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# Transferrin-mediated transcellular transport of 59Fe across confluent epithelial sheets of Sertoli cells grown in bicameral cell culture chambers

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The transferrin-mediated transcellular transport of 59Fe across confluent epithelial sheets of Sertoli cells grown on Millipore filters was investigated. These filters had been impregnated with reconstituted basement membrane and suspended in bicameral (two houses) culture chambers. After five days of culture, Sertoli cells from 10-day-old rats formed basally-located tight junctional

complexes. Concomitantly, there was an increase in electrical resistance and the epithelial sheet became impermeable to lanthanum nitrate. The rate of passage of [3H]inulin across the epithelial sheet was considerably less than passage across a filter alone, a filter impregnated with reconstituted basement membrane or an epithelial sheet pretreated with 2 mM EGTA. We conclude from these permeability studies that the tight junctional complexes between Sertoli cells formed an effective transepithelial permeability barrier. Following addition of human serum [59Fe]transferrin to media bathing the basal cytoplasm of the cells, rat testicular [59Fe]transferrin was immunoprecipitated from apical media overlying the Sertoli cells. Cross-reactivity of the rabbit anti-rat transferrin antibody with human serum transferrin was less than 0.001%. Substitution of the primary antibody with normal rabbit serum reduced the amount of immunoprecipitable rat testicular [59Fe]transferrin to 20% of normal levels. Prior fixation of the Sertoli cell epithelial sheet in 2.5% glutaraldehyde, addition of a 100-fold excess of holotransferrin to the basal media, and incubation of the Sertoli cell epithelial sheet at 4 C all reduced the immunoprecipitable rat testicular [59Fe]transferrin in apical media to levels below that for the non-specific binding of the primary antibody. From these studies we conclude that 59Fe is shuttled across Sertoli cells by two different forms of transferrin. Serum transferrin delivers the 59Fe to the basal cytoplasm of the Sertoli cells. The 59Fe dissociates from the serum transferrin, is delivered to testicular transferrin, and is subsequently secreted from the apical surface of the epithelial sheet of Sertoli cells as testicular [59Fe]transferrin.

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