In vitro Conversion of Testosterone to 17β-Hydroxyandrostan-3-one.* (22337)

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In schemes for the metabolism of testosterone, a theoretical intermediate which has been postulated but up to now not demonstrated is 17β-hydroxyandrostan-3-one(1). In this paper evidence is presented for the formation of this steroid when testosterone was incubated with a homogenate of liver from female rats.

Method. Two incubations were carried out. In both, female Sprague-Dawley rats weighing 200-250 g were decapitated, and the livers removed and homogenized immediately in a Potter homogenizer. The homogenizing medium was that described by Samuels and West (2). The incubations were carried out in a Dubnoff shaking incubator, in air, at 38°C. 1.5 ml of homogenate were added to 20 ml beakers, containing 1 mg testosterone in 0.06 ml propylene glycol, and 3 mg diphosphopyridine nucleotide (DPN) were added just before incubation.

Incubation No. 1. One gram of liver was homogenized per one ml of medium and the gauze-filtered homogenate was diluted with an equal amount of homogenizing fluid. There was approximately one-half gram of tissue per incubation flask. Twenty beakers were incubated for 90 minutes. After incubation, the reaction was stopped by addition of 0.15 ml 3 M acetate buffer, pH 5. The incubation mixtures were pooled and extracted 3 times with ethyl acetate. The ethyl acetate extract was dried over sodium sulfate, filtered, and the solvent removed *in vacuo*. Without any further purification, this extract was chromatographed on a 14 cm paper strip, in the ligroin-propylene glycol system(3) for 27 hours. A zone giving a blue Zimmermann color, having a mobility slower than that of androsterone, was eluted. This eluted material was taken up in chloroform, washed with a small volume of water, and dried over sodium sulfate. After removal of the chloroform, *in vacuo*, it was analyzed by infrared spectroscopy† and found to contain 17β-hydroxyandrostan-3-one as the major component. The crude material weighing 8 mg was rechromatographed in ligroin-propylene glycol for 24 hours, with crystalline 17β-hydroxyandrostan-3-one as standard on an adjacent strip. The mobilities of the isolated material and standard were the same. The eluted material was crystallized from acetone and methanol and melted at 175-178°C. When mixed with an authentic sample melting at 178°, 182-3° the mixture melted at 175-8°C. The melting point was depressed to 148°-167°C when mixed with androsterone melting at 181-4°C. The infrared spectrum of the isolated crystalline material was identical to that of the authentic sample.

Incubation No. 2. 13 g of liver were homogenized to a total volume of 35 ml. This homogenate contained about 0.56 g of tissue per 1.5 ml. Twenty beakers were incubated. After 2 hours incubation, 4 groups of 5 incubation mixtures were pooled, and 150 ml of acetone were added to each pool. After shaking for an hour, the acetone extracts were filtered and evaporated to dryness, *in vacuo*. The acetone extracts were dried overnight in a vacuum desiccator, over calcium chloride. The extracts were chromatographed for 20 hours in ligroin-propylene glycol. The zones whose mobilities were slower than that of androsterone were pooled and rechromatographed for 20 hours and the major Zimmermann-reacting (blue) zone was eluted for infrared analysis. The major component was identified as 17β-hydroxyandro-

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stan-3-one.

Summary and conclusion. Incubation of testosterone with an homogenate of liver from female rats led to formation of 17β-hydroxyandrostan-3-one. This steroid has been postulated as a possible intermediate in the metabolism of testosterone but up to now has been neither isolated from natural sources nor detected in metabolism experiments *in vivo* or *in vitro*.

1. Dorfman, R. I., and Ungar, F., *Metabolism of Steroid Hormones*, 1953, Burgess Publishing Co., Minneapolis.

2. Samuels, L. T., and West, C. D., *Vit. and Horm.*, 1952, v10, 251.

3. Savard, K., *J. Biol. Chem.*, 1953, v202, 457.

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Clinical, Bacteriological, and Serological Observations of two Human Volunteers Following Ingestion of *Escherichia coli* O127:B8. (22338)

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An epidemic of infantile diarrhea due to *Escherichia coli* O127:B8 reported by Stulberg and co-workers(1) has been further studied serologically by Stulberg, Zuelzer and Page(2). In an effort to produce specific antibodies in humans to be used as positive control sera for the serological portion of their studies, 2 adult volunteers ingested O127:B8 organisms following the methods employed by Ferguson and June(3) and June, Ferguson and Worfel(4) in their classical feeding experiments using *E. coli* O111:B4 and O55:B5. Since volunteer studies using O127:B8 have not been published, and because clinical, bacteriological and serological results of seeming interest were obtained, this experience is being reported.

Methods. Two adults, white males, age 28 and 32, participated in this study. Each man was in general good health and had been free of diarrhea of any nature for at least one year prior to this experiment. *Inocula*: *E. coli* O127:B8 cultures obtained from 3 infants during their diarrheal disease(1) were inoculated separately on brain heart infusion agar slants. After 18 hours incubation at 37°C, the growth from each slant was harvested in saline. The 3 suspensions were pooled and washed once in saline. The pooled suspension was diluted in saline and colony counts estimated the inocula for the two volunteers to contain 4,000,000 and 8,000,000 organisms respectively. In each instance the inoculum was contained in 1 ml. Each inoculum was added to one-half pint of pasteurized milk which was kept on ice until ingested, approximately one hour later. A challenge inoculum of 8,000,000 organisms prepared in a similar manner was ingested by volunteer A 32 days following the initial ingestion. One pre-ingestion blood sample and 3 pre-ingestion rectal swabs were obtained from each volunteer. Following ingestion, rectal swabs and venous blood samples were collected daily for about 2 weeks and later at approximately 3 day intervals. A detailed record of symptoms was kept by each volunteer. *Bacteriological and serological examinations*: Rectal swabs were inoculated immediately on blood agar and MacConkey's bile salts agar plates. After 20 to 24 hours, colonies appearing on the blood plates were tested for agglutinability with OB rabbit antiserum prepared with *E. coli* O127:B8, strain 9782 obtained from Dr. William Ferguson. Standard bacterial agglutination tests using OB and O antigens prepared with *E. coli* O127:B8 culture No. 2220 were performed according to the method described by Kauffmann(5). Saline suspensions of the organisms grown for 18 hours on brain heart infusion agar were used as antigens. Hemagglutination tests were performed