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The influence of interferon alpha on the rat liver injured by chronic administration of carbon tetrachloride

Due to their complex and not fully known etiopathogenesis as well as difficulties with treatment, chronic hepatitis and cirrhosis still remain one of the main problems hepatologists deal with.

The use of IFN-alpha in the treatment of chronic viral hepatitis B and C has initiated a new stage in the therapy of those ailments (1,2,7). An interesting issue is the use of IFN in patients with cirrhosis. The findings of several studies carried out recently suggest that IFN-alpha administered in small doses improves the liver function and reduces the frequency of cancer in patients with stage A post-inflammatory cirrhosis (according to the Child score) (9,15). Similarly, the results of the experimental studies show that IFN reduces fibrosis in liver cirrhosis induced in rats by ligating the common bile duct (11,13).

The aim of the study was to determine whether IFN-alpha improved the function of the rat liver injured by carbon tetrachloride. This substance shows specific hepatotoxic properties. Single doses of CCl_4 result in rapid development of liver fatty degeneration and cirrhosis. Fatty degeneration of the liver is caused by the CCl_4–induced inhibition of lipoprotein synthesis or synthesis of lipoprotein defective forms. Prolonged administration of CCl_4 (14 and more doses) for several weeks causes fibrosis and cirrhosis similar to that observed in human cirrhosis.

MATERIAL AND METHODS

The studies were performed in white Wistar male rats whose initial body weight ranged from 240 to 250g. The animals were randomly chosen according to the rule of simultaneity of the examined and control groups. The substances administered were carbon tetrachloride and interferon alpha (Wellferon, Wellcome). The rats were divided into 9 groups, 10 rats each. Group I received Ringer's solution 3 times a week for 2 weeks. Group II received intraperitoneal CCl_4 in the dose of 0.5mg/kg b.w. 3 times a week for 3 weeks. In the group III intraperitoneal CCl_4 was administered in the dose of 0.5mg/kg b. w. 3 times a week for 6 weeks. Group IV received subcutaneous IFN-alpha in the dose of 100,000 IU 3 times a week for 2 weeks while in the group V this dose was 300,000 IU, administered 3 times a week for 2 weeks. Group VI first received CCl_4 in the dose of 0.5mg/kg b.w. 3 times a week for 3 weeks and then subcutaneous IFN-alpha in the dose of 100,000 IU 3 times a week for 2 weeks. Group VII received CCl_4 in the same dose as mentioned above while the dose of IFN-alpha was 300,000 IU 3 times a week for 2 weeks. Group VIII was given CCl_4 in the dose of 0.5mg/kg b.w. 3 times a week for 6 weeks followed by IFN-alpha in the dose of 100,000 IU for 2 weeks. In group IX CCl_4 was administered in the same way as above while the dose of subcutaneous IFN-alpha was 300,000 IU given 3 times a week for 2 weeks. After the CCl_4 administration, the mortality rate in rats was 60%. The administration of IFN-alpha did not result in any further mortality increase.

The liver function was evaluated using the test of aminophenazone elimination in the isolated, perfused rat liver according to Miller modified by Hafte. The examinations were performed for 2 days after the administration of substances (CCl_4 or IFN-alpha) had been discontinued. Moreover, the activities of alanine and aspartate aminotransferase in serum were determined according to Reitman and Frankl. In each group, 3 rats were randomly chosen whose livers were histopathologically tested under the light and electron microscope–Zeiss, type EM 900 (Fig.1). The specimens were stained with hematoxylin and eosin according to van Gieson and the reticular fibres were impregnated with silver according to Gomory. Additionally, the immunohistochemical studies with monoclonal antibodies against desmin and actin of smooth muscles (DAKO) were performed. The LSA/Peroxidase technique was used (Fig. 5 and 6).

RESULTS

IFN alpha administered after the 3-week CCl_4-induced liver damage does not significantly affect AlAt and AspAt activities, irrespective of the dose used. IFN-alpha administered after the 6-week CCl_4-induced liver damage significantly affects AlAt and AspAt activities when its doses are high.

Carbon tetrachloride in the doses and periods used in the experiment does not result in evident features of cirrhosis, however it activates Ito cells and causes fibrosis and focal retraction of the stroma (Fig. 2 and 3).

An increased number of Ito cells in Disse's space observed immunohistochemically and ultrastructurally is indicative of the activation of fibrotic processes in rat livers after administering CCl_4 in both variants used (Fig. 5).

IFN-alpha inhibits the processes of fibrogenesis in the liver damaged by CCl_4, which is visible in decreased number of Ito cells and weaker expression of the stroma retraction.

It appears that the early phase of fibrogenesis is more effectively blocked by higher IFN doses. However, to confirm this the morphometric studies should be performed (Fig. 4 and 6).

IFN-alpha administered after the CCL_4-induced damage of the liver increases aminophenazone clearance, particularly when used in higher doses.

DISCUSSION

IFN-alpha is the main drug used in the treatment of chronic viral hepatitis B and C (1, 2, 7). However, its use in liver cirrhosis remains controversial. There are doubts as to the dose and duration of the treatment (15). Some researchers pointed out the intensified decompensation function of the liver after the IFN-alpha administration in patients with cirrhosis (3, 6). Perrillo et al. studied the effects of small doses of IFN in patients with HBV-induced cirrhosis. All the patients with stage A cirrhosis according to the Child score responded in a permanent decrease in serum HBV-DNA, reduced aminotranspherases and showed clinical stability. However, only 33% of the patients with stage B showed such results. Similar findings were reported by Dimopoulou et al. who used IFN in patients with cirrhosis induced by B and C viruses (4).

Beside biochemical aminotransferase normalization, an interesting issue is the effect of IFN on fibrosis. Manabe et al. studied the effects of IFN on fibrosis in patients with chronic hepatitis C. Biopsy was performed before and after the 24-week treatment with IFN administered in the doses of 1 and 3 IU three times a week. A statistically significant decrease in periportal cirrhosis was observed with 3 IU. However, the Knodell rate of fibrosis did not change. Nevertheless, the total collagen level determined by colorimetry and morphometry was significantly lower after 3 IU compared to that after 1 IU (9).

The studies performed in clinical and experimental conditions in which IFN was used in the treatment of cirrhosis show that small doses of IFN (1 IU) brought about good results and minor side-effects. The doses of 3IU, however, improved biochemical parameters to a higher degree and more significantly reduced fibrosis. Our findings, particularly improved aminophenazone clearance and reduced aminotransferase activities after higher IFN doses may speak in favour of administering higher doses of this drug but their use in clinical conditions requires further studies.

It seems worthy to perform histopathological examinations, including ultrastructural and immunohistochemical ones, and particularly to find Ito cells in Disse's space, which in physiological conditions store vitamin A in fatty vacuoles while in pathological ones are responsible for fibrosis (5, 8). We are extremely cautious about our ultrastructural pictures since there are no reports on histological changes after the CCl_4-induced liver damage and the use of IFN. The findings about the changes in the rough-and smooth-surfaced endoplasmic reticulum, the increased number of Browicz-Kupffer cells containing apoptic bodies should be confirmed by further studies.

CONCLUSIONS

The comparison of all the research methods used reveals inhibitory effects of IFN on early processes of fibrogenesis in rat livers. To confirm this phenomenon, determinations of collagen levels must be performed using morphometry.

Favourable effects of IFN on liver tissue justify the use of this drug in patients with chronic hepatitis and early stages of cirrhosis since it may not only eliminate the virus but also improve the liver function and reduce fibrosis.

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SUMMARY

Due to their complex and not fully known etiopathogenesis as well as difficulties in treatment, chronic hepatitis and cirrhosis still remain one of the main problems of hepatologists. Nowadays, the use of IFN alpha is considered the most effective method of treatment in chronic hepatitis. Recently, a new property of IFN, i.e. its effects on the reduction of fibrosis, has been discovered.

The aim of the paper was to examine the effects of IFN alpha on biochemical parameters (AlAt and AspAt activities), on the metabolic function of the liver and its morphologic picture observed under the light and electron microscope after the 3-and 6-week CCl_4-induced damage.

The experiments were carried out in Wistar male rats. To evaluate the liver function, the test of aminophenazone elimination in the isolated perfused rat livers was used according to Miller modified by Hafte. Additionally, AspAt and AlAt activities were determined. The liver specimens were analysed under the light and electron microscope and using immunohistochemical methods.

The findings show that after the 3-week CCl_4-induced liver damage, IFN alpha does not significantly affect AlAt and AspAt activities, irrespective of the dose used. IFN alpha administered after the 6-week damage significantly changes those activities when the doses used are high. It was found that carbon tetrachloride does not result in evident cirrhotic changes, however it activates Ito cells, causes focal retraction of the stroma and fibrosis. The increased number of Ito cells in Disse's space observed in immunohistochemical and ultrastructural examinations is indicative of the activation of liver fibrotic processes following CCl_4 administration in both variants used. IFN alpha substantially weakens fibrogenesis of the CCl_4-damaged liver which is visible in the decreased number of Ito cells and weaker expression of the stroma retraction. Moreover, IFN alpha administered to the experimental animals after the CCl_4-induced injury of the liver increases aminophenazone clearance, especially when used in higher doses.

Positive effects of IFN confirmed in the studies suggest that the drug may be used in patients with chronic hepatitis and early cirrhosis since it is likely not only to eliminate the virus but also to improve the liver function and reduce fibrosis.

Wpływ interferonu alfa na uszkodzoną czterochlorkiem węgla wątrobę szczura

Celem pracy było badanie wpływu IFN alfa na parametry biochemiczne (aktywność AlAt i AspAt) wątroby, wpływu na funkcję metaboliczną wątroby i jej stan morfologiczny badany w mikroskopie świetlnym i elektronowym po wcześniejszym uszkadzaniu CCl_4 przez 3 i 6 tygodni.

Doświadczenie przeprowadzono na szczurach samcach rasy Wistar. Do oceny funkcji wątroby używano testu eliminacji aminofenazonu w izolowanej, perfundowanej wątrobie szczura według metody Millera w modyfikacji Hafte'a. Oznaczano również aktywność AspAt i AlAt. Fragmenty wątrób badano w mikroskopie świetlnym, elektronowym oraz przy pomocy metod immunohistochemicznych.

Uzyskane wyniki wskazują na to, że po 3-tygodniowym uszkodzeniu wątroby CCl_4 interferon alfa nie ma istotnego wpływu na aktywność AlAt i AspAt, niezależnie od stosowanej dawki. IFN alfa podawany szczurom po 6-tygodniowym uszkadzaniu wątroby CCl_4 zmienia w istotnym zakresie aktywność AlAt i AspAt, gdy jest stosowany w dużej dawce. Stwierdzono, że czterochlorek węgla w dawkach i przez czas stosowany w naszym doświadczeniu nie wywołuje wyraźnych cech marskości wątroby szczurów, aktywuje jednak komórki Ito, powoduje ogniskowe zapadnięcie się zrębu i włóknienie. Zwiększenie liczby komórek Ito w przestrzeniach Dissego, wykazane immunohistochemicznie i ultrastrukturalnie, przemawia za aktywacją procesów włóknienia wątroby szczurów po podawaniu CCl_4 w obu stosowanych wariantach. IFN alfa znacząco osłabia fibrogenezę w uszkodzonej CCl_4 wątrobie, co wyraża się zmniejszeniem liczby komórek Ito i słabiej wyrażonymi cechami zapadania się zrębu. IFN alfa podawany zwierzętom doświadczalnym po uprzednim uszkodzeniu wątroby CCl_4 zwiększa też klirens aminofenazonu, szczególnie gdy jest stosowany w większych dawkach.

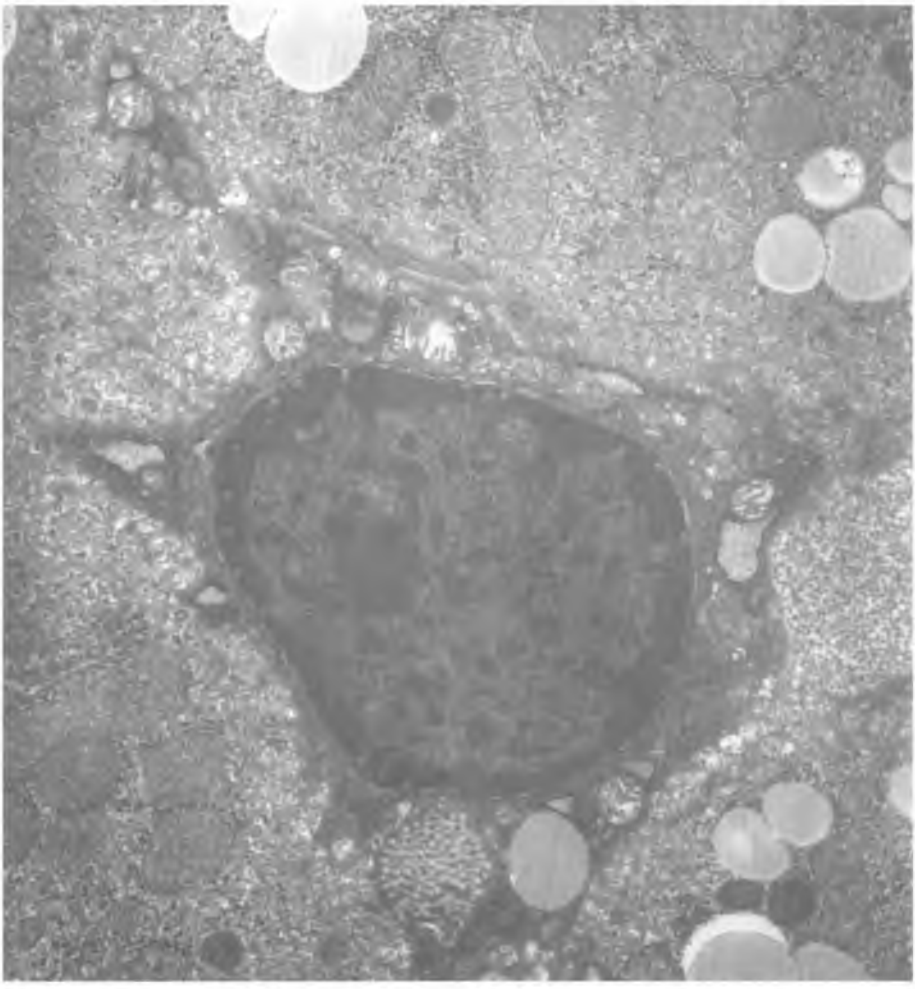

Fig. 1. Ito cell with fat drops lying in Disse's space.
TEM. Group I. Magn. 7000x

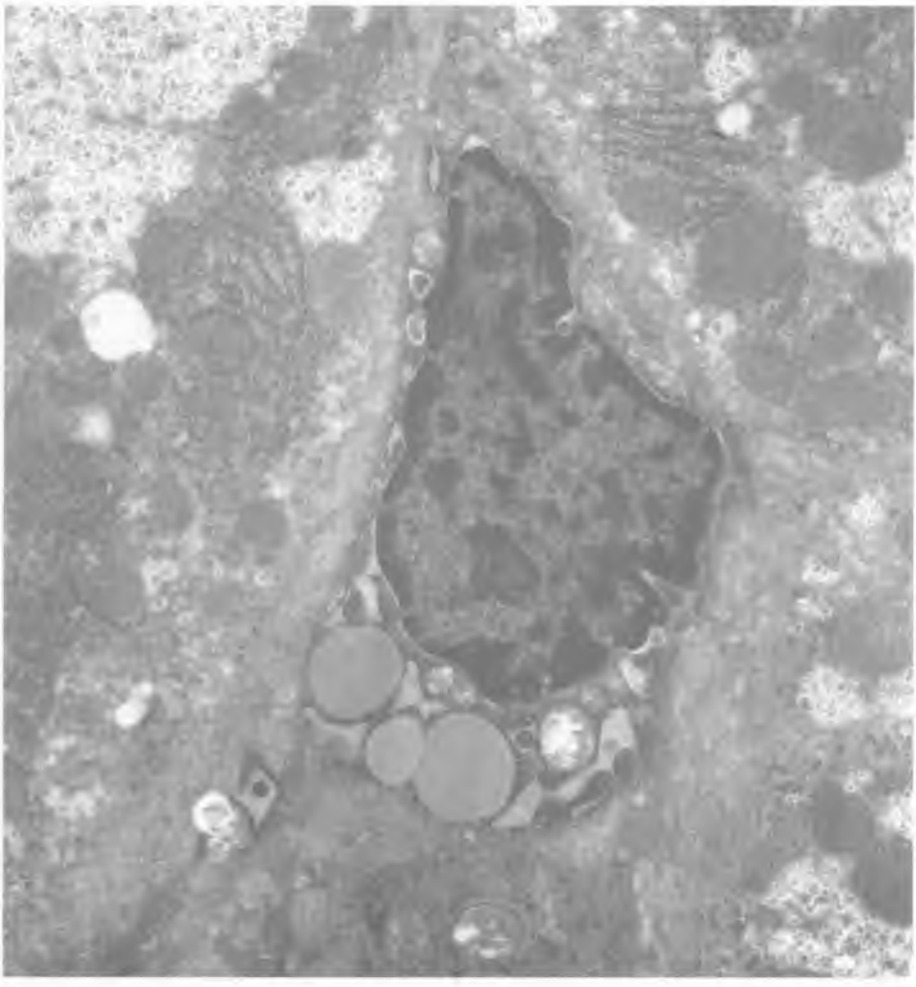

Fig. 2. Ito cell after damage by CCl_4
TEM. Group III. Magn. 7000x

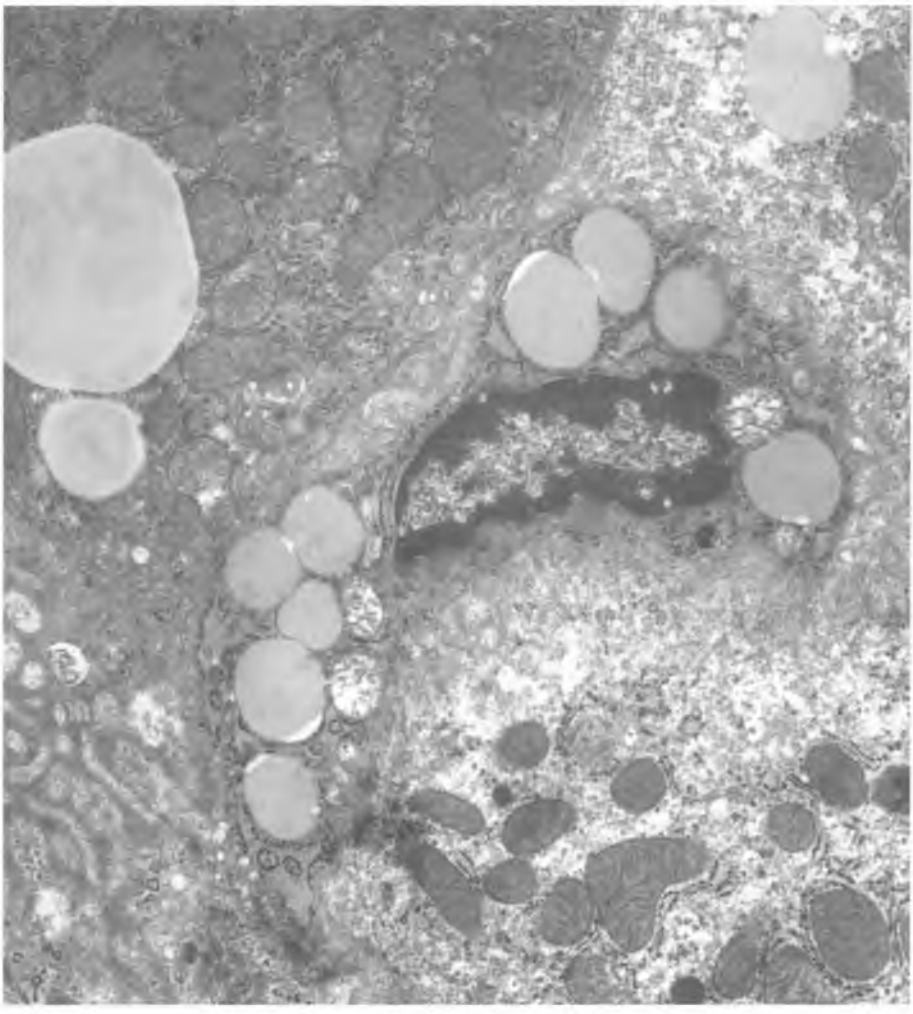

Fig. 3. Ito cell with extended rough endoplasmatic reticulum with floccular materials. TEM. Group III. Magn. 20000x

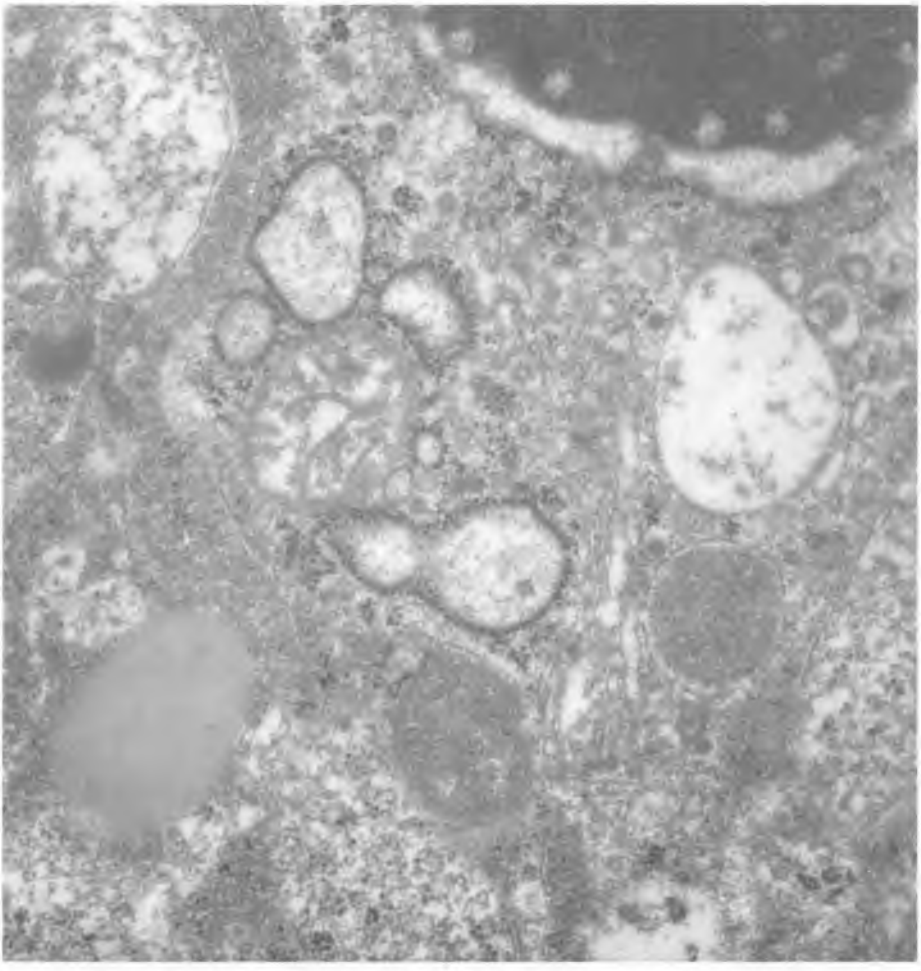

Fig. 4. Ito cell after IFN administration. We again observed numerous fat drops in Ito cell. TEM. Group IX. Magn. 7000x

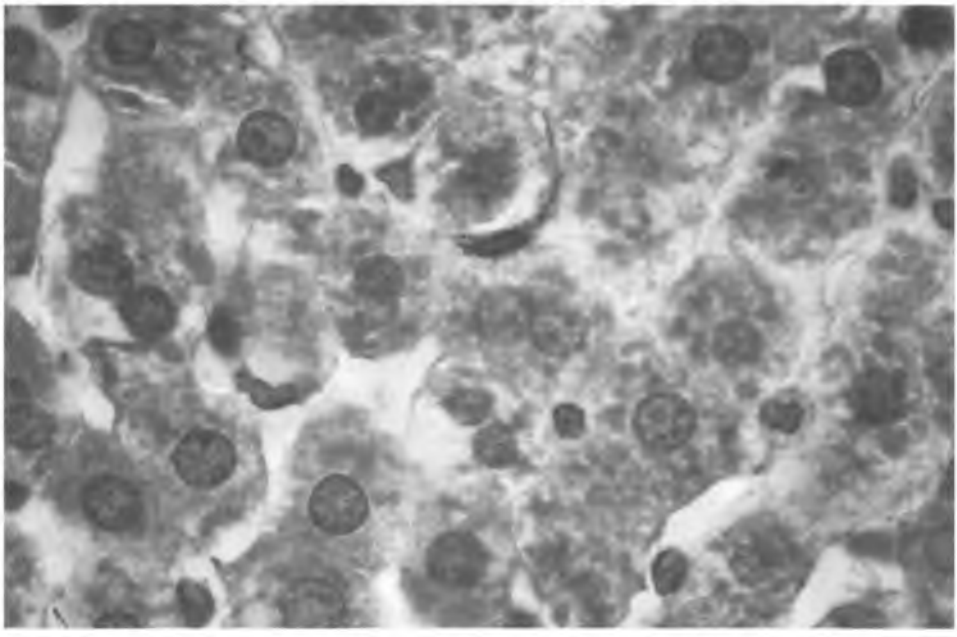

Fig. 5. An increased number of Ito cells after CCl_4 administration.
The LSA/peroxidase technique. Magn. 200x

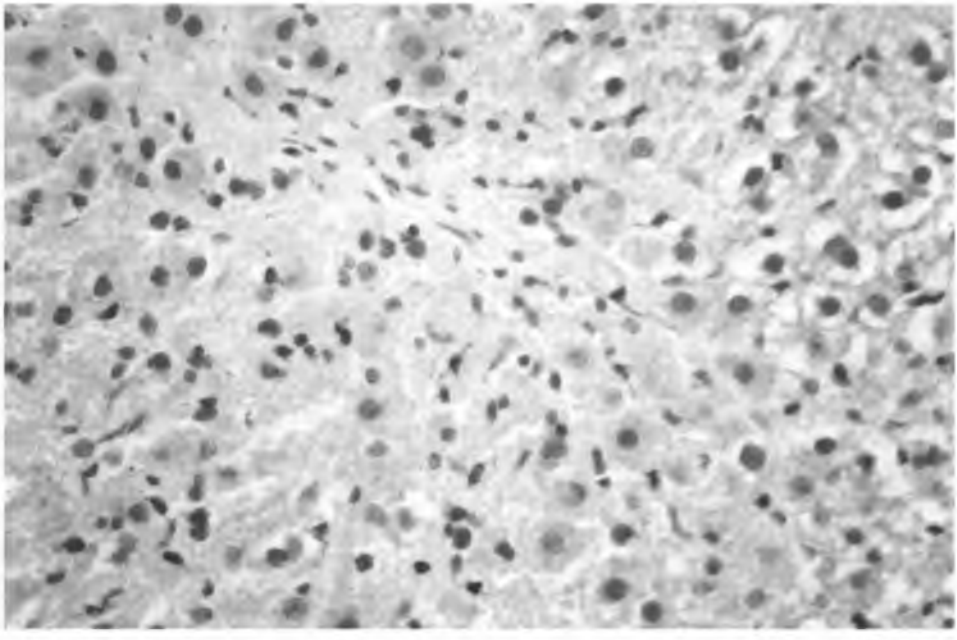

Fig. 6. The decreased number of Ito cell after IFN alfa administration.
The LSA/peroxidase technique. Magn. 200x