reaction as yet unknown. The mechanism presented here, however, is in line with the current concept for the biogenesis of cortisol.

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Note added in proof. We recently found that acetone-washed beef adrenal mitochondria convert [4-^{14}C, 4-β-^{3}H]cholesterol to pregnenolone with tritium retention and to progesterone with loss of tritium. These findings support the view that steroid Δ-isomerase mediates the tritium loss from pregn-5-ene-3,20-dione.

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[1] O. HECHTER, A. ZAFFARONI, R. P. JACOBSEN, H. LEVY, R. W. JEANLOZ, V. SCHENKER AND G. PINCUS, *Recent Progr. Hormone Res.*, 6 (1951) 215.
[2] L. T. SAMUELS, M. L. HELMREICH, M. B. LASATER AND H. REICH, *Science*, 113 (1951) 490.
[3] P. TALALAY AND M. M. DOBSON, *J. Biol. Chem.*, 205 (1953) 823.
[4] P. TALALAY AND V. S. WANG, *Biochim. Biophys. Acta*, 18 (1955) 300.
[5] R. E. IRELAND, T. I. WRIGLEY AND W. G. YOUNG, *J. Am. Chem. Soc.*, 81 (1959) 2818.
[6] E. REICH AND A. L. LEHNINGER, *Biochim. Biophys. Acta*, 17 (1955) 136.
[7] E. G. BLIGH, R. D. L. HEARD, V. J. O'DONNELL, J. L. WEBB, M. SAFFRAN AND E. SCHONBAUM, *Arch. Biochem. Biophys.*, 58 (1955) 249.
[8] N. L. R. BUCHER, *J. Am. Chem. Soc.*, 75 (1953) 498.
[9] G. T. OKITA, J. J. KABARA, F. RICHARDSON AND G. V. LEROY, *Nucleonics*, 15 (1957) 111.
[10] H. WERBIN, I. L. CHAIKOFF AND E. E. JONES, *J. Biol. Chem.*, 235 (1960) 1629.
[11] I. E. BUSH, *Biochem. J.*, 50 (1952) 370.
[12] R. B. BURTON, A. ZAFFARONI AND E. H. KEUTMANN, *J. Biol. Chem.*, 188 (1951) 763.
[13] J. W. CORNFORTH, *J. Lipid Res.*, 1 (1959) 3.
[14] B. W. AGRANOFF, H. EGGERER, U. HENNING AND F. LYNEN, *J. Biol. Chem.*, 235 (1960) 326.
[15] B. E. NORCROSS, P. E. KLINEDINST, Jr. AND F. H. WESTHEIMER, *J. Am. Chem. Soc.*, 84 (1962) 797.

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Gas-chromatographic identification of progesterone in human pregnancy plasma

Methods for the microquantitative detection of steroids have improved greatly in recent years. The association of thin-layer adsorption and gas–liquid chromatography offers the advantages of rapidity, simplicity, sensitivity as well as specificity. In the present report evidence is indicated that progesterone can be qualitatively detected in peripheral plasma by gas-chromatographic techniques.

A Research Specialties gas chromatograph (model-600) equipped with a hydrogen flame detector was used in association with a 1-mV recorder. Three columns were packed and prepared according to the methods of HORNING, MOSCATELLI AND SWEELEY[1]. The use of a solids injector device[2] allowed for the introduction of 1–30-μl aliquots of sample without significant solvent interference. The latter was kept against the "flash heater" (330°) for 30 sec.

5–10 ml of heparinized pregnancy plasma were extracted with a mixture of chloroform–ether as described by FINKELSTEIN *et al.*[3]. The extract containing free steroids was placed on a 20 × 20 cm glass plate containing the adsorbant silica gel G, and run in a benzene–ethyl acetate (3:2) solvent system. The time of development varied from 70 to 100 min. The R_F values for progesterone and related steroids in this system are presented in Table I. The progesterone zones were eluted with ethyl

TABLE I

R_F STEROIDS ON SILICA GEL G

R_F, values estimated in benzene–ethyl acetate (3:2).

Compound	R_F
Testosterone	0.47
20α-Hydroxypregn-4-en-3-one	0.53
17α-Hydroxypregn-4-en-3-one	0.55
Pregnenolone	0.64
Androst-4-en-3,17-dione	0.69
Progesterone	0.77

TABLE II

RELATIVE RETENTION TIMES

Compound	*SE-30** 240°	*Polydiethylene glycol adipate*** 213°	*Neopentyl glycol adipate**** 213°
Cholestane	1.00	1.00	1.00
Progesterone	0.94	7.90	10.05

* Column, 6.8% silicone SE-30 on 110–120 mesh Anakrom ABS 3 ft × 1.9 mm U-tube; 47.5 lb/in²; cholestane time, 6.0 min.

** Column, 1.1% polydiethylene glycol adipate on 130–140 mesh Anakrom ABS 2½ ft × 2.0 mm U-tube; 55.5 lb/in²; cholestane time, 1.30 min.

*** Column 1.34% Neopentyl glycol adipate on 130–140 mesh Anakrom ABS 3 ft × 2 mm U-tube; 45.5 lb/in²; cholestane time, 1.9 min.

acetate, and transferred to small conical tubes. The samples were then dissolved in 50–75 μl of acetone and aliquots of the latter placed onto the solids injector with a Hamilton microliter syringe. Standard solutions containing 0.02–0.15 μg progesterone were injected, and a weight *vs.* area graph obtained after integration with a Royson planimeter. The area of the extract peak corresponding to a known amount of plasma was then obtained and the quantity of progesterone estimated from the graph. Several other extracts were only purified on a 3-g silica gel column[4]. The ethyl acetate eluant was similarly dried and transferred to a small conical tube for chromatographic analysis.

Table II contains the retention times for progesterone, relative to cholestane, in the 3 columns packed with silicone SE-30, neopentyl glycol succinate and polydiethylene glycol adipate. In each instance the extract of pregnancy plasma showed a peak identical in retention time to that of authentic progesterone. An augmented symmetrical peak was obtained when 0.03–0.1 μg of progesterone was added to an aliquot of plasma presumed to contain a similar quantity of progesterone. A cleaner

extract was obtained with thin-layer chromatography than with simple silica gel defatting of the crude extract.

The response/mass ratio was very much less with the selective polyester phases than with silicone SE-30. The latter demonstrated a minimal sensitivity of 0.015 μg of progesterone at an attenuation $\times$ 100. Although [7α-^{3}H]progesterone (specific activity 30 mC/mg) was added to the original plasma extract to determine extraction losses, accurate quantitation of progesterone on the silicone SE-30 phase was hampered by the presence of a relatively small nonsteroidal contaminant, which appeared in several of the extracted water blanks as well. This contaminant, however, was not detected on a polyester column. The source of this contaminant is presently under investigation.

The use of polydiethylene glycol adipate resulted in less sensitivity than obtainable with SE-30 presumably due to excessive steroidal adsorption. Using the former column three plasma samples yielded the following values for progesterone expressed as μg/100 ml plasma: 4th month pregnancy, 6.9; 5th month pregnancy, 11.6; 8th month pregnancy, 29.7. These values are in agreement with previously published values[5].

20α-Hydroxypregn-4-en-3-one is present in the peripheral plasma of pregnant women[6]. This progestationally active steroid is not efficiently separated on the preferable silicone SE-30 phase but preliminary purification on silica gel G affords a rapid way of separating this steroid from progesterone and other closely related compounds such as pregnenolone. In a recent report, KUMAR *et al.*[7] isolated progesterone from human pregnant myometrium. Their procedure included preliminary purification on a silicic acid column and final gas chromatographic separation using a 2% silicone SE-30 phase.

The results of this investigation indicate that a method for gas-chromatographic analysis of plasma progesterone is feasible especially when coupled with the advantages of capacity, speed and resolution afforded by preliminary purification on thin-layer adsorption chromatography. This technique has been used for the determination of testosterone in urine[8].

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[1] E. C. HORNING, E. A. MOSCATELLI AND C. C. SWEELEY, *Chem. Ind. London*, (1959) 751.
[2] N. L. MCNIVEN, W. FUTTERWEIT AND K. M. BERENKEY, *J. Gas Chromatography*, unpublished data.
[3] M. FINKELSTEIN, E. FORCHIELLI AND R. I. DORFMAN, *J. Clin. Endocrinol. Metab.*, 21 (1961) 98.
[4] J. W. GOLDZIEHER, R. A. BAKER AND E. C. RIHA, *J. Clin. Endocrinol. Metab.*, 21 (1961) 62.
[5] G. W. OERTEL, S. P. WEISS AND K. B. EIK-NES, *J. Clin. Endocrinol. Metab.*, 19 (1959) 213.
[6] R. V. SHORT, *J. Endocrinol.*, 20 (1960) XV.
[7] D. KUMAR, J. A. SOODNO AND A. C. BARNES, *Nature*, 195 (1962) 1204.
[8] W. FUTTERWEIT, N. L. MCNIVEN, L. NARCUS, C. LANTOS AND R. I. DORFMAN, *Steroids* submitted for publication.

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