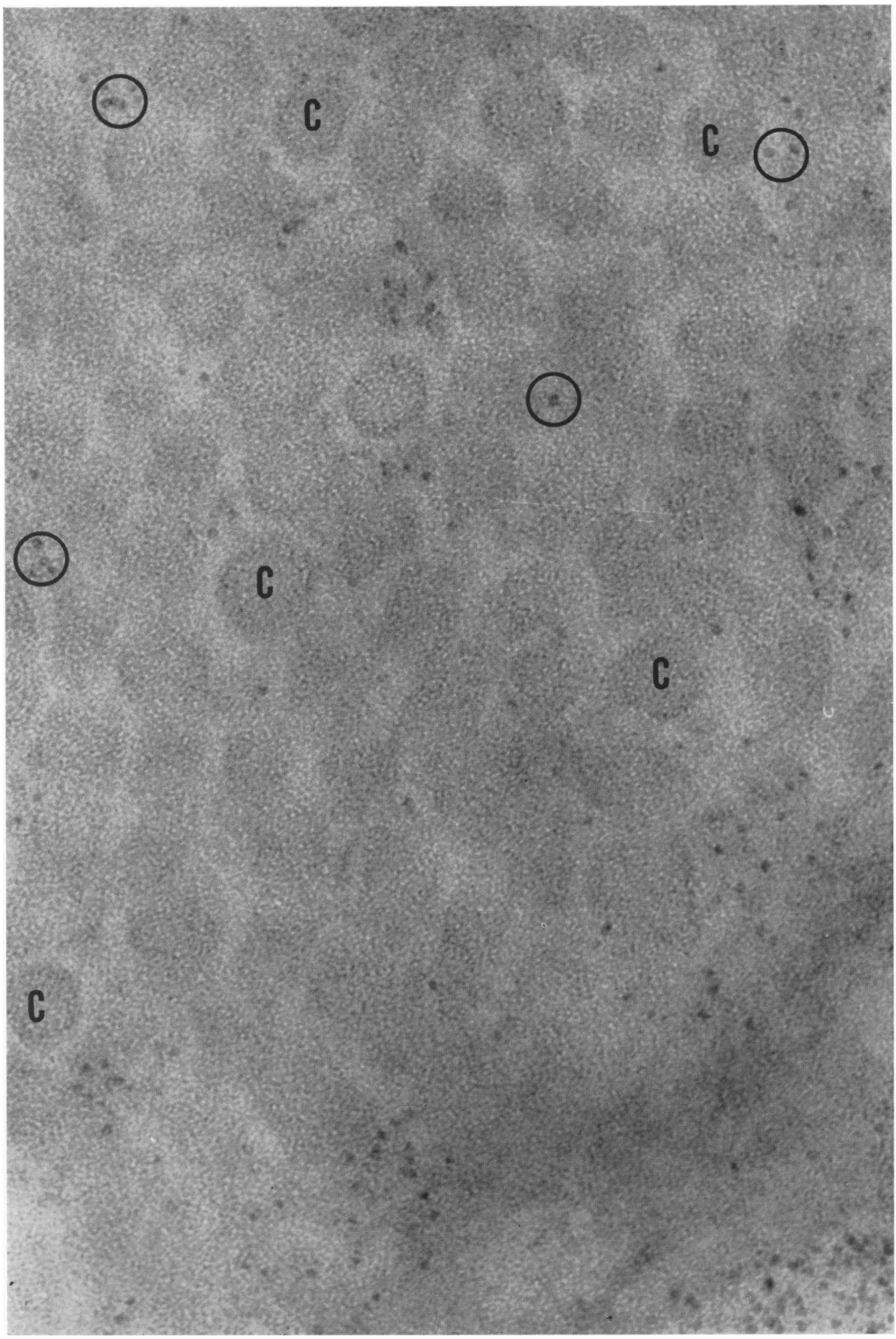
C
C
C
C
C
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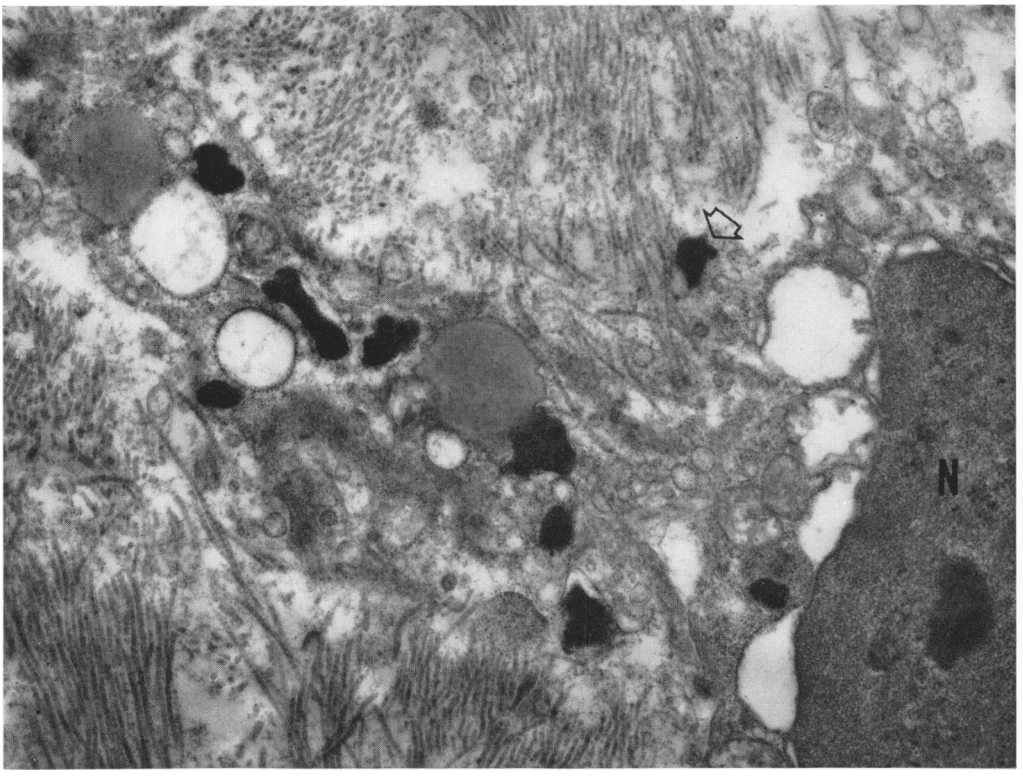
FIG. 16. A nucleus (n) and a cytoplasmic process of a fibroblast appear amidst collagen fibrils. One iron-containing body (arrow) is apparently extracellular in location. Uranyl acetate stain. × 15,000.

FIG. 17. Another iron-containing body, very close to collagen (arrows), is apparently extracellular in location. Phosphotungstic acid followed by uranyl acetate. × 28,000.

FIG. 18. A cell is interpreted as a fibroblast because of the conspicuous rough-surfaced endoplasmic reticulum (arrows), but otherwise resembles a macrophage. Lead citrate stain. × 21,000.



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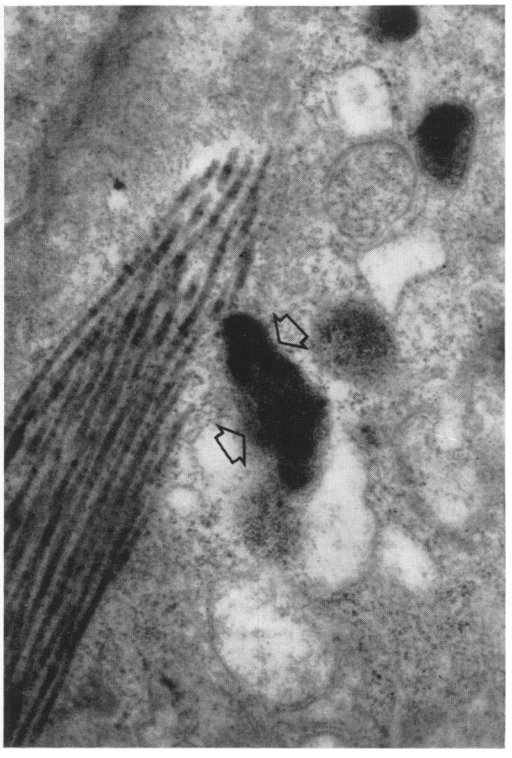

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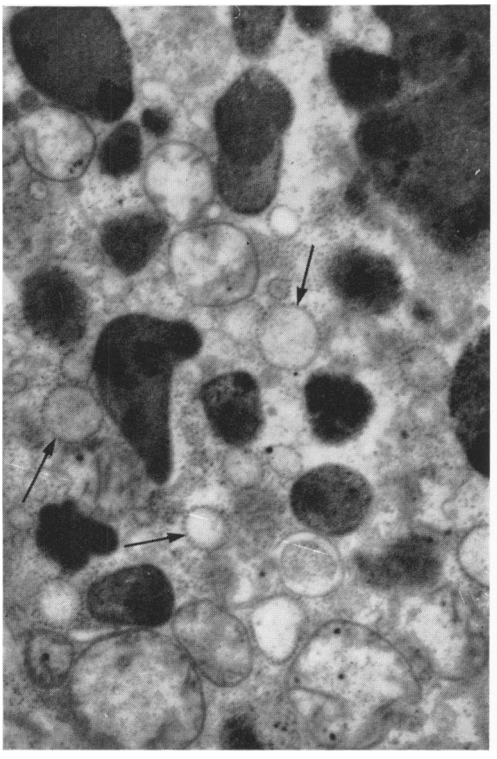

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