Lactobacillus pentosus, and has suggested that L-ribulose is phosphorylated in the presence of adenosine triphosphate (ATP). Similarly, Volk[2] tentatively identified heptulose, arabinose, ribose, and ribulose phosphates following incubation of L-arabinose and ATP with an extract of *Propionibacterium pentosaceum*.

Extracts of *Aerobacter aerogenes* also isomerize L-arabinose to L-ribulose. We now wish to report the finding of an enzyme that catalyzes the reaction

L-ribulose + ATP → L-ribulose-5-phosphate (L-Ru-5-P) + adenosine diphosphate (ADP)

This enzyme, L-ribulokinase, occurs in cells of *A. aerogenes* grown on L-arabinose, but not in significant amounts in cells grown on D-xylose. A 200-fold purified preparation is devoid of L-arabinose isomerase, phosphoketopentoepimerase[3] and transketolase,[4] but is contaminated with a small amount of phosphoriboisomerase.[5] Neither ADP nor UTP serve as phosphate donors.

The product of phosphorylation was prepared by incubating 0.25 mg. of purified enzyme (612 μM. phosphorylated per hr.), 1000 μM. ATP, 1000 μM, L-ribulose, 1000 μM. $MgCl_2$, 100 μM. sodium glutathione and 80 μM. Versene (20 ml. volume) at room temperature until alkali was no longer required to maintain the *p*H at 7.4. This was then chromatographed on a Dowex-1 formate column.[6] A very small peak identified as adenylic acid and a large symmetrical peak were located by the orcinol assay for pentoses.[6] The phosphorylated sugar was recovered (700 μM) as the alcohol insoluble barium salt.[6] The salt was redissolved and the barium precipitated as sulfate. The phosphate group was removed from an aliquot with acid phosphatase[7] and the solution deionized with a mixed bed of IR120(H^+) and IR45(OH^-).

In the carbazole method[8] the reaction with the free sugar was complete in 10 min., as is characteristic of ribulose.[9] Both the free and the phosphorylated sugars produced a red color with a maximum at 540 mμ. Both failed to give the carbazole color after incubation in 1 *N* NaOH for 20 min. as is characteristic of ribulose and D-ribulose-5-phosphate (D-Ru-5-P). In the orcinol method the ratio of absorbancy at 540 to 670 mμ for the free sugar was 0.80 as compared to 0.89 for L-ribulose. Paper chromatography of the free sugar revealed a single component having the same R_f and color reactions as ribulose[10] (Table I).

The phosphorylated product contained, per mole, 1 mole of organic phosphate. The rate of inorganic phosphate formation at 90° in 1 *N* H_2SO_4

TABLE I

CHROMATOGRAPHY OF THE DEPHOSPHORYLATED REACTION PRODUCT

	R_f		Color of Spot	
	Acetate-H_2O[a]	Phenol[b]	Orcinol-TCA[c]	Dimethyl-phenaline[c]
Xylose	0.14	0.51	Colorless	Brown
Fructose	.10	.56	Olive green[d]	Brown
Arabinose	.11	.57	olorless	Pink
Ribose	.19	.64	Colorless	Pink
Xylulose	.21	.65	Steel gray	Purple
Ribulose	.22	.68	Brownish gray	Rose[e]
Reaction product	.23	.68	Brownish gray	Rose[e]

[a] Three parts ethyl acetate, 1 part acetic acid, 3 parts water (upper phase). [b] Water saturated phenol. [c] As modified by M. I. Krichevsky, and W. A. Wood.[10] [d] Fluoresces green under ultraviolet. [e] Fluoresces orange under ultraviolet.

parallels that published for D-Ru-5-P.[6] The phosphorylated product resisted oxidation by bromine.[6,11] Two μM. of periodic acid were reduced[12] per μM. of phosphorylated product while under the same conditions 1.7 μM. were reduced per μM. of D-Ru-5-P.[13] $[\alpha]^{20}D$ +28° (c = 0.265 in 0.2 *N* HBr)[14] compared to $[\alpha]^{20}D$ −28.5° reported for D-Ru-5-P.[5,6] The phosphorylation product is there fore believed to be L-Ru-5-P.

In a system composed of crystalline glyceraldehyde-3-phosphate dehydrogenase from muscle and a purified preparation from spinach containing transketolase, epimerase and phosphopentoisomerase, diphosphopyridine nucleotide (DPN) was not reduced with L-Ru-5-P as the substrate although reduction was rapid with D-R-5-P. However, when an extract from *A. aerogenes* was added to the system, DPN was reduced with L-Ru-5-P. Thus, L-Ru-5-P appears to be the first phosphorylated intermediate in the metabolism of L-arabinose by *A. aerogenes*.

(2) W. A. Volk, *Fed. Proc.*, **15**, 1229 (1956).

(3) B. L. Horecker, J. Hurwitz and P. Z. Smyrniotis, THIS JOURNAL, **78**, 692 (1956).

(4) B. L. Horecker, P. Z. Smyrniotis and H. Klenow, *J. Biol. Chem.*, **205**, 661 (1953).

(5) J. Hurwitz, A. Weissbach, B. L. Horecker and P. Z. Smyrniotis, *ibid.*, **218**, 769 (1956).

(6) B. L. Horecker, P. Z. Smyrniotis and J. E. Seegmiller, *ibid.*, **193**, 383 (1951).

(7) R. M. Hochster, *Canad. J. Microbiol.*, **1**, 346 (1955).

(8) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

(9) B. L. Horecker, J. Hurwitz and A. Weissbach, *ibid.*, **218**, 785 (1956).

(10) P. J. VanDemark and W. A. Wood, *J. Bacteriol.*, **71**, 385 (1956).

(11) S. Mitsuhashi and J. O. Lampen, *J. Biol. Chem.*, **204**, 1011 (1953).

(12) A. C. Neish, "Analytical Methods for Bacterial Fermentations," National Research Council, Ottawa, Ontario, 1952, pp. 20-31.

(13) Prepared from R-5-P with phosphoriboisomerase[5] by Dr. M. Wolin and Dr. W. Volk.

(14) Calculated from the pentose content based on a ribulose-5-PO_4:aldopentose ratio of 0.57 in the orcinol method.

(15) On educational leave of absence from Prairie Regional Laboratory, National Research Council, Saskatoon, Saskatchewan, Canada.

DEPARTMENT OF DAIRY SCIENCE
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS
F. J. SIMPSON[15]
W. A. WOOD

RECEIVED SEPTEMBER 12, 1956

THE *IN VITRO* SYNTHESIS OF 17α-HYDROXYPROGESTERONE AND Δ⁴-ANDROSTENE-3,17-DIONE FROM PROGESTERONE BY BOVINE OVARIAN TISSUE

Sir:

Almost 20 years after the suggestion was made[1] that exogenously administered testosterone might serve as a precursor for the estrogens, Baggett, *et al.*,[2] proved conclusively that human ovarian

(1) E. Steinach, H. Kun and O. Peczenik, *Wien. Klin., Wschr.*, **49**, 899 (1936).

(2) B. Baggett, L. L. Engel, K. Savard and R. I. Dorfman, *Fed. Proc.*, **14**, 175 (1955).

slices could effect this conversion *in vitro*. Heard, *et al.*,[3] have added confirmatory *in vivo* evidence and West, *et al.*,[4] have shown that this conversion can occur in humans even in the absence of ovaries and adrenals. We now wish to report evidence for the formation of 17α-hydroxyprogesterone and Δ^4-androstene-3,17-dione from progesterone by ovarian tissue, thereby providing a link between progesterone and estradiol biogenesis. Recent publications by Slaunwhite and Samuels[5] and Lynn[6] have reported the conversion of progesterone to 17α-hydroxyprogesterone and to androstenedione by testes homogenates.

Bovine ovarian tissue was homogenized according to the procedure of Hayano, *et al.*,[7] and incubated aerobically at 37° for four hours with 4.8 mg. of progesterone-4-C^{14} (5.5×10^6 c.p.m.) and 10 mg. each of 17α-hydroxyprogesterone and androstenedione. A neutral, ketonic extract was prepared[7] and chromatographed on alumina. The recovered 17α-hydroxyprogesterone, eluted with ether–benzene (1:9), was mixed with unlabeled progesterone and submitted to two "washout" chromatograms on alumina to remove traces of progesterone-4-C^{14}. The specific activity of the isolated 17α-hydroxyprogesterone was 1190 c.p.m./mg. after the first recrystallization (m.p. 220–222° from acetone) and 1250 c.p.m./mg. after the second (m.p. 222–223°)[8]. After dilution with 11 mg. of carrier, the 17α-hydroxyprogesterone (4 mg.) was converted by hydrogenation over Pt catalyst in acetic acid and subsequent oxidation with periodic acid to 3β-hydroxyandrostan-17-one,[8] m.p. 177–177.5°, having a specific activity of 309 c.p.m./mg. (theoretical 369 c.p.m./mg.).

The androstenedione fraction, eluted from the alumina chromatogram by ligroin–benzene (2:8), was mixed with unlabeled progesterone and submitted to a "washout" 25 transfer countercurrent distribution using the system of Pearlman.[9] The fractions containing androstenedione were combined, diluted with carrier and purified by two additional countercurrent distributions followed by two chromatograms on paper using the propylene glycol–ligroin C system of Savard.[10] These procedures succeeded in eliminating two radioactive contaminants. The androstenedione eluted from the second paper chromatogram was purified through a column of alumina and recrystallized twice from ether–ligroin C. The specific activity after the first recrystallization (m.p. 170–172°) was 90 c.p.m./mg. and after the second[8] (m.p. 172–173°) 97 c.p.m./mg. The latter sample when repartitioned by a 50-transfer countercurrent procedure was distributed, as determined both by radioactivity and absorbance at 240 mμ., in a manner characteristic of pure androstenedione. The ultraviolet absorbing material from this distribution was recombined, purified over alumina and recrystallized from ether to yield androstenedione,[8] m.p. 171–173°, having 93 c.p.m./mg.

Thus the biogenesis of the two principal secretory products of the ovary, progesterone and estradiol, has been interrelated. In similar *in vitro* experiments with *corpora lutea* cholesterol has been shown[11] to be a precursor of progesterone and therefore a biosynthetic scheme in the ovary appears to be: cholesterol → progesterone → androstenedione (testosterone) → estradiol. The intermediacy of androgens on the biogenetic pathways leading to the estrogens affords a new approach to the understanding of the origin and role of ovarian androgens. The occurrence of 17α-hydroxylase in ovarian tissue makes it likely that 17α-hydroxyprogesterone is an intermediate between progesterone and androstenedione in ovarian biosynthesis. Definitive proof of this is now being sought.

Acknowledgment.—The authors gratefully acknowledge the partial support given them by a grant from the National Science Foundation.

(3) R. D. H. Heard, P. H. Jellinek and V. J. O'Donnell, *Endocrinology*, **57**, 200 (1955).

(4) C. D. West, B. L. Damast, S. D. Sarro and O. H. Pearson, *J. Biol. Chem.*, **218**, 409 (1955).

(5) W. R. Slaunwhite, Jr., and L. T. Samuels, *ibid.*, **220**, 349 (1956).

(6) W. S. Lynn, *Fed. Proc.*, **15**, 305 (1956).

(7) M. Hayano, M. C. Lindberg, M. Wiener, H. Rosenkrantz and R. I. Dorfman, *Endocrinology*, **55**, 326 (1954).

(8) The identity was confirmed by infrared analysis and mixed melting point with an authentic sample.

(9) W. H. Pearlman, *Rec. Prog. Hor. Res.*, **9**, 27 (1954).

(10) K. Savard, *J. Biol. Chem.*, **202**, 457 (1953).

(11) S. Solomon, R. Vande Wiele and S. Lieberman, unpublished results.

DEPARTMENTS OF BIOCHEMISTRY AND
OBSTETRICS AND GYNECOLOGY
COLLEGE OF PHYSICIANS AND SURGEONS SAMUEL SOLOMON
COLUMBIA UNIVERSITY RAYMOND VANDE WIELE
NEW YORK, N. Y. SEYMOUR LIEBERMAN

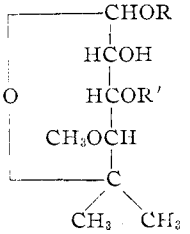
RECEIVED AUGUST 8, 1956

NOVOBIOCIN. V. THE CONFIGURATION OF THE ALDOSE MOIETY

Sir:

We have assigned the configuration of L-lyxose to 3-O-carbamyl-4-O-methylnovobiose (I).[1,2] This assignment is based in part on rules of optical rotation.

Hydrolysis of methyl 4-O-methylnovobiopyranoside (III)[1,2] with 0.1 *N* hydrochloric acid followed by reaction with N-benzyl-*p*-methoxyphenylhydrazine has yielded an N-benzyl-*p*-methoxyphenylhydrazone IV, m.p. 111–113°, $[\alpha]^{28}D$ −39° (*c*, 1 in methanol). The negative optical rotation of IV allows assignment[3] of the C-2 hydroxyl group to the right in the Fisher projection formula.



I, R = H, R′ = $CONH_2$
II, R = CH_3, R′ = $CONH_2$
III, R = CH_3, R′ = H

(1) C. H. Shunk, C. H. Stammer, E. A. Kaczka, E. Walton, C. F. Spencer, A. N. Wilson, J. W. Richter, F. W. Holly and K. Folkers, THIS JOURNAL, **78**, 1770 (1956).

(2) H. Hoeksema, E. L. Caron and J. W. Hinman, *ibid.*, **78**, 2019 (1956).

(3) It has been shown by E. Votoček, *Collection Czechoslov. Chem. Communs.*, **3**, 259 (1931), that N-benzylphenylhydrazones of aldoses having the C-2 hydroxyl located on the right have negative optical rotations.