INTRANUCLEAR AGGREGATES OF FERRITIN IN LIVER CELLS OF MICE TREATED WITH SACCHARATED IRON OXIDE

Their Possible Relation to Nuclear Protein Synthesis

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ABSTRACT

Several months following parenteral injections of saccharated iron oxide into DBA/2J mice, granules rich in iron were found in nuclei of scattered parenchymal liver cells as well as in the cytoplasm. As seen in the light microscope, the intranuclear granules were brown; most of them measured between 0.5 μ and 1 μ in cross-section. They gave positive Prussian blue tests, and were not selectively stainable with pyronine. Electron micrographs of the granules showed closely packed aggregates of ferritin molecules, occasionally in paracrystalline order. The intranuclear collections were often surrounded by bands of material of moderate opacity. Scattered ferritin molecules and collections of such molecules were also present in the cytoplasm of many liver cells, but there seemed to be no quantitative relationship between intranuclear and cytoplasmic ferritin. Liver cells from untreated control mice failed to reveal intranuclear deposits of ferritin. Although the site of origin of the intranuclear aggregates of ferritin is unknown, the findings suggest the possibility that under suitable circumstances ferritin synthesis may take place within nuclei of liver cells—perhaps induced by the presence of colloidal iron.

Intranuclear granules containing iron have been demonstrated in liver cells of various species of laboratory animals (rabbits, rats, guinea pigs, mice) following parenteral injections of saccharated iron oxide (1). These granules have the appearance of hemosiderin, and react positively when Perls' test is applied. That various sorts of cells will take up colloidal iron compounds, such as iron-dextran and saccharated iron oxide, has been known for some time (1–6), and the transformation of colloidal iron-dextran into precipitates of hydrous ferric oxide within cells has been studied to some extent with the aid of electron microscopy (7).

The present report deals with observations on the nature and significance of iron deposits in nuclei of parenchymal liver cells of mice following parenteral administration of saccharated iron oxide (SIO). The observations concern large, intranuclear granules that are composed mainly of closely packed aggregates of ferritin molecules. Such intranuclear granules were found in livers of animals that had been given several injections of SIO some months prior to necropsy. The findings may be relevant to the problem of protein synthesis in the nucleus for reasons that will be pointed out.

MATERIAL AND METHODS

Ten female DBA/2J mice, weighing 20 to 30 grams, were given four intraperitoneal injections of 1 ml. of

a 17.5 per cent solution of saccharated iron oxide (N.F. VII). The four injections were given at intervals ranging from 2 days to 2 weeks during a period of 1 month. No further injections were given. The animals were fed a diet of Purina mouse pellets, and given tap water *ad libitum*. Animals were kept at least 5 months following cessation of treatment with SIO and were then killed at intervals, the last two mice being sacrificed nine months after the last injection of SIO. They remained healthy throughout the period of observation. Fifteen female DBA/2J mice that had not received any injections were used as controls. These controls received the same diet and also weighed 20 to 30 grams. Tissue from liver, spleen, and kidney was taken, fixed in Palade's fluid containing 3 per cent sucrose, dehydrated in graded concentrations of ethyl alcohol, and embedded in methacrylate (9 parts *n*-butyl, 1 part methyl methacrylate) containing 0.07 per cent uranyl nitrate and luperco. Sections were prepared for light and electron microscopy with a Porter-Blum microtome. Perls' Prussian blue method for iron was applied to the thick sections after removal of most of the methacrylate with xylol (7–8). The thin sections were mounted on carbon-coated specimen grids and examined in an RCA electron microscope, model EMU-3b. For comparison, blocks of tissue were also fixed in neutral formalin and embedded in paraffin. Sections from these blocks were stained for iron, and with pyronine and methyl green (9).

OBSERVATIONS

This description will be limited essentially to observations on parenchymal liver cells. Some other findings have been reported previously (7). Grossly, the livers had a reddish-brown color, but were not otherwise remarkable. On examination in the light microscope, the liver architecture was well preserved. The most definite pathological alteration was the presence of "hemosiderin" granules in cytoplasm and nuclei of many, but not nearly all parenchymal liver cells; also in the cytoplasm of many Kupffer cells and of endothelial cells lining intrahepatic centrolobular and portal veins. One other noteworthy finding was the prominence of large nucleoli (often several) in many liver cell nuclei (Fig. 1). Their presence was often strikingly evident in nuclei that also contained iron-positive granules. The nucleoli were selectively stainable with pyronine in sections treated with pyronine and methyl green according to standard methods (9). As seen in sections with a thickness of 0.5 to 1 μ, the number of iron-containing granules within nuclei varied from one to three or four (Fig. 1). Some of these granules measured up to 2 μ in diameter, while others were much smaller. The intranuclear iron-positive granules could not be stained selectively with pyronine. In this respect observations on livers taken from animals 5 months after the last injection and on livers from animals killed after 9 to 10 months were similar. Examination of liver cells in the electron microscope revealed (Figs. 2 and 3) that the intranuclear iron-positive granules contained closely packed aggregates of dense particles with dimensions and profiles characteristic of the iron hydroxide micelles of ferritin molecules as previously described (7, 10–17). In some instances these intranuclear aggregates contained regions of paracrystalline order (Fig. 3), the center-to-center distances of adjacent dense particles being uniform and measuring approximately 105 A, thus corresponding to the diameters of entire ferritin molecules as seen in the electron microscope. Material appearing as a zone or band of moderate density was often noted at the periphery of the intranuclear ferritin aggregates (Fig. 3). Similar material of unknown identity was present in the interior of some ferritin aggregates.

The hydrous ferric oxide present in SIO as well as in other iron compounds (*e.g.* iron-dextran) has a micellar structure unlike that present in ferritin; it can be distinguished from the latter in the electron microscope (7). Micelles of $Fe_2O_3 \cdot x\ H_2O$ can be found in the nuclei of a variety of cells within a few days following administration of SIO (7). By contrast, the intranuclear iron-positive granules described in this report appeared to contain iron as part of ferritin molecules. On the other hand aggregates of SIO iron hydroxide micelles were frequently found in the *cytoplasm* of liver cells, often in close proximity to ferritin molecules. Such aggregates were often enclosed by single membranes. This finding was reported earlier (7).

Many sections of liver cells from fifteen untreated control mice were examined in the electron microscope. No intranuclear aggregates of ferritin were found in these though cytoplasmic aggregates were occasionally encountered. Prussian blue tests revealed no intranuclear iron.

DISCUSSION

The presence of intranuclear aggregates of ferritin in liver cells seems unusual for it has been shown

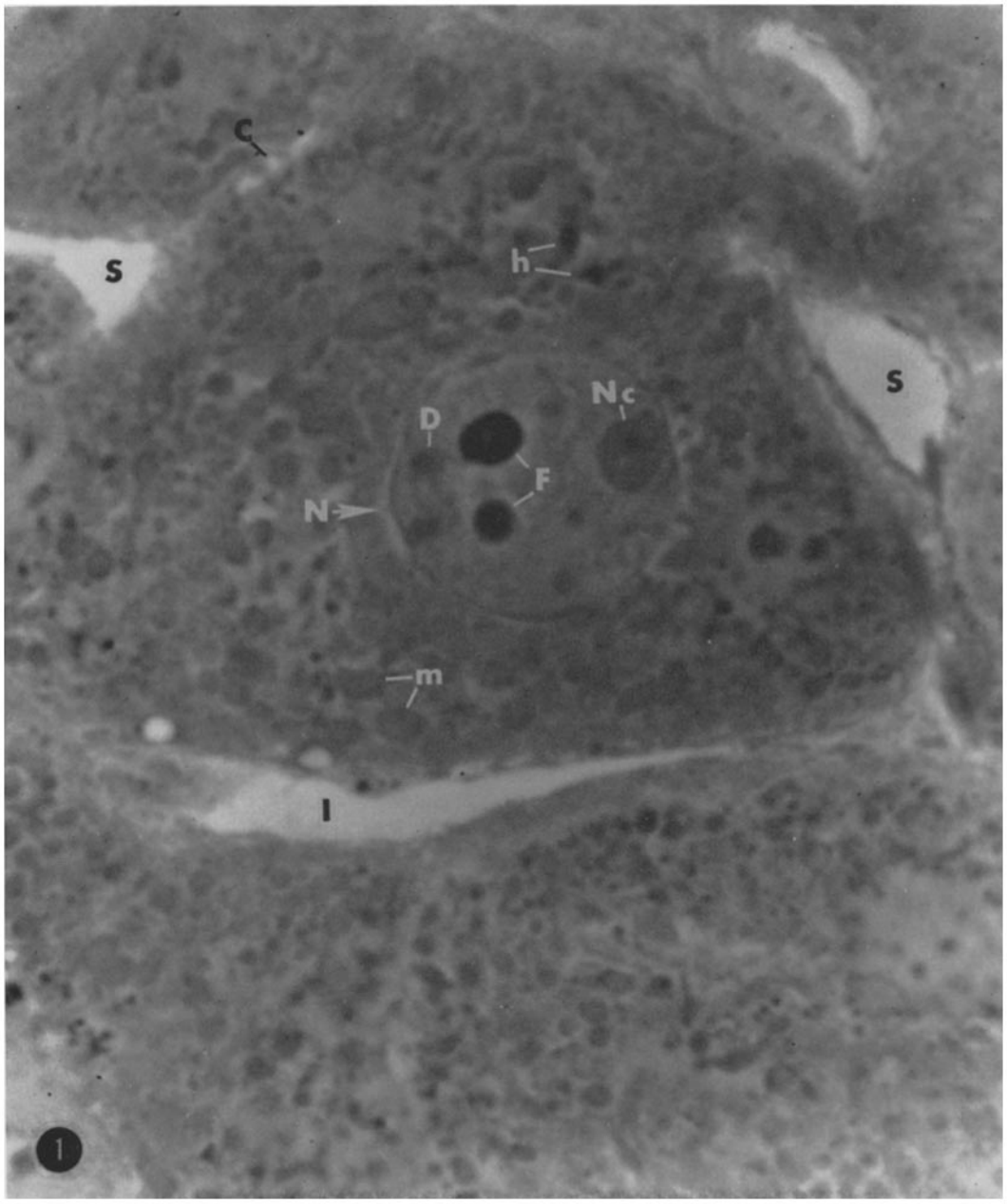

FIGURE 1

Photomicrograph of liver cells from mouse treated with saccharated iron oxide 6 months prior to necropsy. The cell in the center has a nucleus (*N*) with two granules (*F*) that gave strongly positive Prussian blue tests. There is a nucleolus (*Nc*). Several smaller intranuclear granules—such as that labeled *D*—probably represent condensed chromatin (chromosomal?). Cytoplasmic hemosiderin granules (*h*), and mitochondria (*m*), are also indicated. *S*, sinusoids; *C*, bile canaliculus; *I*, intercellular space, probably part of a sinusoid. Potassium ferrocyanide reaction with basic fuchsin counterstain. × 3,250.

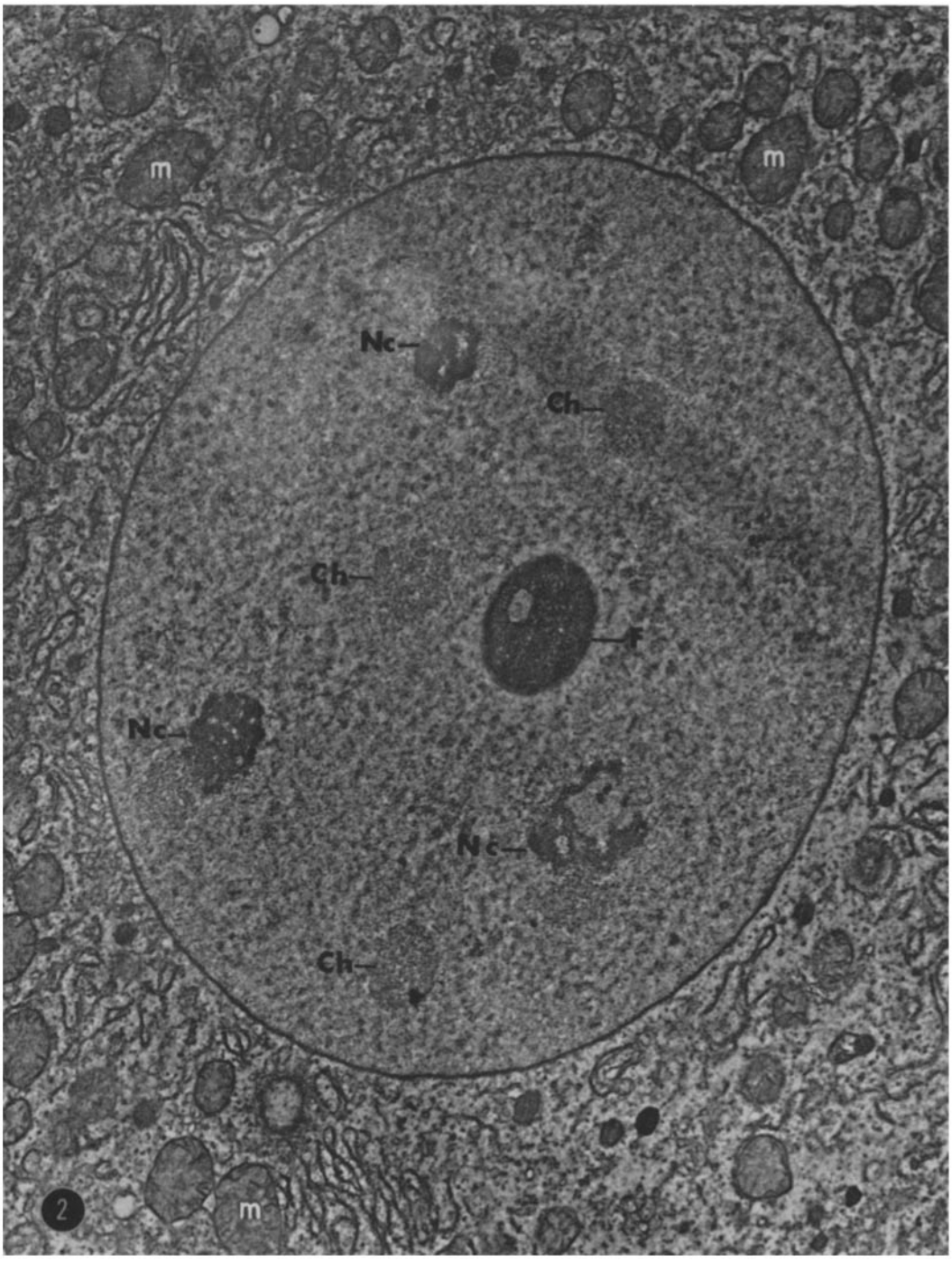

FIGURE 2

Electron micrograph of part of liver cell from mouse treated with saccharated iron oxide 6 months prior to necropsy. Within the confines of the nucleus the profiles of several structures can be seen. The profile at *F* represents an iron-positive granule; this is shown at higher magnification in Fig. 3. *Nc*, nucleoli; *Ch*, condensed chromatin (chromosomal?); *m*, mitochondria. × 9,100.

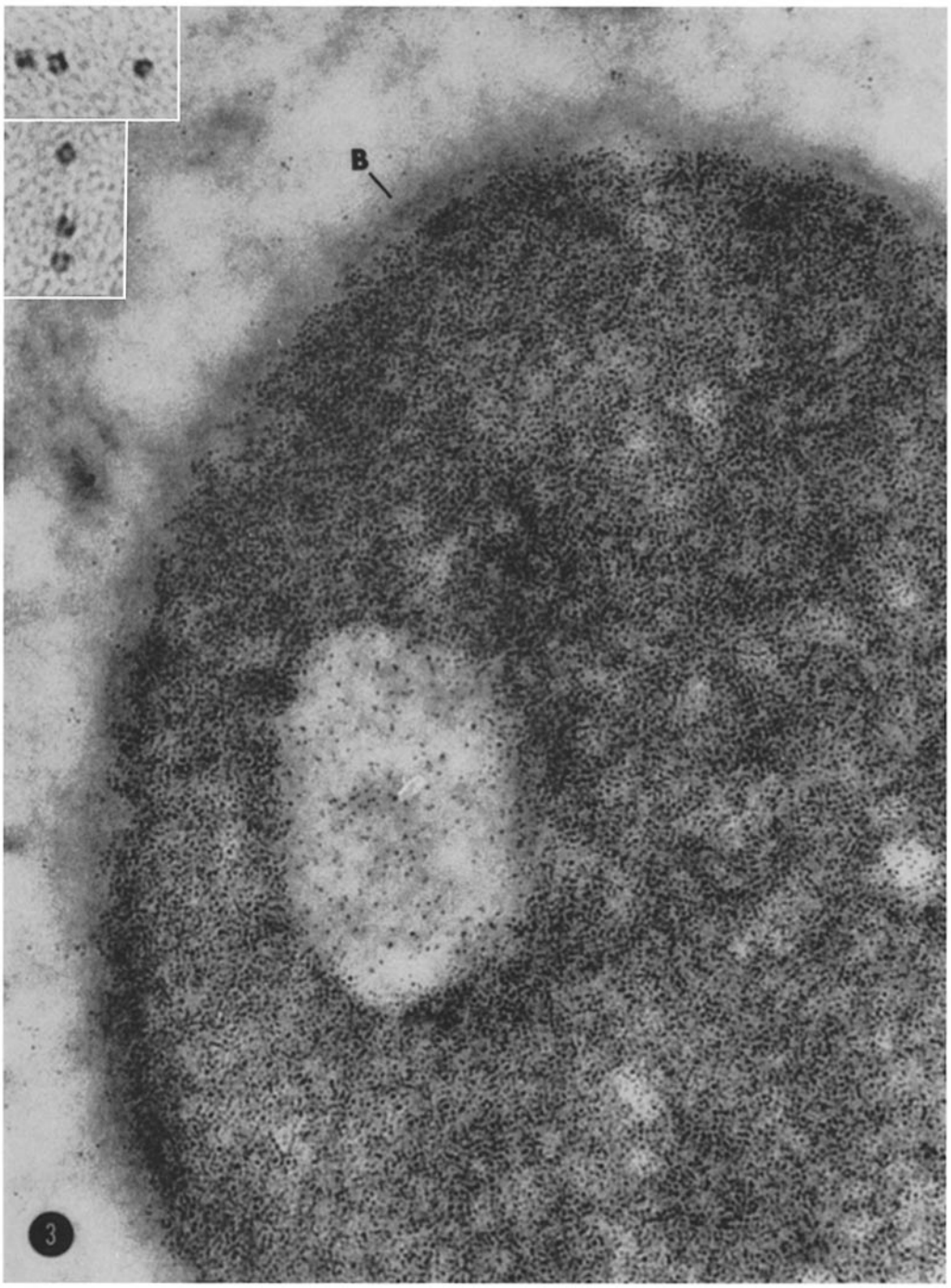

FIGURE 3

Detail of body labelled *F* in Fig. 2. Note that this body contains innumerable dense particles that are closely packed. These particles represent the cores of ferritin molecules, as previously described, having the following attributes: Their average diameter is about 60 A; many are arranged in paracrystalline order, with center-to-center distances averaging about 105 A; at higher magnification they show the substructure that characterizes the iron hydroxide micelles of ferritin molecules (see insets). Note the opaque zone (*B*) that surrounds the aggregate of dense particles. × 100,000. Inset, × 417,000.

previously that ferritin is generally a cytoplasmic constituent of liver cells (11, 14, 16–19). Judging from previous work with the electron microscope (7, 11–19, 25–26), and in line with current concepts of protein synthesis (30, 35), one would think that ordinarily ferritin is synthesized in the cytoplasm. Also, it is clear from much experimental work that an increased uptake of iron (in various forms) by liver cells stimulates the production of ferritin, and that repeated parenteral administration of iron results in a large increase of extractable ferritin in the liver (20–22). Electron micrographs clearly substantiate these biochemical findings (7, 11, 12). One can say, therefore, that various iron compounds, including SIO, can induce synthesis of a specific protein, apoferritin. For diverse reasons it is highly probable that in the process of ferritin synthesis apoferritin is synthesized first; iron is then incorporated into apoferritin molecules (21, 23, 24).

Although the evidence presented does not provide an answer to the question of where the intranuclear ferritin was synthesized, three possibilities will be considered briefly.

One might postulate that the ferritin molecules in intranuclear granules were synthesized in the cytoplasm, transported into the nucleus, and then somehow brought together through the nuclear meshwork to form closely packed aggregates. But such a sequence seems improbable for mechanical reasons; and it is not supported by present knowledge of the functional interaction of cell components. The two other possibilities are: (*a*) incorporation of cytoplasmic ferritin aggregates into the nucleus during cell division, and (*b*) intranuclear synthesis of ferritin. If the "incorporation" hypothesis were correct, one would expect to find cytoplasmic organelles containing ferritin (such as siderosomes) with appropriate dimensions and architecture in nuclei of liver cells. But such organelles have not been found in this study. Furthermore, in hemosiderosis or hemochromatosis in man, intranuclear iron-positive granules are relatively infrequent in liver cells that are laden with ferritin. To make the "incorporation" hypothesis more plausible, one might assume that extraordinary numbers of liver cells undergo division as a result of a few injections of SIO. However, there is no evidence for such an effect.

That ferritin might—under certain conditions—be synthesized in the nucleus seems possible in the light of biochemical evidence on intranuclear protein synthesis provided by Allfrey and Mirsky and their coworkers (27–29). In extensive studies these investigators have shown that isolated nuclei (*e.g.* from calf thymus) can incorporate amino acids into proteins under the influence of DNA. The mechanism whereby this synthesis occurs is not known at present. On the basis of current hypotheses on the relation of nuclear DNA to cytoplasmic RNA, one may suppose that RNA "coded" for apoferritin synthesis originates in the nucleus and is subsequently moved to the cytoplasm (36–38). Thus, one of the early events in the induction of apoferritin synthesis by iron may be the induction of RNA template synthesis in the nucleus, or, alternatively, the activation of pre-existing RNA templates in the nucleus. It may also be recalled that certain adenoviruses, which are composed of DNA and protein, form intranuclear crystals (31–34). In this instance the synthesis of viral protein in the nucleus is highly probable.

The complete synthesis of ferritin might occur in the nucleus only when there are sufficiently large sources of iron (in a suitable state) in the nuclear matrix. However, it is clear that much more evidence is required in order to determine the genesis of the intranuclear collections of ferritin. In particular, more knowledge is needed on the rapidity of turn-over of iron in liver cell nuclei and on the time required for the appearance of the intranuclear aggregates of ferritin.

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