SHORT PAPER

Short Title: Hepatic Myelolipoma in a Swan Goose

Hepatic Myelolipoma and Amyloidosis with Osseous Metaplasia in a Swan Goose (*Anser cygnoides*)

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Summary

An adult Swan Goose (*Anser cygnoides*) kept in a zoological garden had gross hepatic enlargement with extensive ill-defined white foci. Microscopically, the hepatic lesions were composed of a mixture of adipocytes and myeloid cells. The goose was also affected with systemic amyloidosis and there were areas of osseous metaplasia associated with deposition of amyloid within the liver.

Keywords: Swan Goose; liver; myelolipoma; amyloidosis; osseous metaplasia

Myelolipomas are unusual neoplasms that are composed of a mixture of mature lipocytes and haemopoietic cells and usually occur in the adrenal gland, spleen and liver in man, non-human primates, *Felidae*, cattle and other animals (Lack, 2000; Capen, 2007; Stalker and Hayes, 2007; Valli, 2007). There are only three case reports describing myelolipomas in birds (Andreasen *et al.*, 1995; Latimer and Rakich, 1995; Ozaki *et al.*, 1996) and hepatic myelolipoma has been reported in one saffron toucanet (*Baillonius bailloni*) (Latimer and Rakich, 1995).

Amyloidosis is a disorder that is characterized by extracellular deposition of amyloid, which is composed of non-branching fibrils that form β-pleated sheets as identified by X-ray crystallography and infrared spectroscopy (Snyder, 2007). Amyloid

appears as an amorphous hyaline material in haematoxylin and eosin (HE)-stained sections and is specifically stained by Congo red. Amyloid stained with Congo red also exhibits a characteristic apple-green birefringence by polarized light microscopy. Cartilaginous and osseous metaplasia within deposits of amyloid has been reported to occur in man and dogs (Ramos-Vara *et al.*, 1998), but never in birds. The present report documents the pathological features of myelolipoma and amyloidosis with osseous metaplasia in the liver of a Swan Goose (*Anser cygnoides*).

An adult female Swan Goose kept in a zoological garden became debilitated and was later found dead. On necropsy examination the carcass showed no appreciable autolysis. The liver was diffusely enlarged, firm in consistency and had locally extensive mottled, ill-defined white foci in both hepatic lobes (Fig. 1). There were small deposits of chalky white material on the serosal surface. The spleen was moderately enlarged and firm and the cut surface had a waxy appearance. The kidneys were also mildly enlarged, pale and firm and their cut surfaces were glossy with diffuse accumulation of white viscous material. The pericardium had a light covering of white chalky flakes consistent with visceral gout. Obstruction by an egg, rupture of the oviduct and ulcerative pododermatitis (“bumblefoot”) of the right footpad were also found. The organs, including the liver, spleen, kidneys, heart, lung, brain, gastrointestinal tract, pancreas, thyroid glands, parathyroid glands, adrenal glands,

ovaries and right footpad were fixed in 20% neutral buffered formalin and embedded in paraffin-wax. Sections (4 μm) were stained with HE. Immunohistochemistry (IHC) was performed on selected sections using the streptavidin-biotin (SAB) peroxidase method with a commercial kit (Nichirei Corp., Tokyo, Japan). The primary antibodies were anti-human muscle actin (HHF35; diluted 1 in 10, ENZO Diagnostics Inc., NY) and anti-human amyloid A (mc1; diluted 1 in 1000, DakoCytomation, Denmark).

Microscopically, hepatic lesions consisted of a locally extensive proliferation of mature adipocytes with an accumulation of haematopoietic cells, including erythroblasts, myelocytes, eosinophilic myelocytes and heterophils (Figs. 2A, B). These foci showed infiltrative growth with an indistinct border and often interdigitated with the surrounding hepatic cords. Intracytoplasmic granules and non-segmented round nuclei of eosinophilic myelocytes and heterophils were clearly observed by Giemsa stain. Osseous metaplasia was observed within the foci of myelolipoma (Fig. 2C). Immunohistochemically, hepatic stellate cells and a few proliferative adipocytes were positively labelled by antibody HHF35 (Fig. 2D).

Additionally, amorphous eosinophilic material was deposited throughout the liver, spleen, kidneys, heart, lung, proventriculus, duodenum, ileum, colon, pancreas, thyroid glands and parathyroid glands. In the liver, these deposits were mainly found in the connective tissue of hepatic triads, vascular walls, subcapsule and the space of Disse.

The eosinophilic material stained weakly with Congo red. Direct fast scarlet (Muto Pure Chemicals, Tokyo, Japan), which has been used previously for amyloid staining in man and a vervet monkey (Fujita *et al.*, 2006; Nakamura *et al.*, 2008), stained the deposits orange-red (Fig. 2E) and showed apple-green birefringence under polarized light. In a further section pre-treated with potassium permanganate, the deposits were not stained with Congo red and lost birefringence under polarized light. Immunolabelling with anti-human amyloid A antibody was negative. In addition, occasional foci of osseous metaplasia associated with the regions of amyloid deposition occurred in the liver (Fig. 2F).

Marked deposition of amyloid was also observed in the splenic cord, interstitium and trabeculae of the spleen. Mild to moderate amyloidosis was found in the interstitium and arterial walls of the kidneys, proventriculus, duodenum, ileum, colon, thyroid glands, parathyroid glands and adrenal glands. A small amount of amyloid was sometimes deposited in the walls of arterioles of the heart and in the interstitium, arterial wall and subcapsule of the pancreas. The lesion of the right footpad was composed of mild heterophilic and lymphoplasmacytic infiltration, fibrovascular proliferation, acanthosis and hyperkeratosis with ulceration.

Myelolipomas in mammals are composed of well-differentiated adipocytes with a variable admixture of myeloid cells (Capen, 2007; Stalker and Hayes, 2007). The

tumour frequently has an irregular edge with adipocytes interdigitating with normal hepatocytes (Head *et al.*, 2003). Metastasis of myelolipomas to other organs has not been reported, and the tumours are typically found as incidental lesions unassociated with clinical signs. All three previous avian cases of myelolipoma had heterophil predominance in the haematopoietic component of the tumour (Andreasen *et al.*, 1995; Latimer and Rakich, 1995; Ozaki *et al.*, 1996). The findings in the liver of the present case were consistent with those of hepatic myelolipoma. Human adrenal myelolipoma is usually associated with endocrine disorders such as Conn's syndrome, 21-hydroxylase deficiency, hormonally active adrenal neoplasms and adrenocortical hyperplasia (Weiss and Goldblum, 2001a). Myelolipoma is considered to be a hormonally induced metaplasia of the adrenal stromal cells or primitive mesenchymal cells (Bishop *et al.*, 2006; Weiss and Goldblum, 2001a). Chang *et al.* (2002) reported that adipocytes and myeloid cells in human adrenal myelolipoma show the same clonal cytogenetic abnormality which suggests that adipocytes and myeloid cells may proliferate in a clonal manner. In addition, nonrandom X-chromosome inactivation was found in both myeloid elements and the adipose tissue of human adrenal myelolipomas, suggesting that the haemopoietic components and the adipose tissue of myelolipomas clonally proliferate and originate from common, pluripotent stem cells (Bishop *et al.*, 2006). However, the origin of hepatic myelolipoma is obscure. Hepatic stellate cells, also

termed fat-storing cells, hepatic lipocytes and Ito cells, express muscle actin in mammals (Hines *et al.*, 1993; Grinko *et al.*, 1995; Ijzer *et al.*, 2006). A report concerning broiler chickens also stated that hepatic stellate cells in normal livers were reactive for HHF35 (Handharyani *et al.*, 2001). In the goose reported here, hepatic stellate cells and proliferative adipocytes were positively labelled by anti-HHF35. These findings suggest that proliferative adipocytes may either have differentiated from hepatic stellate cells or originated from a pluripotent stem cell which is common to both hepatic stellate cells and haematopoietic cells.

Caged wild waterfowl, especially the *Anatidae*, to which Swan Goose belongs, are prone to systemic amyloidosis (Landman *et al.*, 1998). The predilection organs of amyloid deposition are the liver, spleen, kidneys and small intestine. In addition to these organs, amyloid is also distributed in the thyroid and pancreas in waterfowl (Sato *et al.*, 1981; Tanaka *et al.*, 2008). In the present case, amyloid treated with potassium permanganate lost reactivity for Congo red and birefringence consistent with amyloid A (AA). However, the amyloid of this goose was not labelled with anti-human amyloid A antibody. Tanaka *et al.* (2008) considered the possibility that the amyloid deposits of swans were admixed with another type of amyloid because in several cases of amyloidosis of mute swans (*Cygnus olor*) birefringence was not completely lost in sections pre-treated with potassium permanganate despite a positive reaction with AA

antibody. In addition, compared with human AA, duck AA has a major difference in its first 20 amino acid residues, including five additional amino acids at the C-terminus (Gorevic *et al.*, 1977). Therefore, avian amyloid protein should be thought of as different to human AA. In birds, most amyloidosis is found in association with chronic infections (Landman *et al.*, 1998). Systemic amyloidosis in waterfowl is frequently coincident with bumblefoot, a chronic inflammatory disease involving bacterial infection (Sato *et al.*, 1981; Tanaka *et al.*, 2008). Although the precise relationship between amyloid deposition and chronic inflammation remains unclear, the present case was considered to show systemic amyloidosis secondary to bumblefoot.

Two possibilities may explain the presence of osseous metaplasia in the liver of this case. Firstly, metaplastic bone formation is observed in adrenal myelolipoma of man and other mammals (Lack, 2000; Capen, 2007). Secondly, metaplastic cartilage and bone within amyloid deposits have been found in pulmonary amyloidosis, nodular amyloidosis in the breast ("breast amyloid tumour") and amyloid-producing plasmacytoma in man (Karasick *et al.*, 1996; Kuhn III *et al.*, 1996; Weiss and Goldblum, 2001b). Two dogs with rectal plasmacytoma also had an associated intestinal amyloidosis (Ramos-Vara *et al.*, 1998). The mechanism of osseous metaplasia in amyloidosis and myelolipoma remains to be elucidated. Several authors have discussed the possibility that ossification associated with amyloid is probably a non-specific,

reactive lesion (Yokoo and Nakazato, 1998).

In summary, the present case was considered to be one of severe hepatic myelolipoma and systemic amyloidosis associated with bumblefoot. The amyloid deposition in the kidneys led to the development of visceral gout. Two distinct lesions, myelolipoma and amyloidosis, were present in the liver. Both myelolipoma and osseous metaplasia in amyloid are rare conditions in avian species. This report presents the first case of each lesion in a Swan Goose.

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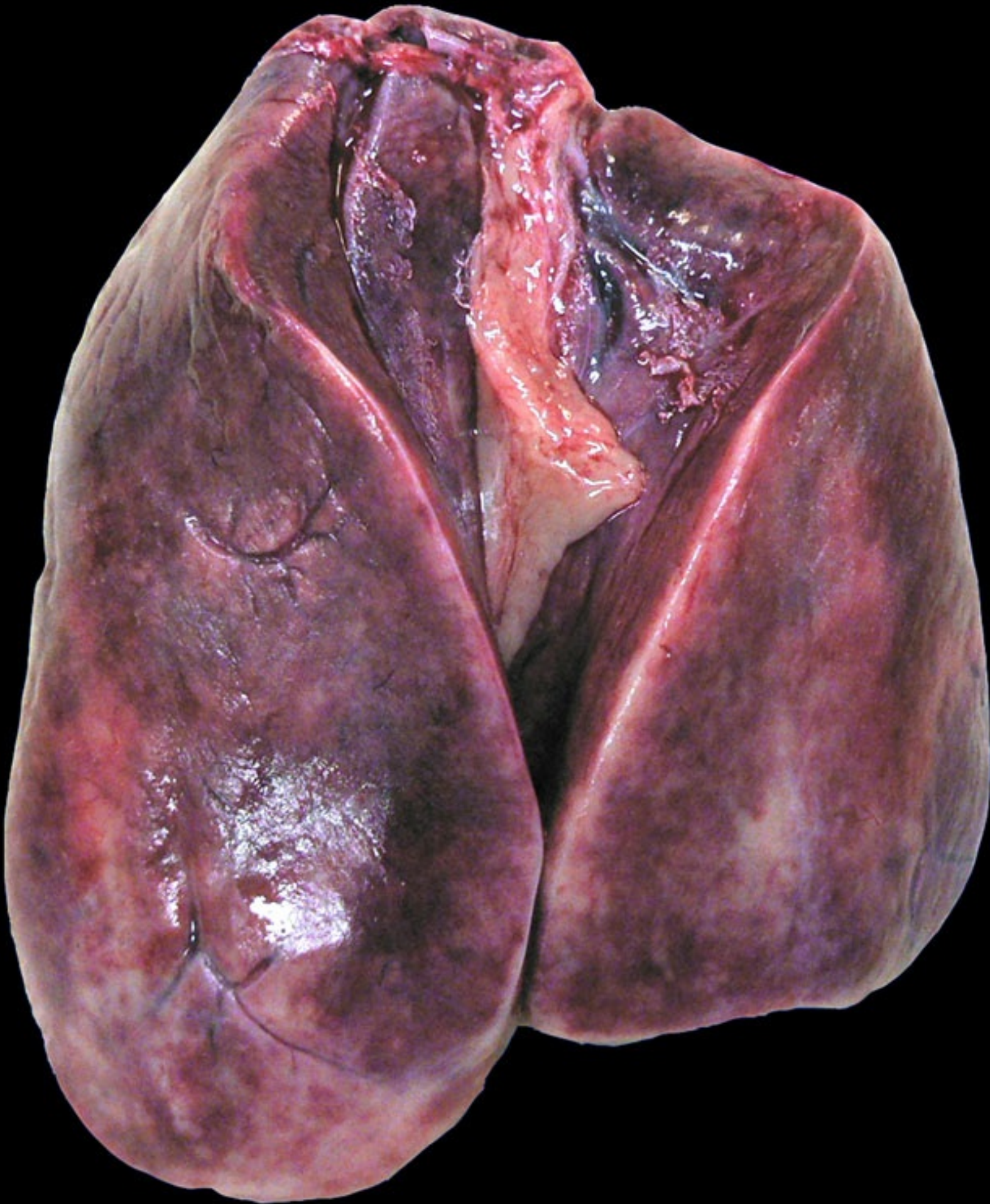
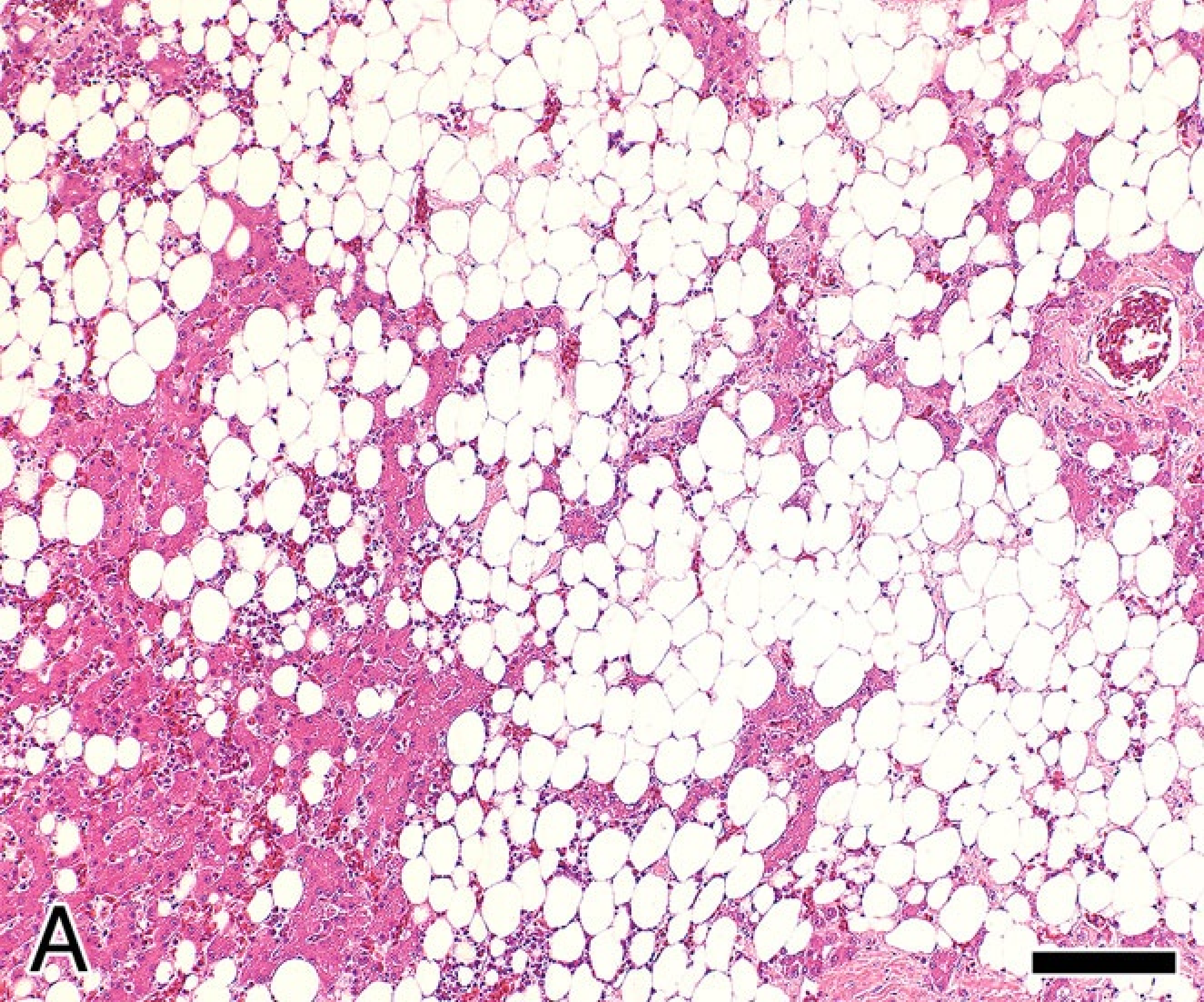
Figure Legends

Fig. 1. Diffuse enlargement of the liver with ill-defined locally extensive white foci.

Fig. 2. (A) Infiltrative proliferation of adipocytes in hepatic parenchyma. HE. Bar, 100 μm. (B) Myeloid cell lineage, including erythroblasts (arrowheads), myelocytes (small arrowheads) and eosinophilic myelocyte (arrow). HE. Bar, 15 μm. (C) Multifocal bone formation in the parenchyma and subcapsule. Bar, 60 μm. (D) An adipocyte is positive for HHF-35 antigen (arrowhead) as are hepatic stellate cells (arrows). IHC. Bar, 15 μm. (E) Amyloid deposition in the vascular wall and adjacent fibrous connective tissue. Direct fast scarlet stain. Bar, 45 μm. (F) Osseous metaplasia (arrowheads) in amyloid. Direct fast scarlet stain. Bar, 60 μm.

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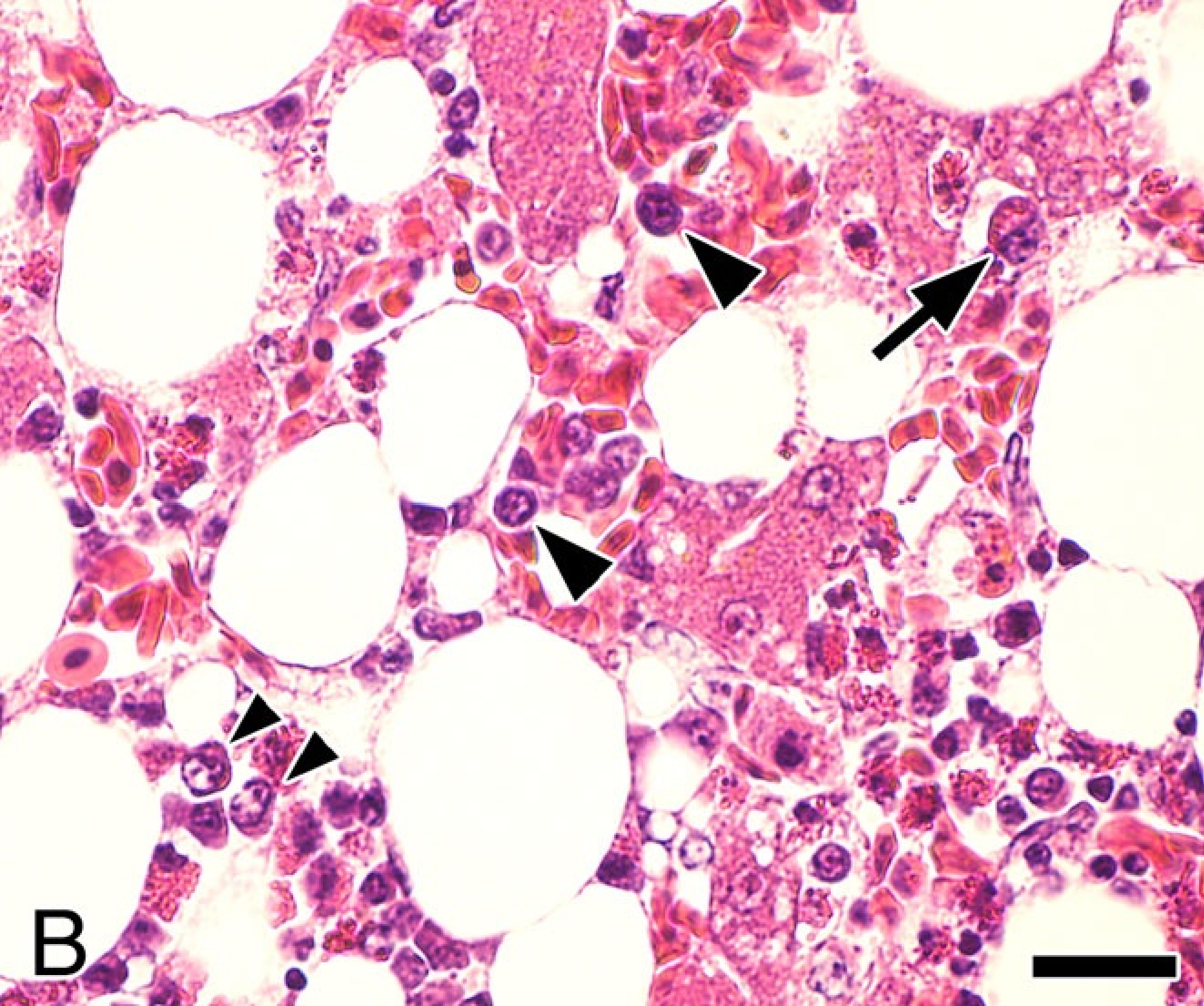
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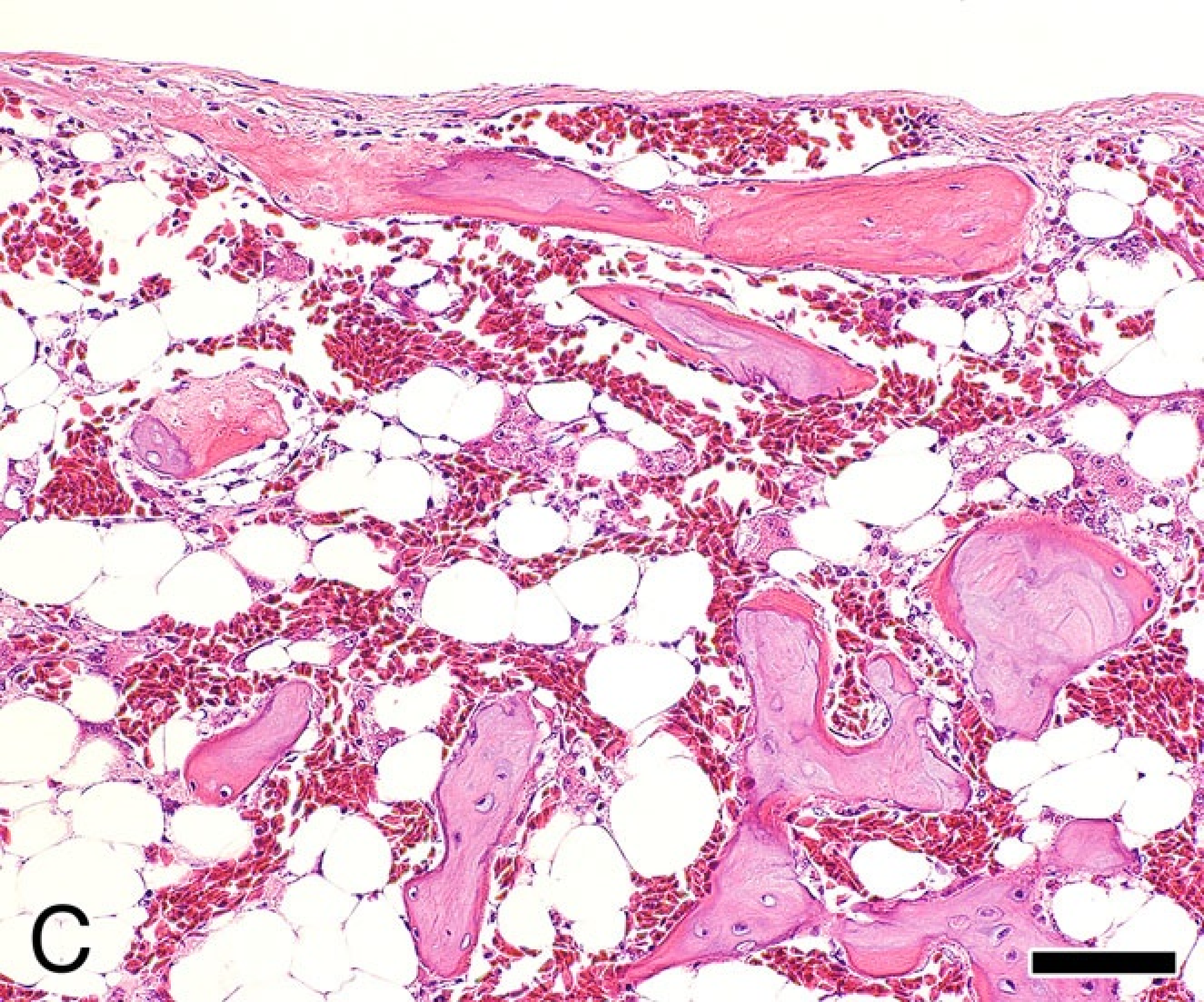
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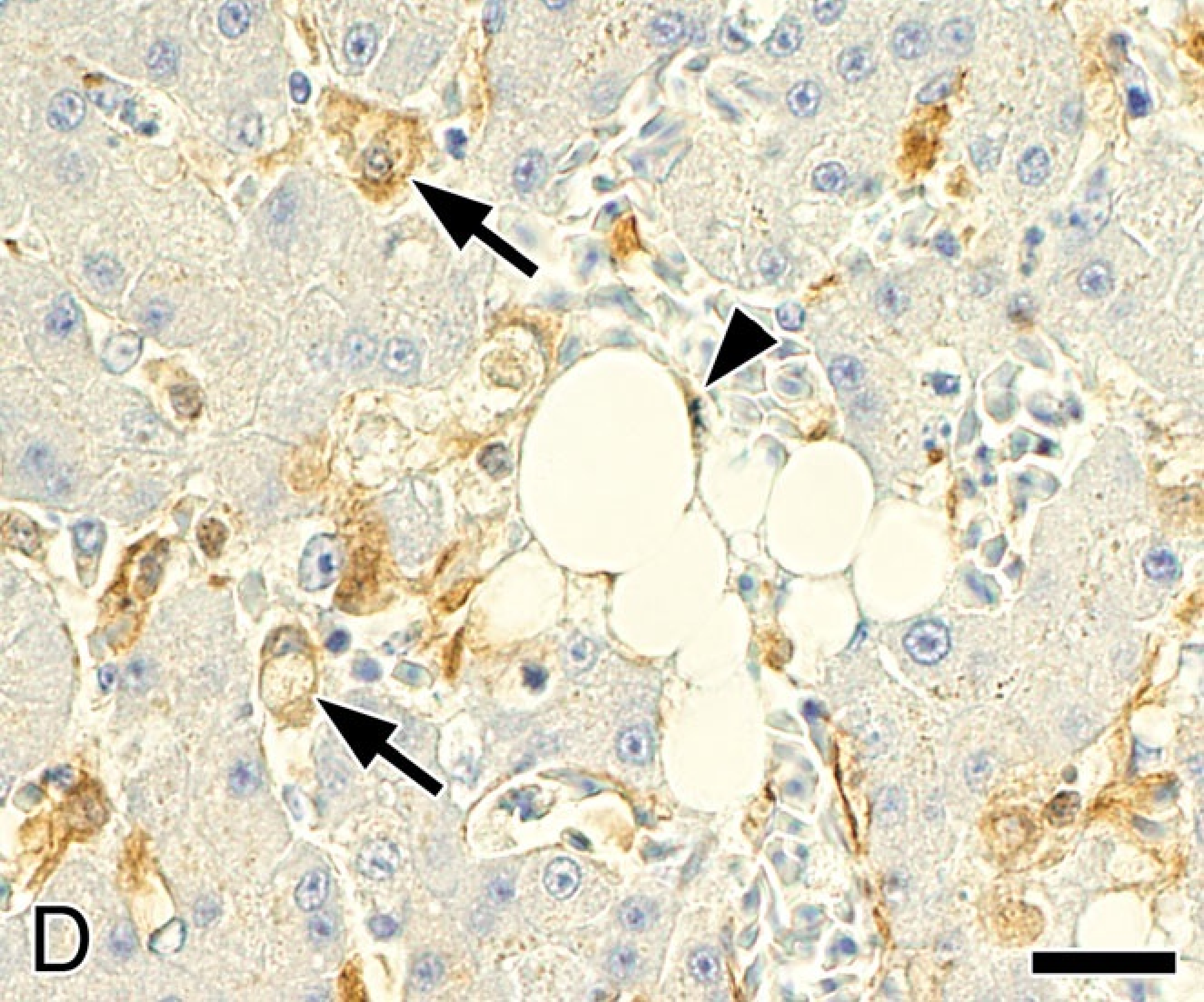
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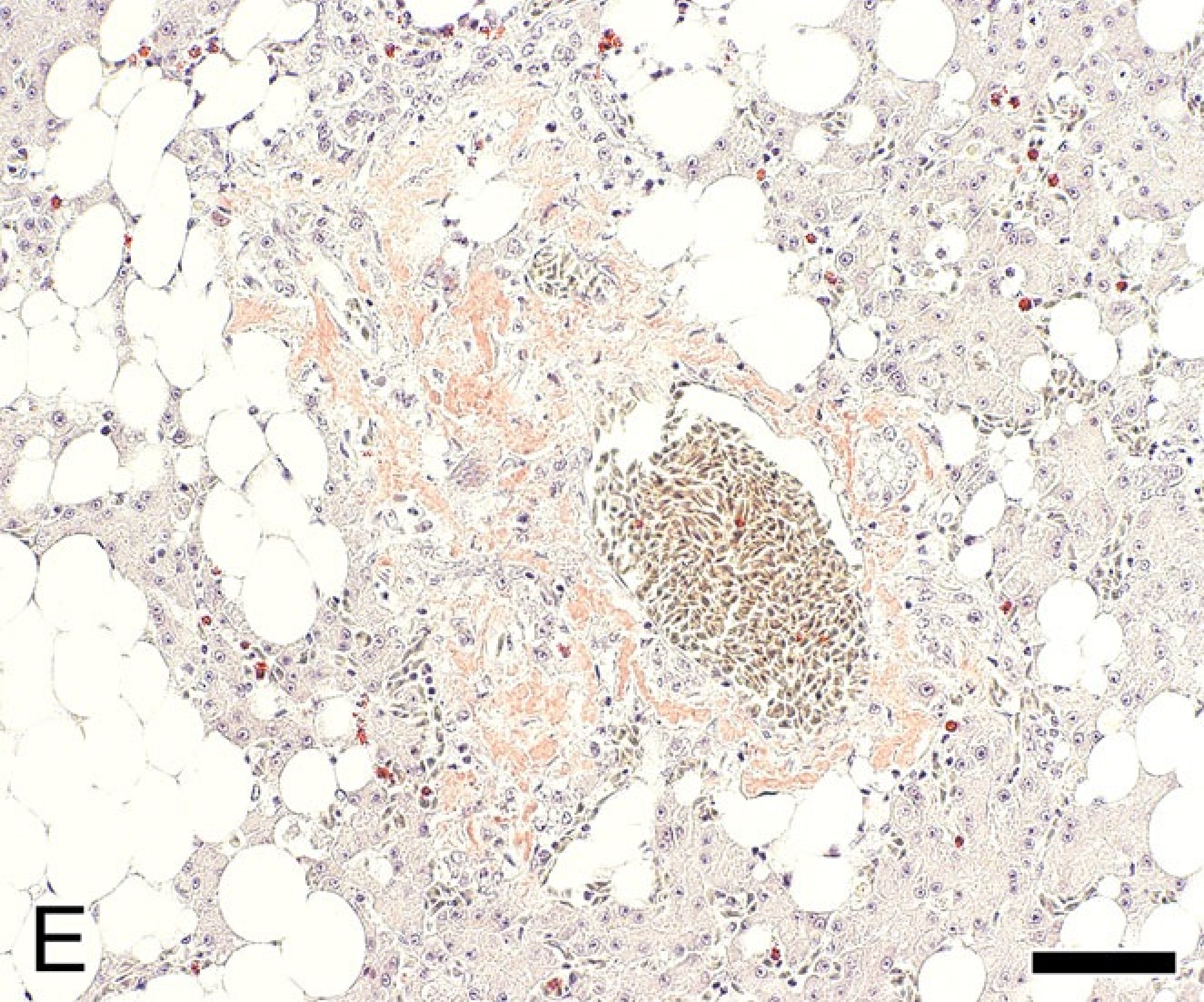
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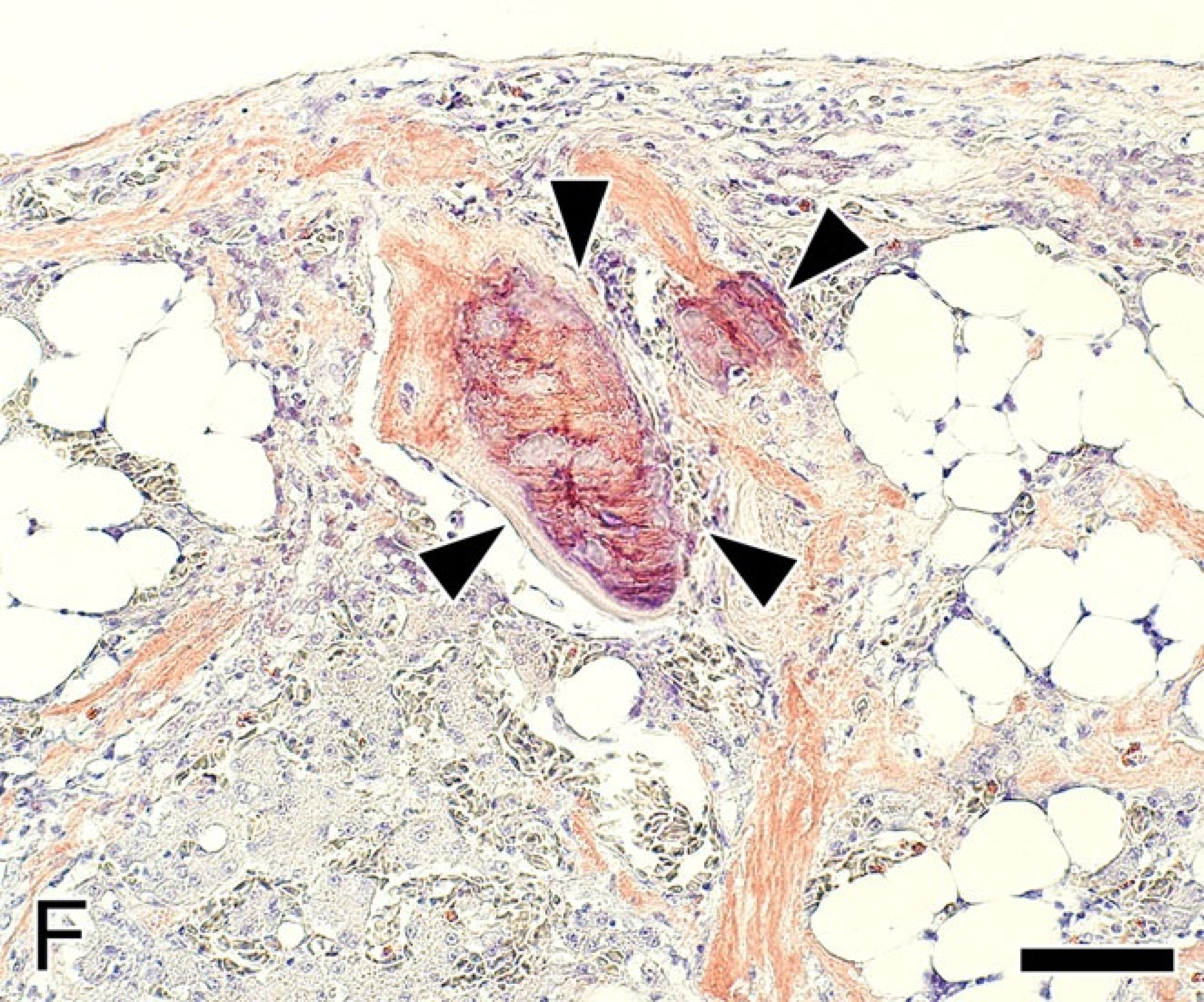
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