Protoporphyrinic Disorder in Livers of Broiler Chickens

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ABSTRACT. Two cases of protoporphyria (PP) disorder detected in a 60- and 65-day old female chicken were described. The gross lesions were restricted to the livers which were enlarged and dark green to black in color. Histologically, dark brown granules were found in hepatocytes, Kupffer cells, macrophages, sinusoids, bile canaliculi, and bile ducts. These granules, as seen in smears and sections of livers, were red under a fluorescence microscope and exhibited bright birefringence with a centrally located dark Maltese-cross by polarized light. Ultrastructurally, these granules consisted of aggregates of needle-like crystals in a radial arrangement. Fluorometrically, extracted level of PP in the affected liver was determined to be 390.6 μg per gram of wet tissue. Spectrofluorometric scans of liver extract and PP standard were almost identical. -- KEY WORDS: broiler chicken, protoporphyric disorder.

Porphyria has been reported in various animals and humans [1, 6, 7], and has been seen periodically in broiler chickens in U.S.A. [5]. Drug-induced protoporphyria (PP) accumulations have been described in mice [4], and pigs [11]. Protoporphyric livers contain dark brown granules within hepatocytes, Kupffer cells, and bile ducts [1, 4, 5, 16, 17]. These granules show red fluorescence and birefringence [4-6, 17], and ultrastructurally consist of aggregates of needle-like crystals [1, 4, 5, 17]. This note describes the pathology and the identification of pigment accumulating in the livers of two female broiler chickens in Japan.

Greenish-blackish livers were collected from two cases of broiler chickens subjected to meat inspection. Case 1 was one of 3,005 sixty-five-day-old Chunky chickens which were examined at a processing plant on August 16, 1993. Case 2 was one of 4,074 sixty-day-old Cobb chickens detected on April 28, 1994. The livers of affected chickens were enlarged and dark green to black in color. The gall bladder in each case was black in color and contained coarse black precipitates. In unfixed, unstained smears of the livers and precipitates, pigment granules were demonstrated as starlight with a Maltese-cross under polarized light. Other tissues were grossly unremarkable, and other birds in the same flock were unaffected. Tissues from each liver were fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, and sectioned at 5 μm. Sections were stained with hematoxylin and eosin (HE), Mallory’s iron, rhodanine, or Hali’s bile method. Selected sections were bleached with potassium permanganate. Histological examination of HE-stained liver sections of two cases showed dark brown pigment masses occupying dilated bile canaliculi (Fig. 1) and sinusoids, and bile ducts. This pigment was commonly present as a group of large round granules measuring 15 μm in diameter. Smaller pigment granules were observed in the cytoplasm of hepatocytes, Kupffer cells (Fig. 2) and macrophages in portal triads. Foci of necrosis of hepatocytes were scattered in the parenchyma with infiltration of mononuclear cells. Proliferation of bile ducts with heterophil and lymphocytic infiltrates were also present in portal triads. The granules seen in HE-stained sections exhibited red fluorescence by fluorescence microscopy (excitation 405 nm light range, emission 490-740 nm range). Examination of large globular pigments by polarization microscopy in stained sections revealed a bright orange-red birefringence with a centrally located dark Maltese-cross (Fig. 3) as described for PP [5, 7, 11, 16]. Smaller granules were presented as a pin point birefringence. These pigment granules were negative for bile, iron, or copper, but were bleached by potassium permanganate.

Pieces of formalin-fixed liver from case 2 were refixed in 2.5% glutaraldehyde and 1% osmium tetroxide and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a JEM-1200 EX electron microscope. Ultrastructurally, the granules consisted of aggregated hair, needle-like or curved needle-like crystals, often radially arranged in clusters within hepatocyte cytoplasm and bile canaliculi (Fig. 4), and were found in Kupffer cell lysosomes. These were morphologically identical to those described for PP [4, 5, 17].

Extracts obtained from case 2 and normal livers in the same flock were solubilized in 1.5 N HCl and compared with a PP standard (porophyrin IX, Sigma Chemical Co., U.S.A.) by fluorescence spectroscopy (excitation 405 nm light range, emission 490-740 nm range) using a spectrofluorometer (Shimadzu RF-1500 PC, Kyoto, Japan) to quantitate the hepatic PP content. The PP level of the affected liver was 390.6 μg/g of wet tissue, as compared with the control liver PP content of 1.64 μg/g of wet tissue. The fluorescence spectra of the affected liver and the PP standard were almost identical, with the highest peak occurring at 604 nm and a secondary peak at 655 nm (Fig. 5).

Porphyropoikia is a genetic disorder in which there is a defect in heme synthetase (ferrochelatase), the enzyme that catalyzes the formation of heme from PP and iron [1, 6, 12]. In animals, porphyrins and porphyrin precursors are usually formed in the liver and excreted in the urine and feces [9]. Whether the major site of PP overproduction is liver or bone marrow has been a controversial point [10, 14, 15]. Several studies have suggested that some of...
Fig. 1. Liver of case 1. Dark brown, grouping pigments (arrow) and solitary granule (arrowhead) in dilated bile canaliculi. HE-stain, × 400.

Fig. 2. Liver of case 2. Pigment granule in distended sinusoid (arrow) (a) and within Kupffer cell (arrowhead) (b). HE-stain, × 1,000.

Fig. 3. Liver of case 1. Most of the globular pigments exhibit striking birefringence with a centrally located dark Maltese-cross pattern. HE-stain, Polarized light micrograph, × 400.

Fig. 4. Liver of case 2. Electron micrograph of large pigment granule showing a compact mass within a distended canaliculus (arrow) and aggregated hair-like crystals in the cytoplasm of a hepatocyte (arrowhead). × 7,500.

Fig. 5. Spectrofluorometric wavelength were identical to those of the affected liver and protoporphyrin IX standard.

the increased PP are hepatic in origin [2, 3, 10, 13, 14].

Porphyria in domestic animals can be broadly classified on the basis of their tissue of origin, erythropoietic system or the liver, depending on the fundamental biochemical defect, and more recently on the basis of their enzymatic defects [6]. Pathologically, porphyria is based on the affected tissue of origin, erythropoietic and hepatic.

The present cases occurred in two flocks of apparently clinically healthy chickens. In addition, chickens in these flocks were not treated with drugs or other hepatotoxic agents. Hepatic pigment deposits have been reported previously [5], although these authors did not mention of pigment accumulation in macrophages and sinusoids. PP
which has accumulated in the hepatobiliary system is excreted into the intestine via bile, while sinusoidal PP seems to arise from the erythropoietic system. Hepatic pigment accumulation in field or experimental cases of ethoxyquin toxicosis have been described in young chickens [8], although these pigment granules were not analyzed. They did not stain for bile or iron but exhibited birefringence under polarized light [5, 7, 8]. The nature of protoporphyric granules in the present cases was consistent with those described previously. In our cases, protoporphyric granules were intimately associated with hepatocellular injury, and it can be conceded that the accumulation of pigment within hepatocytes led to their destruction.

Presumably, PP accumulation in the liver results from the retention of material cleared in greater quantities than normal from that synthesized within the liver itself. Liver PP content was determined fluorometrically from extracts of the affected liver of the present case. The PP content and the fluorescent spectra of this liver were similar to those described elsewhere [5]. Protoporphyrin has been reported in animals and in humans, and it is commonly believed that this disease is an autosomal hereditary disorder [6, 12]. Bovine erythropoietic protoporphyria is inherited by a recessive trait and to date it has been seen only in females [12]. The protoporphyric chicken liver reported previously may have resulted from a congenital deficiency of ferrochelatase [5]. It is noteworthy that the cases encountered were both females. This report provides evidence for the presence of protoporphyric liver disorder in chickens in Japan, and so any further cases suspected of this disorder should be carefully examined by polarization microscopy.

REFERENCES