sulphur amino-acids. Further studies are needed to establish the response of urinary sulphate excretion to the 'biological availability' of sulphur amino-acids in various foods.

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Metabolism of 6β-Hydroxy-3,5-*cyclo*androstan-17-one by Rabbit Liver *in vitro*

6β-Hydroxy-3,5-*cyclo*androstan-17-one is related to the naturally occurring steroid dehydro*epi*androsterone in that it can be readily converted to dehydro*epi*androsterone under acidic conditions and can be produced by hydrolysis of solutions of dehydro*epi*androsterone sulphate at elevated temperatures at neutral *p*H[1]. In addition it has been suggested that the 3,5-*cyclo*steroids have an 'anti-fatigue' effect[2]. For these reasons it seemed of interest to examine the metabolism of this steroid.

Incubations were performed with rabbit liver homogenate; a 50 per cent homogenate was prepared in 0·2 M nicotinamide in 0·25 M sucrose. Each incubation flask contained 12 ml. homogenate and 3 ml. each of 0·04 M potassium dihydrogen phosphate, *p*H 7·4 ; 0·01 M potassium citrate, *p*H 7·4; 0·005 M manganese sulphate and water. The *cyclo*steroid (25 mg) was added to the mixture in 0·5 ml. propylene glycol and incubated at 37° for 1 or 2 h. After incubation acetone was added to precipitate the proteins and the protein precipitate washed twice with hot acetone. The combined aqueous acetone extracts were evaporated to a small volume, diluted with water and extracted with benzene. The benzene extract was washed twice with 0·1 N sodium hydroxide, twice with water, dried with anhydrous sodium sulphate and evaporated to dryness. The extract was chromatographed on alumina[3] and eluted first with 0·3 per cent ethanol in benzene to give a less polar fraction and then with 10 per cent ethanol in benzene to give a more polar fraction. The less polar fraction was chromatographed on paper ; the major Zimmermann reacting spot was less polar (R_F value 0·72) than 6β-hydroxy-3,5-*cyclo*androstan-17-one (R_F value 0·55). The positive Zimmermann reaction suggested the presence of a 17-oxo group ; the compound obtained by elution from paper gave a negative Pettenkofer reaction showing that it did not contain either a Δ^5-3β-hydroxy group or the 6β-hydroxy-3,5-*cyclo* group which is isomerized to the Δ^5-3β-hydroxy grouping under the acidic conditions of the reaction. Reaction with acetic anhydride and pyridine did not produce a change in the R_F value of the compound showing the absence of an acetylatable hydroxyl group. It seemed likely, therefore that the metabolite was 3,5-*cyclo*androstane-6,17-dione. Authentic 3,5-*cyclo*androstane-6,17-dione was prepared by chromic acid oxidation from the 6β-hydroxy compound[4] and the synthetic compound was similar to the metabolite in the foregoing respects. Reduction of the metabolite with sodium borohydride in methanol gave a product with the same chromatographic properties as the compound obtained by reduction of 6β-hydroxy-3,5-*cyclo*androstan-17-one or of the synthetic dione. In addition, the sulphuric acid spectra of the isolated steroid and synthetic 3,5-*cyclo*androstane-6,17-dione were identical (λ_{max} 255, 300 mμ, minor peak at 405 mμ; λ_{min}, 280 mμ). There was no doubt, therefore, that the isolated metabolite was 3,5-*cyclo*androstane-6,17-dione.

Yields of the 3,5-*cyclo*androstane-6,17-dione were maximal at incubation times of 1 or 2 h, compared with 15 or 30 min. Paper chromatography of the more polar fraction in the solvent system light petroleum, benzene, methanol, water (5 : 5 : 4 : 1, by vol.) consistently showed the presence of three Zimmermann reacting spots with R_F values of 0·06, 0·17 and 0·26. These latter spots were also obtained by incubation of 3,5-*cyclo*androstane-6,17-dione with liver homogenate, but no 6β-hydroxy-3,5-*cyclo*androstan-17-one was produced under these conditions. Whereas oxidation of 6-hydroxylated oestrogens to 6-oxo ones has been reported[5], the oxidation of a neutral steroid 6-hydroxyl group has not been described previously although 6-oxoprogesterone was produced by perfusion of cow placenta with progesterone[6].

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Simple Determination of Heart-specific Lactic Dehydrogenase Isoenzyme in Serum

The denaturation of proteins by urea is a well-known phenomenon, the mechanism of which has been investigated by Lauffer[1].

Enzymes, like other protein entities, are subject to the denaturating activity of urea, which can be measured by the loss of enzyme activity. It was first shown by Plummer *et al.*[2] that 2 M urea in a pyruvate/phosphate buffer/substrate system completely inhibited the lactic dehydrogenase (LDH) activity of human liver extract, and it also completely inhibited the LDH activity of crystalline preparations of rabbit skeletal muscle, whereas that of human heart and crystalline beef heart preparations was inhibited only to the extent of about 20 per cent. Brody[3], studying the effect of various enzyme inhibitors on organ specific isoenzymes of LDH, found in an electrophoretic experiment that urea specifically inhibited the LDH isoenzymes, which were travelling with those fractions slower than albumin. The specific inhibitory property of urea has been used here to develop a simple spectrophotometric method for the determination of heart-specific LDH isoenzyme levels in human sera for clinical application.