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Oxidation of Steroids by *Alcaligenes faecalis.*

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Previous studies[1, 2] have shown that a strain of *Alcaligenes faecalis,* isolated from the cecum of a guinea pig, oxidizes cholic acid (3,7,12-trihydroxycholanic acid) to ketocholanic acids—the end product being 3,7,12-triketocholanic acid. It seemed of interest to determine whether this organism could also oxidize other bile acids and such steroids as estradiol, estriol and dehydroisoandrosterone.

Experimental. Methods. Duplicate quantities of desoxycholic, hyodesoxycholic and lithocholic acids and estradiol, estriol and dehydroisoandrosterone* were placed in 250 cc Erlenmeyer flasks and sterilized by autoclaving, after which 10 cc of sterile sheep serum were added to each flask. One cc of a heavy suspension of *Alcaligenes faecalis*† in infusion broth was added to one of each pair of flasks; 1 cc of sterile broth was added to the other flask, which served as a control. All flasks were incubated at 37.5° for 6 days and were shaken 3 to 5 times daily throughout this period. At the end of incubation, the contents of each flask were extracted with 200 cc ethyl alcohol and the extracts analyzed for ketosteroids according to a modification‡ of the Hughes procedure.[3, 4]

Results. Keto-derivatives were formed when *Alcaligenes faecalis* was cultured in serum containing desoxycholic, hyodesoxycholic and lithocholic acids and dehydroisoandrosterone (Table I). Under the same experimental conditions no keto-derivatives were formed from estradiol and estriol.

The weights of mercuric iodide hydrazones derived from digests of desoxycholic acid and dehydroisoandrosterone correspond closely to the theoretical weights required for conversion of the hydroxyl groups of these compounds to carbonyl groups. Since previous experiments[2] showed that *Alcaligenes faecalis* could oxidize hydroxyl groups at C_3 and C_{12} to carbonyl groups, it seemed probable that this organism converted desoxycholic acid to 3,12-diketocholanic acid and dehydroisoandrosterone to androstenedione. Bacterial oxidation of dehydroisoandrosterone to androstenedione was reported previously by Mamoli and coworkers,[5] who found that an organism similar to *Corynebacterium helvolum* accomplished this conversion.

The weights of mercuric iodide hydrazones obtained from digests of hyodesoxycholic and lithocholic acids were considerably smaller than those theoretically required for oxidation of the hydroxyl groups of these compounds to carbonyl groups. Incomplete conversion of lithocholic acid was probably due in part to the insolubility of this compound in serum. Incomplete conversion of hyodesoxycholic acid may have been due to the limitation of the action of *Alcaligenes faecalis* to the C_3 hydroxyl—the weight of mercuric

1 Schmidt, L. H., and Hughes, H. B., *J. Biol. Chem.*, 1942, **143**, 771.

2 Schmidt, L. H., Hughes, H. B., Green, M. H., and Cooper, E., *J. Biol. Chem.*, 1942, **145**, 229.

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† The strain of *Alcaligenes fœcalis* used in this study was isolated from a guinea pig cecum in November, 1940, and has been passed through infusion broth daily since that time. The organism suspension was obtained from agar slant cultures that had been incubated for 18 hours, the organisms from each slant being washed off with 2 cc of infusion broth.

‡ The original Hughes procedure[3] was modified to eliminate the lipids contained in serum extracts. These lipids were removed by ether extraction of the lipid-hydrazone mixtures at pH 7.

3 Hughes, H. B., *J. Biol. Chem.*, 1941, **140**, 21.

4 Hughes, H. B., *J. Biol. Chem.*, 1942, **143**, 11.

5 Mamoli, L., Koch, R., and Teschen, H., *Naturwissenschaften*, 1939, **27**, 319.

TABLE I.
Oxidation of Steroids by *Alcaligenes fæcalis.*

Steroid tested	Amt steroid added to 10 cc serum, mg	Reaction mixture	Mercuric iodide hydrazone*	
			Derived from digest after incubation, mg	Theoretical for conversion of hydroxyl to carbonyl groups, mg
Desoxycholic (3,12-dihydroxycholanic) acid	20	Inoculated	79.8	82.2
	20	Control	0	
Hyodesoxycholic (3,6-dihydroxycholanic) acid	20	Inoculated	41.6	82.2
	20	Control	0	
Lithocholic (3-hydroxycholanic) acid	20	Inoculated	14.6	52.2
	20	Control	0	
Dehydroisoandrosterone	16	Inoculated	78.8	77.4
	16	Control	47.5	

*The theoretical weights of hydrazone were calculated according to equations described previously.[3] The conversion factors for the various hydroxysteroids are: 4.110 for desoxycholic and hyodesoxycholic acids; 2.599 for lithocholic acid; 4.836 for dehydroisoandrosterone.

iodide hydrazone obtained being approximately 90% of that required for conversion of just one hydroxyl to a carbonyl group.

Summary. *Alcaligenes faecalis,* cultured in serum, oxidized desoxycholic acid, hyodesoxycholic acid, lithocholic acid and dehydroisoandrosterone to keto-derivatives but had no action on estradiol and estriol.

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Electrophoretic Studies on New-Born Calf Serum.

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The composition of new-born calf serum before the ingestion of colostrum is completely different from that of the adult, and different as well from that of the calf after a short period of nursing. The very rapid changes in serum composition that take place during nursing were first studied by Howe[1] by salting-out methods. He found that the blood of a new-born calf "does not contain euglobulin nor pseudoglobulin I," and that after ingestion of colostrum it contains "relatively large" amounts of these globulins. We have confirmed and extended his observations by the electrophoretic study of the serum of new-born, nursing, and mature calves. Phase-rule studies have also been carried out by one of the authors.[2]

Fig. 1 shows schlierren pictures of the proteins separated by electrophoresis in new-born calf serum, and serum from 18-hour, 3-day, 5-day, and 2-year-old calves. The great difference in composition is quite obvious, both the number of fractions present and the concentration of the protein fractions change during nursing. Using the terminology first proposed by Tiselius and now commonly used in electrophoretic work, we can say that the γ fraction is absent in the new-born serum, appears when colostrum is first ingested, increases very rapidly during early nursing, and

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1 Howe, P. E., *J. Biol. Chem.,* 1921, **49**, 115.

2 Jameson, E., and Roberts, D. B., *J. Gen. Physiol.,* 1937, **21**, 249.