Before a definitive placenta is established, the rat visceral yolk sac functions as the nutritive organ of the conceptus, capturing various macromolecules and transferring them whole or as degraded products to the embryo (Beck et al., 1984). It is now possible to bypass the visceral yolk sac, and thus, assess the embryonic capacity to deal with macromolecules using a technique which involved the cannulation of the vitelline vessels of 11.5 day embryos. At this time these vessels function in transporting nutritive and informational products to the embryo (Mensah-Brown & Beck, 1985; Beck et al., 1987).

The fate of various radiolabelled and colloidal-gold-bound macromolecules, as well as iron dextran, has been studied. Radiolabelled polyvinylpyrrolidone, formaldehyde denatured bovine serum albumin (BSA) and rat IgG were injected into embryos via the vitelline vessels, the conceptuses cultured for various times, and processed for radioactivity as described in Fig. 1. In the case of proteins, samples were also assayed for the presence of digestion products. Colloidal gold particles (18 nm) bound to these proteins were introduced into the vitelline circulation; the conceptuses incubated for 30 min or 2 h and after processing, ultrathin sections of yolk sacs and embryos were examined by transmission electron microscopy.

Abbreviations used: BSA, bovine serum albumin; IgG, immunoglobulin G.

For comparison, conceptuses injected with colloidal gold, colloidal gold in the presence of unbound IgG (1 mg/ml), colloidal gold bound to BSA and colloidal gold bound to immunoglobulin in the presence of a ten thousandfold excess unbound immunoglobulin were examined. Iron dextran (100 mg iron/ml) was injected and conceptuses cultured for 30 min.

Fig. 1 shows that using immunoglobulin (or polyvinylpyrrolidone) the embryo-associated radioactivity increased progressively with time while that of BSA increased slightly for 2 h and then fell. The yolk-sac-associated radioactivity remained low throughout the period of study for all three markers.

The radioactivity in the exocoelomic fluid decreased progressively from immediately post-cannulation — at the end of incubation 16% of immunoglobulin, 20% BSA and 40% polyvinylpyrrolidone were associated with this compartment.

Trichloroacetic-acid-soluble radioactivity was released into the culture medium after injection of BSA (0.10 µl at 2 h and 0.21 µl at 4 h). Whereas for immunoglobulin-injected conceptuses very low levels were detected.

After 30 min post-injection culture gold particles were detected in coated pits and vesicles, smooth vesicles and the juxta-nuclear area of endothelial cells. Very few particles were detected in the intracellular spaces of the yolk sac.

After 2 h incubation and examining sections randomly taken from embryos, 105 vacuoles and 3454 particles were detected in colloidal-gold–immunoglobulin-injected embryos, 92 vacuoles and 982 particles using gold-BSA, 18 vacuoles and 92 particles with gold-immunoglobulin plus excess unbound immunoglobulin, 6 vacuoles and 56 particles for...
colloidal gold alone and 9 vacuoles and 72 particles in the presence of unbound immunoglobulin.

Most of the particles were present in endothelial cells, some, especially when bound to albumin, were present in mesenchymal cells around the vessels.

Paraffin-wax sections of embryos injected with iron dextran, stained histochemically for iron by the Prussian Blue reaction, showed reaction products mainly in the mesenchymal cells, but also in the endodermal cells of the gut and the neural tube.

From these results we have concluded that: (i) the embryo is capable of endocytosing and digesting degradable macromolecules. This digestion occurs in the lysosomal system of the mesenchymal cells, the endodermal cells of the gut and the neural tube; (ii) IgG is endocytosed probably by a receptor mediated pathway which involves coated vesicles.

That it is not digested to an appreciable level is important in view of the fact that it is transported into the embryo to provide passive immunity.

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The effect of clomipramine on the concentration of plasma lipoproteins and the distribution of high-density lipoprotein subspecies

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Clomipramine is used extensively in the treatment of depressive illness, which, it has been suggested, is associated with an increased incidence of vascular disease. Since the incidence of coronary heart disease correlates positively with low-density lipoprotein (LDL) and negatively with high-density lipoprotein (HDL) concentration (Gordon et al., 1977), a knowledge of the effect of clomipramine on plasma lipoprotein concentrations and on the distribution of HDL subspecies could be of importance and formed the purpose of the present investigation.

Blood samples were taken into EDTA (1 mg/ml) after an overnight fast from five normal, healthy, male volunteer subjects, aged between 24 and 42 years (week 0) who then took 50 mg of clomipramine daily for 2 weeks with blood sampling at the end of each (weeks 1 and 2). One week after withdrawal from the drug, a final blood sample was taken (week 3).

Very-low-density lipoprotein (VLDL) and LDL were isolated from the plasma by ultracentrifugation at densities 1.006 and 1.063 g/ml, respectively (Skinner et al., 1983). The LDL infranatant was divided into two portions: from one, HDL, and HDL, were separated by ultracentrifugation at densities 1.25 and 1.21 g/ml, respectively, and the other was used for the isolation of the total HDL fraction at density 1.21 g/ml. The latter was used for determining the HDL subspecies profile by gradient gel electrophoresis (Blanche et al., 1981) with subsequent scanning and peak integration using the Bio-Rad Model 620 Video Densitometer. Lipoproteins were quantified by cholesterol measurements using enzymic assays. Plasma apolipoprotein (apo) A-I concentrations were determined by electroimmunoassay (Laurell, 1972).

There was no significant change in the plasma concentration of VLDL–cholesterol (C) or of total plasma triacylglycerol throughout the four weeks. The concentration of LDL-C decreased during the two weeks of clomipramine treatment (89.1 ± 23.9 (mean ± s.d.) and 76.2 ± 14.2 mg/100 ml on weeks 0 and 2, respectively), and increased during the week after clomipramine withdrawal (77.6 ± 15.9 mg/100 ml on week 3). The total plasma cholesterol concentration followed a similar pattern: in both cases the changes did not quite reach statistical significance at the 5% confidence level. The concentration of HDL-C, on the other hand, increased by a small amount during weeks 1 and 2, and decreased after clomipramine withdrawal on week 3. This change was due to an increase (p < 0.01) in the concentration of HDL-C with drug treatment (12.8 ± 5.4, 18.1 ± 4.5, 19.6 ± 3.0 and 14.3 ± 8.4 mg/100 ml on weeks 0, 1, 2 and 3, respectively), while the level of HDL-C remained essentially unchanged. A marked increase in HDL-C concentration during weeks 1 and 2 was observed in all five subjects, though the magnitude of the decrease that occurred during week 3 varied considerably between subjects. Variability between different subjects in response to a given stimulus on changes in lipoprotein concentrations is a well-recognized phenomenon (Skinner et al., 1985). These changes were confirmed by analysis of the total HDL fraction by gradient gel electrophoresis which revealed that clomipramine administration was accompanied by a shift in the distribution of HDL subspecies towards subspecies of a larger particle size. Integration of peak areas showed that the relative concentration of HDL3 and HDL3 (of diameters 8.44 and 7.97 nm, respectively; Blanche et al., 1981) were lower, while that of HDL2 (9.16 nm) was higher (p < 0.001) on weeks 1 and 2 than on either week 0 or 3. The plasma concentration of apo A-I increased between week 0 and week 2 and showed a further increase in week 3 (184.4 ± 38.9, 191.7 ± 33.0, 222.1 ± 42.9 and 235.7 ± 57.7 on weeks 0–3, respectively), though the increase did not reach statistical significance.

On the basis of the relationships between plasma lipoprotein levels and the incidence of coronary heart disease observed in epidemiological studies (Gordon et al., 1977), the increase in the concentration of HDL-C and HDL-C and the decrease in LDL-C and total plasma cholesterol observed during clomipramine treatment in the present study would suggest that this drug might have an antiatherogenic effect. The ratio of total plasma cholesterol to HDL-C, which has been found to be a sensitive indicator of coronary risk (Castelli, 1984) decreased significantly with clomipramine administration (3.70 ± 0.34, 3.54 ± 0.29, 3.14 ± 0.61 and 3.94 ± 0.98 on weeks 0–3, respectively).

Abbreviations used: LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; apo, apolipoprotein; C, cholesterol.

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