Dehydroepiandrosterone in the fetal gonads of the horse

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Early studies suggested the placenta as the source of oestrogens in the pregnant mare (Catchpole & Cole, 1934). Ainsworth & Ryan (1966), however, were unable to demonstrate oestrogen formation from C_{21} -steroids by placental preparations from the mare, although C_{19} -steroids were aromatized to oestrogens. A feto-placental unit for oestrogen biosynthesis is suggested from these findings and from the inactive appearance of the maternal ovaries in mid-pregnancy, when oestrogen production increases rapidly to very high levels (Catchpole & Cole, 1934). At this time the fetal gonads show a remarkable growth, mainly of interstitial tissue (Amoroso & Rowlands, 1951) with ultrastructural features indicative of active steroidogenesis (Gonzalez-Angulo, Hernandez-Jauregui & Marquez-Monter, 1971).

The present study was to examine extracts of fetal gonads for potential precursors in the formation of oestrogens by the placenta. Gonads were taken from fetuses in 6 Pony mares at surgery in the 5th–8th months of pregnancy. Testes and ovaries were immediately weighed (47.0–81.8 g per pair), minced and homogenized in a Waring blender in 80% aqueous ethanol. Details of the procedure used for extraction and preliminary purification have been given previously for stallion testes (Raeside, 1969).

Neutral steroids of the unconjugated fraction were purified further by thin-layer chromatography (TLC) on silica gel G (Merck), before and after acetylation, using chloroform:ethyl acetate (2:1 v/v) and chloroform:benzene (2:1 v/v), respectively. Dehydroepiandrosterone, pregnenolone, 17 α -hydroxypregnenolone and 5-androstene-3 β ,17 β -diol and their acetates were used as reference standards in TLC. Steroid acetates from zones corresponding to reference standards were hydrolysed in 10% potassium hydroxide in 50% aqueous methanol overnight. Final separations were made by column partition chromatography on Celite.

Text-fig. 1. Column partition chromatography of DHA-like material (----) from extracts of fetal gonads, and DHA standard (—) in the solvent system of *n*-hexane: 55% aqueous methanol (v/v).

Text-fig. 2. Column partition chromatography of DHA-like material and DHA standard, after acetylation with ^{14}C -labelled acetic anhydride. The same Celite column was used for consecutive chromatography of the acetylated DHA-like material (O) followed by the acetylated DHA standard (●). The solvent system was n-hexane:80% aqueous methanol (v/v).

Text-fig. 3. Infrared spectra of DHA-like material from (a) extracts of fetal gonads of the horse and (b) DHA standard. Spectra were recorded using micro-KBr pellets.

Material behaving chromatographically like DHA was seen as the most prominent compound in extracts of fetal testes and ovaries. The elution pattern for this material compared well with that of authentic DHA (Steraloids, Inc.), chromatographed consecutively on the same Celite column (Text-fig. 1), when a colour reaction with sulphuric acid:ethanol (2:1 v/v) was used for measurement (Oertel & Eik-Nes, 1959). Similarly, behaviour as DHA acetate was demonstrated in column chromatography after acetylation (Text-fig. 2); the DHA-like material and some DHA standard had been reacted separately with ^{14}C -labelled acetic anhydride in pyridine. Final identification of DHA in the extracts of fetal gonads was made by infrared spectroscopy (Text-fig. 3) on a composite sample of DHA-like material from the more abundant fetal ovaries (four pairs). Lesser amounts of other compounds were present but the quantities after purification were sufficient for only a very weak spectrum of what appeared to be pregnenolone. No steroids were identified in the conjugated fraction of the extracts. Although no attempts were made to quantify the amounts of DHA or other steroids in any of the extracts, no apparent differences in the steroids were noted between the sexes.

MacArthur, Short & O'Donnell (1967) found radioactive DHA in their study on the formation of steroids by preparations of an equine fetal testis. The yield of DHA from [^{14}C]sodium acetate greatly exceeded that of testosterone and androstenedione. Its isolation from fetal testes and ovaries in the present study, therefore, points to a possible role for dehydroepiandrosterone as a precursor in the placental formation of oestrogens in the mare. The gonads rather than the adrenal glands may be the important fetal component of a feto-placental unit for oestrogen biosynthesis in equids. Preliminary results with bilaterally and unilaterally gonadectomized fetuses (Raeside, Liptrap & Milne, 1973) have suggested a relationship between the fetal gonads and the levels of urinary oestrogen excretion by the pregnant mare.

I thank Dr F. J. Milne and Dr R. M. Liptrap for the surgery, and Dr S. Solomon of McGill University, Montreal, for the infrared spectroscopy.

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Received 17 July 1975