A developmental shift from low- to high-\(M\), copper binders in guinea-pig serum

COLIN D. BINGLE, OWEN EPSTEIN and SURJIT K. S. SRAI

Academic Department of Medicine, Royal Free Hospital School of Medicine, London NW3 2QG, U.K.

Carculoplasmin (EC 1.16.3.1), a blue \(\alpha\)-globulin, is the major serum copper-binding protein [1]. Before birth and in neonates, levels of carculoplasmin and carculoplasmin-bound copper are markedly reduced, but levels of non-carculoplasmin copper are elevated [2]. In the present study we have attempted to identify the form in which this copper is found using gel-filtration and atomic absorption spectroscopy.

Blood samples were collected by cardiac puncture from time mated Dunkin–Hartley guinea-pigs at 60 days of gestation, the day of birth, 24 h after birth and from adults. Prenatal litters were delivered by Caesarean section. Serum was subjected to gel-filtration on Sephacryl S-200 using 10 mM-potassium phosphate, pH 7.4, as an eluant. Copper was determined in the fractions by electrothermal atomic absorption spectroscopy. Carculoplasmin oxidase activity was determined using \(p\)-phenylenediamine [3] and the presence of anti-carculoplasmin and anti-albumin activity was determined by Ouchterlony double diffusion using polyclonal antibodies raised against purified guinea-pig proteins.

After gel-filtration on Sephacryl S-200, adult serum separated into three distinct peaks (Fig. 1). The major copper peak eluted around fractions 28–30 (\(M_r\) 120 000–150 000) and corresponded with the elution of both oxidase and anti-carculoplasmin activity. Pure carculoplasmin also eluted in these fractions. The second major peak eluted in fractions 33–35 (\(M_r\) 60 000–70 000) and was assumed to represent albumin in view of its \(M_r\) and its reactivity with anti-guinea-pig albumin. Some samples also contained a lesser peak in the void volume of the column. The profiles of serum from late gestation animals or from animals soon after birth were markedly different (Fig. 1). In prenatal animals copper appeared only in a low \(M_r\) peak (\(M_r\) < 5000), no copper was associated with either carculoplasmin or albumin. At birth copper was still present in the low-\(M_r\) peak, although a small amount was present in the carculoplasmin and albumin fractions. In day 1 serum, copper was present with carculoplasmin and albumin but a large proportion was still present in the low-\(M_r\) peak.

In conclusion, we have demonstrated that in the foetal and newborn guinea-pig, when carculoplasmin is absent or low, serum copper is bound to low-\(M_r\) components. Similar low-

**Fig. 1. Fractionation of guinea-pig serum on Sephacryl S-200**

Serum samples (0.5 ml) were fractionated on a column of S-200 (1.2 cm x 70 cm) at 10 ml/h using 10 mM-potassium phosphate as eluant. Each fraction was assayed for copper, carculoplasmin oxidase activity, and both anti-carculoplasmin and anti-albumin activity. —— Adult; ———, day 1; ———, day 0; ———, day 60 of gestation.

\(M_r\) components have been proposed (along with copper–albumin) to play an important role in the donation of copper to tissues [4]. The large amount of copper present in this form may contribute to the hepatic accumulation of copper that occurs in the foetus during the later stages of gestation [5, 6].

This project is supported by the Sir Jules Thorn Charitable Trust.