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Separation of Δ^4 -3-Oxo Steroids and Δ^5 -3 β -Hydroxy Steroids by Chromatography on Digitonin-Silica Thin Layers

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In an investigation of Δ^5 -3 β -hydroxy steroid dehydrogenase (EC 1.1.1.51) activity in foetal adrenal glands, a method was required for the complete separation of nanogram amounts of Δ^4 -3-oxo steroids from a mixture also containing Δ^5 -3 β -hydroxy steroids. Digitonin precipitation of Δ^5 -3 β -hydroxy steroids is well known (Dorfman, 1962), but the present author has found that near-quantitative precipitation requires relatively large amounts of Δ^5 -3 β -hydroxy steroids (e.g. quantities of 3 β ,17 α -dihydroxypregn-5-en-20-one greater than 100 μ g), and even after multiple precipitation traces of Δ^5 -3 β -hydroxy steroid may remain in the supernatant.

A chromatographic method has been developed that enables complete separation of the two steroid groups on thin layers of silica gel impregnated with digitonin.

A layer (0.3mm thick) is spread on t.l.c. plates from a slurry containing: silica gel (Chromalux; May and Baker Ltd., Dagenham, Essex, U.K.), 30g; digitonin (Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.), 3g; water, 48ml; ethanol, 2ml. The spread plates are dried at room temperature and then heated for 1 h at 100°C. After application of the steroid mixture, the plates are developed in the solvent system 2-methylpropan-2-ol-water-ethyl acetate (5:2:5, by vol.) for 4.5 h.

Δ^4 -3-Oxo steroids run as a group (R_F 0.86) whereas 1 μ g amounts of Δ^5 -3 β -hydroxy steroids exhibit R_F less than 0.65. The precise R_F value of individual Δ^5 -3 β -hydroxy steroids depends both on structure and the amounts present (e.g. for 10 ng of cholesterol, R_F 0; for 0.1 μ g and 10.0 μ g of 3 β ,17 α -dihydroxypregn-5-en-20-one, R_F 0.5 and 0.05 respectively). A co-operative effect exists whereby the total mass of Δ^5 -3 β -hydroxy steroids present determines the R_F value of each component Δ^5 -3 β -hydroxy steroid and of the group as a whole (e.g. R_F of 0.1 μ g of 3 β ,17 α -dihydroxypregn-5-en-20-one in the presence of 10 μ g of 3 β -hydroxypregn-5-en-20-one is 0.05). With 1 μ g of Δ^5 -3 β -hydroxy steroid present it is normally possible to obtain uncontaminated re-

covery (94–98%) of Δ^4 -3-oxo steroids from a complex mixture.

Elution of the Δ^4 -3-oxo steroid zone of the plate with ethyl acetate yields (digitonin-free) steroids. Digitonides from the Δ^5 -3 β -hydroxy steroid zone are eluted with methanol, dried and subjected to pyridine hydrolysis (Frame, 1944), yielding the free steroids.

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Effect of Oestradiol 3-Benzate on the Bio-synthesis of Egg Proteins by Cod

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The major protein constituents of cod eggs were found to be two similar lipoproteins, of mol.wt. about 400 000 and containing 79% protein and 21% lipid. From its phosphorus content, the lipid consisted of about 65% phospholipid. These two lipoproteins were not separated by gel filtration on Sephadex G-200 but were partly resolved by chromatography on triethylaminoethyl-cellulose (Jared & Wallace, 1968). Antiserum to cod eggs was prepared in rabbits and both immunodiffusion and immunoelectrophoresis gave two precipitin arcs close together with extracts of cod eggs.

For a fish of 4 kg wet wt., containing about 800 g of dry matter, some 100 g of egg proteins are produced during maturation of the ovaries over a period of 3–4 months. Egg proteins were detected in the serum of maturing female cod by immunochemical methods and reached a concentration of 32 mg/ml, but they were not present in the serum of immature fish or of male fish with maturing testes.

Oestradiol-17 β 3-benzoate in oil was injected intramuscularly into immature cod to give a slow release of the hormone. Serum samples taken 10 days after injection were tested for egg proteins, and the minimum dose of oestradiol to give a positive result was 30 μ g/kg; with doses of 300 μ g or 1 mg/kg the concentration of egg proteins was 32 mg/ml. Similar results were obtained with both male and female fish. Egg proteins were also present in the livers of injected fish but not in their ovaries, whereas immature control fish had no egg proteins in their livers. These findings suggested that the egg