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Ultrastructural studies of the portal transport of fat in chickens

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Summary. Dietary fat is transported in the chicken by portomicrons; these large lipoproteins enter the portal blood of the small intestinal villi. We have shown by electron microscopy that avian portomicrons resemble mammalian chylomicrons in size, but their mode of transport differs. Portomicrons enter the intestinal blood vessels through endothelial intracytoplasmic vesicles, whereas chylomicrons enter the intestinal lymphatics through gaps between endothelial cells. We have also shown that the sinusoidal endothelium of the chicken liver, like that of the mammal, is fenestrated. Because the fenestrae are relatively few in number, the endothelium is less porous in the chicken than in the rat. We postulate that this prevents the hepatocytes from being swamped by dietary fat, but makes the chicken susceptible to diet-induced atherosclerosis.

Keywords: atherosclerosis, chylomicron, endothelium, liver, sinusoid

In mammals, absorbed exogenous long chain fatty acids and cholesterol are transported from the small intestine almost exclusively by the lymphatics (Redgrave 1983). These lacteals contain a milky emulsion of lipoproteins rich in dietary lipids, and named 'chylomicrons' by Gage and Fish (1924). Chylomicrons have been shown to range from 50–500 nm in diameter, depending primarily on the amount of fat in the diet (Fraser *et al.* 1968). These measurements have been made both by gradient techniques in the ultracentrifuge and by concurrent electron microscopy (Zilversmit *et al.* 1966; Fraser 1970).

When chickens are fed fat, however, lacteals are inconspicuous, and radioisotope techniques demonstrate the long chain fatty acids enter directly into the portal blood (Noyan *et al.* 1964). These fatty acids were shown to be transported in relatively large lipoproteins with a density of less than 1.006 g/ml. Bensadoun and Rothfeld (1972) termed these 'portomicrons', and showed that the lipid composition of chicken portomicrons closely resembles that of rat chylomicrons. The diameter distribution of portomicrons, as determined by sucrose gradient centrifugation, was also similar to that of the chylomicrons.

In mammals, chylomicrons enter the lumen of intestinal lymphatic capillaries through gaps between adjacent lymphatic endothelial cells which open during fat absorption (Casley-Smith 1962; Yoffey & Courtice 1970; Sabesin & Frase 1977). In the human intestine, intracytoplasmic vesicles in the lymphatic endothelial cytoplasm have

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been shown to play a minor role in chylomicron transport compared to the lymphatic endothelial gaps (Tytgat *et al.* 1971). Consequently, we first examined the ultrastructure of the intestinal villi in chickens, in order to locate the means of entry of portomicrons into the portal blood vessels.

The hepatic sinusoidal endothelium was then scrutinized, as chicken portomicrons circulate directly through the liver, rather than following the complex path of mammalian chylomicrons. The latter reach the blood-stream via the thoracic duct lymph, and most then traverse both the lung and the bowel capillaries, at least, before entering the portal circulation.

The fenestrated hepatic sinusoidal endothelium or 'liver sieve' in mammals is important in lipoprotein metabolism, atherogenesis and the pathogenesis of fatty liver (Wisse *et al.* 1985; Fraser *et al.* 1986*a*). The sieve acts as a barrier to large triglyceride-rich chylomicrons, but smaller triglyceride-depleted, cholesterol-rich and potentially atherogenic chylomicron remnants pass through, to be recognized and catabolized by hepatocytes (Fraser *et al.* 1978; Wright *et al.* 1983).

In humans, the membrane bound liver receptors for chylomicron remnants have been shown *in vitro* to recognize serum chylomicrons and remnants equally well, whereas *in vivo* remnants only are recognized (Florén 1984; 1985). Florén (1984) postulated the cause as a steric hindrance in the space of Disse, which would prevent chylomicrons from binding to hepatocytes *in vivo*. We believe this mechanism to be the 'liver sieve'.

An examination of the ultrastructure of the hepatic sinusoidal endothelium in chickens and the calculation of its porosity was therefore undertaken. This seemed important not only because of the relatively direct access of portomicrons to the liver, but also because cholesterol-fed chickens are known to develop hypercholesterolaemia and atherosclerosis (Horlick & Katz 1949; Pick *et al.* 1965; Jones & Dobrilovic 1969; Patel *et al.* 1982). We have shown previously that the rabbit, a species similarly prone to diet-induced atherosclerosis, has a less porous hepatic endothelium than does the rat, which is relatively resistant to dietary cholesterol (Wright *et al.* 1983). We decided, therefore, to compare the porosity of the rabbit and rat liver sinusoid with that of the chicken.

Materials and methods

Animals. Four meat-hybrid chickens aged 7 weeks and weighing between 1.2 and 1.8 kg, six young female Sprague–Dawley rats weighing 200–250 g, and six young female New Zealand rabbits 1.5–2.0 kg were starved during the night before surgery. Two of the chickens were dosed intragastrically with 5 ml peanut oil, 2 h before anaesthesia. All animals were anaesthetized with parenteral sodium pentobarb (Nembutal) before their liver perfusion.

Perfusion–fixation. A constant pressure perfusion was employed in these experiments (Wright *et al.* 1983), because variations in the diameter of hepatic endothelial fenestrae have been noted following perfusion–fixation of livers at different rates of flow and subsequent pressure (Fraser *et al.* 1980*b*). The flow was designed to approximate physiological conditions of portal flow; pressures of about 10 cm of water were achieved by placing the perfusate reservoir 15 cm above the portal vein. The vein was then cannulated by a needle large enough to deliver a flow which, when not impeded by the liver, exceeded the maximum flow through that organ (Wright *et al.* 1983).

After the animal was anaesthetized, the abdominal cavity was opened to expose the portal vein. In the case of the chicken, a clamp was placed on a large portal-systemic anastomosis, the coccygeomesenteric vein, which joins the portal system to the interiliac anastomosis and the renal vein. The perfusate cannula was then inserted into the portal vein and the flow was initiated while

the abdominal systemic veins were transected to prevent congestion of the liver. In the case of the oil-fed chickens, a retrograde perfusion of the proximal jejunum was also performed by cannulating a mesenteric vein from that area.

The perfusate consisted of 3–10 ml of isosmotic sodium cacodylate buffer (pH 7.3). This was used to flush the liver of blood, and was followed immediately by a fixative of cacodylate buffered 1.5% gluteraldehyde. A successful perfusion was indicated by blanching and rapid hardening of the liver. Perfusion was continued for 3 min, by which time the fluid issuing from the systemic abdominal veins was clear.

Electron microscopy. For transmission electron microscopy (TEM), the livers and a wedge of small intestine were cut with a razor blade into mm^3 blocks under buffered gluteraldehyde fixative. They were then washed in cacodylate buffer, post-fixed in buffered 1% osmium tetroxide for 30 min, and prepared and examined by the method previously described in this journal (Fraser *et al.* 1980*b*). This article also described the methods for scanning electron microscopy (SEM), which consisted of post-fixing tissue in buffered 1% osmium tetroxide for 2 h, dehydration through an alcohol series and critical point drying with carbon dioxide.

The diameters of the osmophilic droplets (portomicrons) seen in the transmission electron micrographs of the intestine and the liver were measured at a known magnification. These were grouped according to anatomical site as follows: the intestinal lamina propria ($n=996$); the lumen of the venous channels in the intestinal villi ($n=174$); the endothelial intracytoplasmic vesicles ($n=9$); the hepatic sinusoidal lumen ($n=26$); and the space of Disse ($n=55$).

Morphometric analysis of the sinusoidal liver endothelium depended on accurate calibration of magnification, and, measurements from scanning electron micrographs magnified ×30000 (Wright *et al.* 1983). The diameters of fenestrae were measured with a ruler, and the number of fenestrae per square micrometre counted under a transparent overlay with a suitable scale of calculated areas. Twenty-four sinusoids and 1418 fenestrae from the four chickens, 18 sinusoids and 1385 fenestrae from the six rats, and 21 sinusoids and 6708 fenestrae from the six rabbits were processed. Only those sinusoids in the 'encentric' or mid-position and containing fenestrae of approximately circular shape were examined (Wisse *et al.* 1985).

The 'liver sieves' of the three species were compared by determining the porosity, or the percentage of endothelial surface perforated by fenestrae (Wisse *et al.* 1983; 1985). This was ascertained from the diameters of fenestrae, enabling the calculation of their collective surface areas, and from the number of fenestrae per unit area of sinusoidal endothelium. For statistical analysis, the measurements for each animal were averaged, then *t*-tests were carried out comparing the species with the animals as the unit of analysis.

Results

Small intestine

Both light microscopy and TEM showed that chickens have blood vessels, but not lymphatics, in the lamina propria of their villi. These vessels form a network of channels often without definite basement membranes, and contain typical nucleated avian red cells.

Osmophilic droplets, the portomicrons, were present in the lamina propria, and varied in diameter from 47–128 nm, with a mean of 83 nm (Figs 1 & 2). Similar droplets were seen in the lumen of portal channels (50–123, mean 92 nm), and in some of the many intracytoplasmic vesicles (60–179, 73 nm). (Figs 2 & 3).

The portomicrons appeared to originate from the epithelial cells of the intestinal villi, and to pass unimpeded through the loose connective tissue of the lamina propria (Figs 1 & 2). They seemed to enter the portal blood directly through the numerous intracytop-

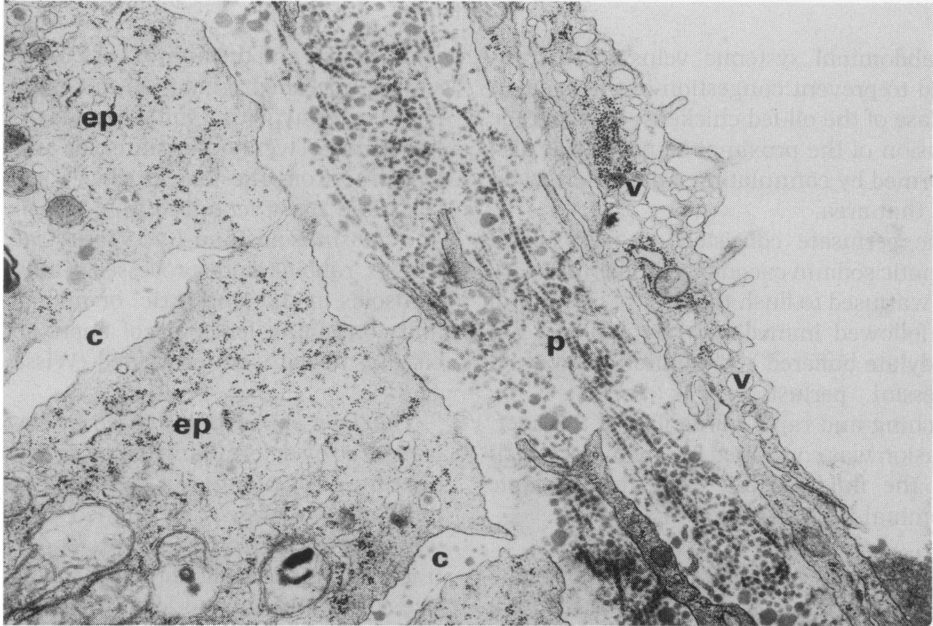

Fig. 1. TEM of a small intestinal villus showing an endothelial cell of a portal capillary containing numerous intracytoplasmic vesicles or channels (v). The endothelium is separated from the epithelial cells (ep) by a wide lamina propria (p). Numerous small portomicrons are shown infiltrating between fibres of collagen within the lamina. Prominent intercellular channels (c), which also contain portomicrons lie between the epithelial cells. Epithelia have active golgi and further portomicrons are visible in vesicles of their smooth endoplasmic reticulum. × 16 750.

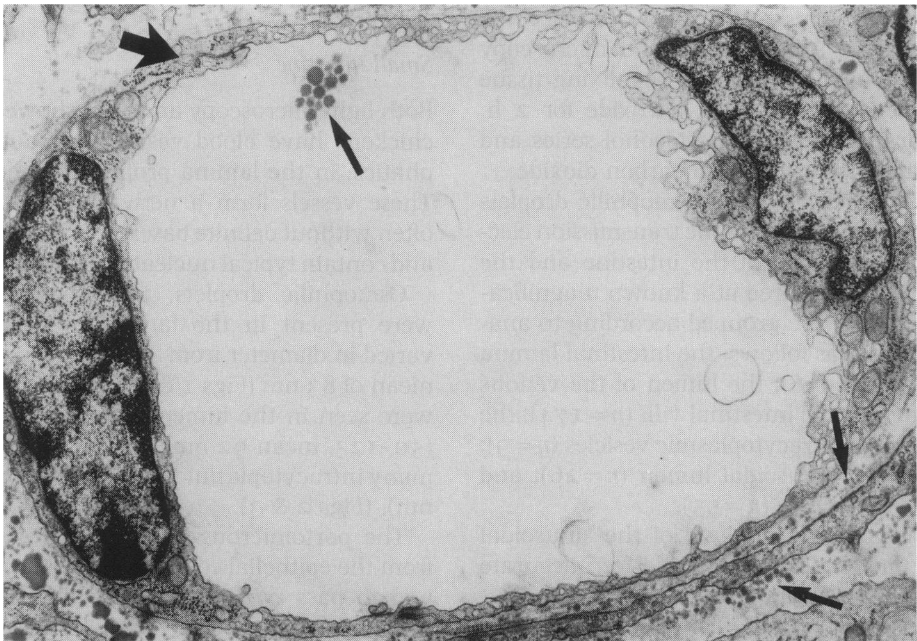

Fig. 2. TEM of a portal capillary lined by endothelial cells. Close apposition between two overlapping cells is visible (broad arrows). Portomicrons can be seen within the capillary lumen and also within the loose connective tissue of the lamina propria (small arrows). × 11 170.

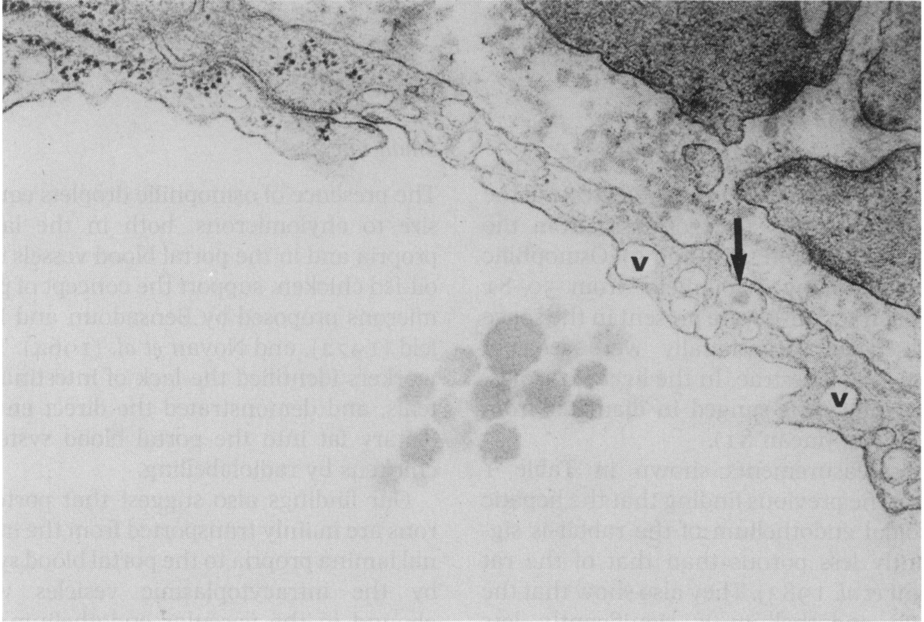

Fig. 3. A higher power view of Fig. 2. Vesicles or channels (v) in the endothelium are clearly visible. A portomicron is shown lying within one such vesicle (arrow). ×44 680.

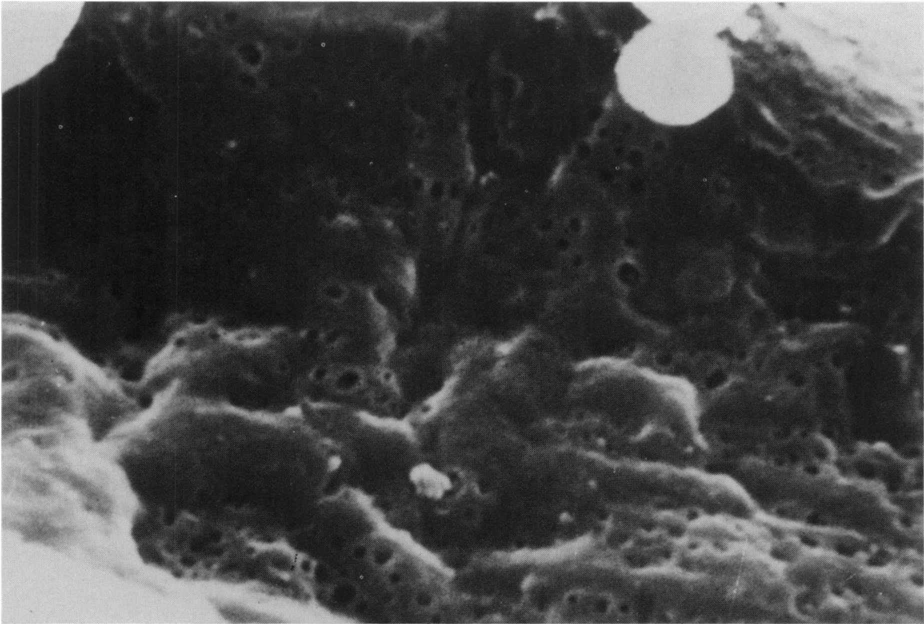

Fig. 4. SEM of the internal surface of a chicken liver sinusoid demonstrating the paucity of fenestrae. ×10 000.

lasmic vesicles in the endothelial cells, rather than through gaps between these cells (Figs 1 & 3).

Liver sinusoidal endothelium

The chicken sinusoidal endothelium is fenestrated, and separates the hepatocytes and the relatively narrow space of Disse from the sinusoidal lumen (Figs 4 and 5). Osmophilic droplets, ranging in diameter from 50–81 nm (mean 63 nm), were present in the space of Disse, and occasionally were seen to traverse the fenestrae. In the liver sinusoids, the portomicrons ranged in diameter from 59–104 nm (mean 81).

The measurements shown in Table 1 confirm the previous finding that the hepatic sinusoidal endothelium of the rabbit is significantly less porous than that of the rat (Wright *et al.* 1983). They also show that the chicken endothelium is significantly less porous than that of both of these mammals particularly because of a paucity of fenestrae per unit area of sinusoid.

Discussion

Small intestine

The presence of osmophilic droplets equal in size to chylomicrons, both in the lamina propria and in the portal blood vessels of the oil-fed chicken, support the concept of portomicrons proposed by Bensadoun and Rothfeld (1972), and Noyan *et al.* (1964). These workers identified the lack of intestinal lacteals, and demonstrated the direct entry of dietary fat into the portal blood system of chickens by radiolabelling.

Our findings also suggest that portomicrons are mainly transported from the intestinal lamina propria to the portal blood system by the intracytoplasmic vesicles which abound in the vascular endothelium. This differs from the mode of entry of chylomic-

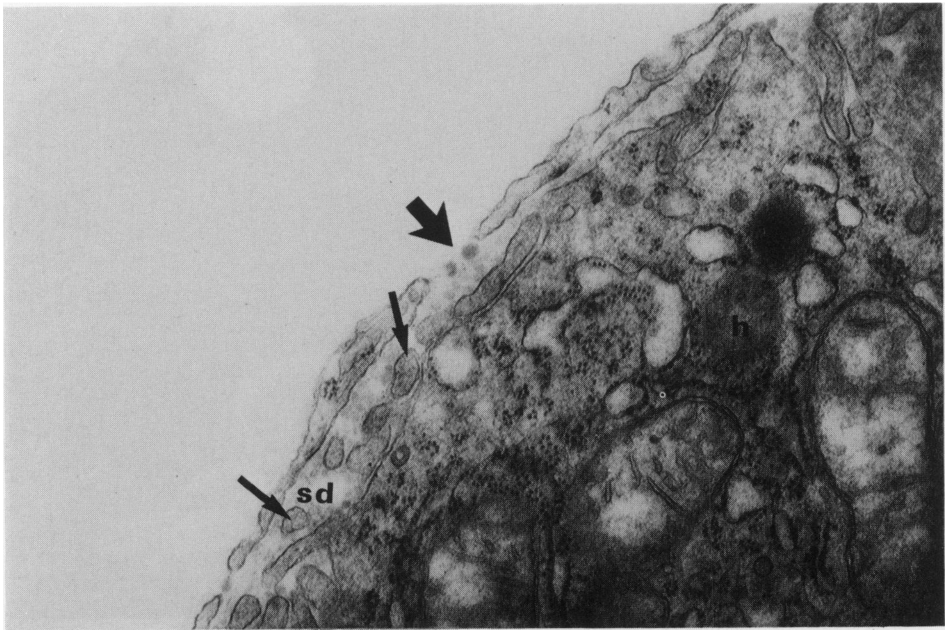

Fig. 5. TEM of the fenestrated endothelium of a chicken liver, illustrating the fenestrae and two small portomicrons within a fenestra (large arrow). Hepatocytes (h) with microvillar projections (small arrows) are separated from the endothelium by a narrow space of Disse (sd). ×33 490.

Table 1. Comparison of rat, rabbit and chicken liver sinusoidal porosity

Species	Diameter of fenestrae (nm)	Fenestrae (μm^2)	Porosity (% area perforated)
Rats ($n=6$)	109.5 ± 7.4^a	12.4 ± 3.6^b	12.0 ± 2.1
Rabbits ($n=6$)	59.1 ± 8.4^d	12.7 ± 2.5^b	$4.0 \pm 1.5^{c,d}$
Chickens ($n=4$)	89.6 ± 17.8^a	2.9 ± 0.3	2.2 ± 0.6^c

Means ± s.d.
$P<0.001$ in rabbits *vs a*, chickens *vs b*, rats *vs c*.
$P<0{\cdot}05$ in chickens *vs d*.

rons into the lacteals of the intestinal villi in rats. These mainly pass through large gaps between the lymphatic endothelial cells (Casley–Smith 1962; Sabesin & Frase 1977). The vesicles may represent intracytoplasmic channels, as suggested by ultrastructural studies of serial sections of rat heart capillaries (Bundgaard *et al.* 1983).

Liver sinusoids

In preliminary studies, Fernando *et al.* (1985) and Fraser *et al* (1986*b*) showed that the hepatic sinusoidal endothelium of chickens is less porous than that of rats. If plasma portomicrons in chickens are recognized directly by receptors on hepatocyte membranes, as are plasma chylomicrons in mammals (Florén 1984; 1985), then an efficient barrier or sieve is of particular importance. This 'sieve' would prevent the relatively large triglyceride-rich portomicrons from swamping the liver, and would redirect them and their lipids to peripheral tissues. This study found that only relatively small particles are present in the space of Disse. Given that the mass of triglyceride carried by these spherical particles is proportional to the cube of their diameters (Fraser *et al.* 1968), hepatocytes are thus shielded from excess dietary fat.

The susceptibility to atherosclerosis of cholesterol-fed chickens when compared with rats may be attributable to the limited porosity of their liver sinusoidal endothelium. We suggest that this increased barrier between the circulating lipoproteins and hepatocytes slows the catabolism of portomicron remnants and their contained dietary cholesterol. This barrier also decreases the negative feedback induced by dietary cholesterol on hepatic cholesterol synthesis. Therefore the uninhibited production of the liver-derived very low density lipoproteins (VLDL) and their remnants, the intermediate density lipoproteins (IDL) and low density lipoproteins (LDL) continues.

The mechanistic view of the liver sieve does not contradict the concept of receptor sites in the liver for specific apolipoproteins (Chao *et al.* 1981; Florén 1984; 1985; Windler & Havel 1985). We believe these two concepts are complementary, thus allowing only certain sized lipoproteins to reach the hepatocytes for recognition or degradation by hepatic lipase (Havel 1985). As well as differences in porosity between species, as shown in this paper, the liver sieve can be altered by drugs such as ethanol (Fraser *et al.* 1980*a*; Mak & Lieber 1984), and hormones such as adrenalin and serotonin (Wisse *et al.* 1980). These changes in porosity are postulated to modulate the receptor-lipoprotein interactions on hepatocytes, thus influencing lipoprotein metabolism and atherogenesis.

Atherosclerosis is characterized by the thickening of the intima of arteries, caused not only by the organization of mural thrombi, cholesterol deposition, and an in-

crease in numbers of macrophages and foam cells, but also by hyperplasia of myointimal cells (Schwartz *et al.* 1985; Wissler 1984). Cholesterol-rich liver-derived lipoproteins, especially LDL, bind through their specific apolipoproteins to several types of intimal cells (Brown & Goldstein 1984). The LDL also cause proliferation of arterial smooth muscle cells in tissue culture (Fischer–Dzoga *et al.* 1976) as do IDL (Kim *et al.* 1985). Cholesterol-rich intestinal lipoproteins, namely chylomicron remnants, increase the cholesterol content of arterial smooth muscle cells to a greater extent than do LDL (Florén *et al.* 1981). Remnants of both the intestinal and liver-derived lipoproteins (the βVLDL) lead to cholesterol uptake by macrophages and the development of foam cells from this source (Schwartz *et al.* 1985).

We postulate that the low porosity of the chicken liver sieve prolongs the circulating time of cholesterol-rich portomicron remnants and also increases the concentration of circulating liver-derived lipoproteins so rendering the chicken susceptible to diet-induced atherosclerosis.

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