Gottschalk, A. (1958). In *Encyclopedia of Plant Physiology*, vol. 6, p. 87. Ed. by Ruhland, W. Berlin: Springer-Verlag.
Halsall, T. G., Hirst, E. L. & Jones, J. K. N. (1947). *J. chem. Soc.* p. 1427.
Hough, L. & Perry, M. B. (1956). *Chem. & Ind.* p. 768.
Hough, L., Powell, D. B. & Woods, B. M. (1956). *J. chem. Soc.* p. 4799.
Manners, D. J. (1955*a*). *Biochem. J.* **61**, xiii.
Manners, D. J. (1955*b*). *Quart. rev. chem. Soc., Lond.*, **9**, 73.
Peat, S., Whelan, W. J. & Hinson, K. A. (1952). *Nature, Lond.*, **170**, 1056.
Peat, S., Whelan, W. J. & Hinson, K. A. (1955). *Chem. & Ind.* p. 385.
Peat, S., Whelan, W. J. & Lawley, H. G. (1958). *J. chem. Soc.* p. 729.
Somogyi, M. (1952). *J. biol. Chem.* **195**, 19.

Progesterone Metabolism: Investigation of the Products of Metabolism with Human Liver *in vitro*

By L. M. ATHERDEN*
Pharmacy Department, Sunderland Technical College

(*Received* 1 *September* 1958)

The metabolism of progesterone *in vivo* in the human subject has been extensively investigated, but only 3α-hydroxy-5β-pregnan-20-one (pregnanolone) (Dorfman, Ross & Shipley, 1948), 5β-pregnane-3α:20α-diol (pregnanediol) (Dorfman *et al.* 1948), 3α-hydroxy-5α-pregnan-20-one (Dobriner, Lieberman, Rhoads & Taylor, 1948; Dobriner & Lieberman, 1950; Davis & Plotz, 1956; Plotz & Davis, 1956) and 5α-pregnane-3α:20α-diol (*allo*pregnanediol) (Kyle & Marrian, 1951) have been shown conclusively to be progesterone metabolites. The metabolism *in vitro* has been investigated in the rat (Taylor, 1954), but progesterone metabolism in the rat appears to be different from that in the human (Wiest, 1956) since the rat does not excrete pregnanediol in the urine (Atherden & Grant, 1957). Taylor (1955) also investigated progesterone metabolism in the rabbit which, like the human, excretes pregnanediol in the urine; but it is well known that steroid metabolism varies with species (see, for example, Brooks & Klyne, 1957).

Thus it seemed desirable that progesterone metabolism *in vitro* in the human should be investigated; Reaven (1955) has shown that αβ-unsaturated ketonic steroids are transformed into substances which do not absorb at 240 mμ when incubated with human-liver slices; therefore progesterone was incubated with a 'suspension' prepared from post-mortem human liver.

EXPERIMENTAL

Materials

All materials were obtained from British Drug Houses Ltd. unless otherwise stated.

Steroids. Progesterone (Ciba Laboratories Ltd.), in the form of prisms ('α'-progesterone), had m.p. 127·5–128° [all melting points were determined on a micro-melting-point apparatus (Gallenkamp and Co. Ltd.) and are uncorrected]; 5α-pregnane-3:20-dione (*allo*pregnanedione) (L. Light and Co. Ltd., Colnbrook, Bucks) had m.p. 199–200°; 5β-pregnane-3:20-dione (pregnanedione) (L. Light and Co. Ltd.) had m.p. 122–123°; 3α-hydroxy-5α-pregnan-20-one and its acetate (m.p. 134–136°) were supplied by the Medical Research Council Steroid Reference Collection; 3β-hydroxy-5α-pregnan-20-one (*allo*pregnanolone) (m.p. 193–196°) and pregnanolone (m.p. 149–151°) were samples from Dr W. Taylor, University of Durham; pregnanolone acetate was prepared from pregnanolone and had m.p. 96–100°; pregnanediol (L. Light and Co. Ltd.) had m.p. 235–238°; pregnanediol diacetate was prepared from pregnanediol and had a double m.p. 162° and 181–182°; cholesterol had m.p. 145–146°.

Reagents. Reduced triphosphopyridine nucleotide (TPNH) (Sigma Chemical Co., Mo., U.S.A.), digitonin (AnalaR) and Girard T reagent (Girard & Sandulesco, 1936) were not purified further; alumina (100–200 mesh, Savory and Moore Ltd.) was screened through a no. 90 sieve, the material not passing through being rejected. It was activated to activity II (Brockmann & Schodder, 1941) by heating at 130° for 12 hr.; some was deactivated by exposure to water vapour for 10 days.

Solvents. Acetone was refluxed for 2 hr. with $KMnO_4$ and NaOH, distilled, refluxed with anhydrous K_2CO_3 and redistilled. Benzene was washed with water, conc. H_2SO_4 and water, dried over $CaCl_2$ and distilled. $CHCl_3$ was prepared freshly as required by washing with water, drying over $CaCl_2$ and distilling. Ether (J. F. MacFarlan and Co.) was treated with $FeSO_4$ soln., washed with water, dried with Na_2SO_4 and Na wire and distilled. Ethyl acetate (AnalaR) was redistilled. Hexane consisted of the fraction from petroleum boiling at 67–70°; this was washed with conc. H_2SO_4, 2N-NaOH and water, dried over $CaCl_2$ and distilled. Methanol (AnalaR) was redistilled. Pyridine was dried over BaO, filtered, distilled and the fraction boiling at 112–117° was collected and stored over BaO.

* Present address: Pharmacy Department, College of Technology, Bristol 7.

Methods

Spectra. For the determination of absorption spectra in H_2SO_4, the sample and an authentic sample (about 100 μg. each) were treated with 5 ml. of conc. H_2SO_4 (AnalaR) for 17 hr. at 25°; spectra were then determined in 1·0 cm. cells over the range 220–520 mμ in a Uvispek spectrophotometer (Hilger and Watts Ltd.).

Incubation medium. This was based on preliminary experiments with rat liver (Atherden, 1956) and contained (final concn.) 0·04M-potassium phosphate (pH 7·4), 0·01M-sodium citrate (pH 7·4), 0·005M-$MnSO_4$ and 0·0003M-TPNH. Progesterone (500 mg.) was added in 50 ml. of propane-1:2-diol to 950 ml. of incubation medium.

Preparation of liver 'suspension'. Post-mortem liver (500 g.) from a 30-year-old woman who had died from bronchial pneumonia was cut into portions and homogenized in an ice-cold Ato-Mix blender (Measuring and Scientific Equipment Ltd.) containing a medium of 0·25M-sucrose and 0·2M-nicotinamide for ½ min. at full speed. The resulting 'suspension' was diluted with the sucrose–nicotinamide medium to give a 50% (w/v) 'suspension' and stored at 0° until all the liver had been treated.

Incubation. The volume (1 l.) of suspension was added to 1 l. of incubation medium in a 3 l. flask and the whole was incubated with manual horizontal shaking in a water bath at 37° for 2 hr. in air.

Protein precipitation. After the incubation, the contents of the flask were added to 2·6 l. of acetone. The mixture was stirred and left overnight at −19°; it was filtered and the residue washed twice with 1 l. volumes of previously chilled acetone. Most of the acetone was removed from the combined filtrates by distillation under reduced pressure.

Extraction of lipids. The aqueous residue from the above distillation was saturated with NaCl and was then extracted with 3 × 500 ml. of ethyl acetate. The extract was washed with 100 ml. volumes of water, 0·2M-$NaHCO_3$, 0·2N-HCl and water until the washings were neutral to litmus; the first water and $NaHCO_3$ washes were combined and back-extracted with 200 ml. of ethyl acetate, and the same 200 ml. was used to back-extract the HCl and water washes individually. The combined ester extracts were dried with Na_2SO_4 and filtered, the residue was washed with ester and the combined filtrates were distilled to dryness *in vacuo* on a water bath at 60°. The residue was partitioned between 430 ml. of 70% (v/v) methanol and 200 ml. of hexane; the lower layer was run off, the upper layer was extracted with 2 × 100 ml. of 70% (v/v) methanol and the three combined methanol extracts were back-extracted with 50 ml. of hexane. Most of the methanol was taken off by distillation *in vacuo*, 150 ml. of water was added and the mixture was extracted with 2 × 500 ml. of $CHCl_3$; the extracts were washed with 100 ml. of N-NaOH, with water until neutral and were then dried over Na_2SO_4. The $CHCl_3$ was evaporated off *in vacuo* leaving a residue which weighed, after vacuum desiccation over P_2O_5 for 24 hr., 923 mg.

Girard separation. The dried residue was taken up in 20 ml. of ethanol, 1·7 g. of Girard T reagent and 1 ml. of acetic acid were added and refluxed for 1 hr. (Girard & Sandulesco, 1936); the contents of the flask were poured quickly into a separating funnel containing 55 ml. of ice-cold water, 30 g. of crushed ice, 15 g. of NaCl and 8·1 ml. of 2N-NaOH. The mixture was extracted with 120 ml. of ether and 3 × 60 ml. of ether and the combined ether extracts were washed with 50 ml. of water; this water wash was added to the aqueous phase. The ether extract was then washed with 2 × 25 ml. of 5% (w/v) $NaHCO_3$ soln., with 2 × 25 ml. of water, dried over Na_2SO_4, filtered and the ether evaporated off to give 199 mg. of non-ketonic fraction. The aqueous phase was acidified with 10 ml. of 18N-H_2SO_4 and left overnight; it was extracted with 100 ml. and 3 × 50 ml. of ether and the combined ether extracts were washed with 2 × 25 ml. of 5% (w/v) $NaHCO_3$ soln. and 2 × 25 ml. of water. The washed ether extract was dried over Na_2SO_4, filtered and the ether evaporated off to give 386 mg. of ketonic fraction.

Digitonin separation. The ketonic and non-ketonic fractions were each separated into digitonin-non-precipitable (α) and digitonin-precipitable (β) fractions (Butler & Marrian, 1938); however, 48 hr. was allowed for complete precipitation (cf. Haslam & Klyne, 1953).

RESULTS

α-Ketonic fraction. This fraction (291 mg.) was taken up in 2 ml. of benzene, 2 ml. of hexane was added and the mixture applied to a column (1 cm. internal diameter) containing 6 g. of alumina activity II. Elution with hexane–benzene (1:1, v/v) yielded a white crystalline mass; this was crystallized from aqueous ethanol and yielded 117 mg. of progesterone, identified by m.p. (128–129°) and mixed m.p. (126–128°) with authentic progesterone (m.p. 127·5–128°).

The mother liquor yielded material which after crystallization from hexane gave 6 mg. of white rods with m.p. 123–124°; mixed m.p. with pregnanedione (m.p. 122–123°) was 123–124°. On admixture of the isolated material with progesterone (m.p. 127·5–128°) the mixed m.p. was 100–104°; the identity of the isolated material as pregnanedione was confirmed by comparison of its ultraviolet-absorption spectra in ethanol and conc. H_2SO_4 with those of authentic pregnanedione.

Elution with hexane–benzene (1:3, v/v) and benzene gave white semicrystalline material with m.p. range 93–132°; rechromatography on alumina yielded material, eluted by benzene–ether mixtures, with m.p. range 122–147°. Repeated crystallization from hexane gave 4 mg. of needles with m.p. 173–175°, the needles re-forming as clusters on cooling. This substance did not depress the m.p. of authentic 3α-hydroxy-5α-pregnan-20-one, and its acetate (m.p. 134–136°) gave a mixed m.p. with authentic 3α-acetoxy-5α-pregnan-20-one (m.p. 134–136°) of 134–136°. Confirmation that the isolated material was 3α-hydroxy-5α-pregnan-20-one was obtained by comparison of its ultraviolet spectrum in conc. H_2SO_4 with that of the authentic steroid.

Elution with methanol gave white material which when recrystallized from methanol gave platelets, m.p. 140–145°; recrystallization from

hexane gave 3 mg. of needles, m.p. 147–150°. Mixed m.p. determination with 3α-hydroxy-5β-pregnan-20-one (m.p. 149–151°) indicated that the isolated material was 3α-hydroxy-5β-pregnan-20-one; it was dissolved in pyridine and acetylated with acetic anhydride overnight. After extraction and crystallization from acetone, crystals were obtained with m.p. 96–100°; admixture with authentic 3α-acetoxy-5β-pregnan-20-one (m.p. 97–100°) gave mixed m.p. 96–99°.

β-Ketonic fraction. This weighed 12 mg.; it was taken up in hexane–benzene (4:1, v/v) and applied to a column containing 2 g. of alumina activity II. Elution with benzene–ether mixtures yielded white material which was crystallized from acetone and the crystals were leached with hexane. They gave m.p. 197–198°; mixed m.p. with *allo*pregnanedione (m.p. 199–200°) was 197–200°. Admixture with *allo*pregnanolone (m.p. 193–196°), which might also be expected to appear in this fraction, gave mixed m.p. 158–176°; mixed m.p. of authentic *allo*-pregnanedione and *allo*pregnanolone was 158–179°. The digitonin-precipitable nature of *allo*-pregnanedione was originally reported by Butenandt & Mamoli (1935).

Elution with methanol gave a small amount of crystalline material which on being twice recrystallized from methanol yielded a few crystals with m.p. 194–195°. No further batch of this material could be obtained from the mother liquors, but it was possibly *allo*pregnanolone, as this had m.p. 193–196° and was also obtained by Taylor (1955) in the same fraction from rabbit liver.

α-Non-ketonic fraction. This weighed 140 mg.; it was dissolved in 2 ml. of benzene and put on a column containing 3 g. of deactivated alumina. Elution with benzene followed by 0·8 % and 3 % (v/v) ethanol in benzene yielded one main fraction eluted chiefly by the later portion of the 0·8 % (v/v) ethanol in benzene (cf. Klopper, Michie & Brown, 1955). Repeated crystallization from methanol yielded white needles with m.p. 224–225°, suspected to be a pregnanediol or an *allo*pregnanediol. The needles were purified by conversion into the diacetate; extraction followed by crystallization from ethanol gave white crystals with a double m.p. 162–163° and 180–182°. Mixed m.p. with pregnanediol diacetate (m.p. 162° and 181–182°) was 160–162° and 178–180°. Hydrolysis for 30 min. with boiling 5 % (w/v) KOH in methanol followed by extraction and crystallization from methanol gave 5 mg. of needles with m.p. 233–235°; mixed m.p. with pregnanediol (m.p. 233–237°) prepared from the diacetate was 234–236°. Comparison of ultraviolet-absorption spectra in conc. H_2SO_4 of the isolated material and of pregnanediol confirmed the isolated substance to be pregnanediol.

Attempts to crystallize the other fractions from this column were not successful, despite acetylation.

β-Non-ketonic fraction. This weighed 27 mg.; crystallization from ethanol yielded white notched crystals with m.p. 145–147°. A mixed m.p. (144–146°) showed that this fraction consisted mainly of cholesterol (m.p. 145–146°).

DISCUSSION

Taylor (1954) carried out a 'blank' incubation of rat-liver homogenate and showed that neither progesterone nor any of its known metabolites were obtained. He also conducted 'control' incubations both with rat-liver homogenate and with rabbit-liver homogenate (1955); these demonstrated that progesterone was not transformed into any known metabolites by the isolation procedures used. Since the isolation procedures used in the present work were similar to those of Taylor (1955), it may be assumed that the progesterone and metabolites obtained resulted from the progesterone added.

Reductive metabolism of progesterone has been discussed by Taylor (1954, 1955). From the work of Tomkins & Isselbacher (1954) it would appear probable that the normal metabolic pathway for the reduction of ring A of progesterone is irreversible hydrogenation of the double bond to give the two pregnanediones, followed by reduction of the 3-oxo group. The weights of metabolites given in Table 1 seem to indicate that, for progesterone, the main metabolic pathway in human liver is

progesterone → pregnanedione
⇌ pregnanolone ⇌ pregnanediol.

Table 1. *Steroids isolated from incubation of* 500 *mg. of progesterone with* 500 *g. of human liver*

Steroid	Wt. (mg.)	As percentage of progesterone added
Progesterone	117	23
5α-Pregnane-3:20-dione	1	0·2
5β-Pregnane-3:20-dione	6	1·2
3α-Hydroxy-5α-pregnan-20-one	4	0·8
3β-Hydroxy-5α-pregnan-20-one	Trace	—
3α-Hydroxy-5β-pregnan-20-one	3	0·6
5β-Pregnane-3α:20α-diol	5	1·0
Totals	136	26·8

The reversibility of the ketonic reductions and the irreversibility of the double-bond hydrogenation have been confirmed by Taylor (1956), who isolated crystalline pregnanediol, pregnanolone and pregnanedione, but not progesterone, after incubation of pregnanediol with rabbit-liver homogenate. The identity of the metabolites obtained with rabbit liver (Taylor, 1955) and human liver suggest that the rabbit is a useful species for biochemical studies, at least for progesterone metabolism. This is confirmed by the observations that pregnanediol is a urinary metabolite of progesterone both in humans (Dorfman *et al.* 1948) and in rabbits (Hoffman, 1942).

Table 2 lists sixteen possible reduction products of progesterone; such compounds as 3-hydroxypregn-4-en-20-ones, 3:20-dihydroxypregn-4-enes, 20-hydroxypregnan-3-ones and derivatives with oxygen functions in positions other than 3 or 20, or a double bond other than in position 4, have not been included. Where applicable, references have been given to studies in which an attempt was made to demonstrate that the steroid reported was a progesterone metabolite. Otherwise, investigations on human pregnancy urine, for work *in vivo*, and on human liver, for work *in vitro*, have been favoured.

It appears that 3-hydroxypregn-4-en-20-ones (and 3:20-dihydroxypregn-4-enes) are not encountered because the 3-oxo group is only relatively slowly reduced if the double bond has not been previously hydrogenated (Talalay, 1957; Talalay & Dobson, 1953), and because the double-bond reduction is irreversible (Tomkins, 1957). However, it is more difficult to explain the non-appearance of 20-hydroxypregnan-3-ones; since the two pregnanediones have been isolated, presumably

Table 2. *Comparison of progesterone metabolites isolated* in vivo *and* in vitro

	In vivo		*In vitro*	
Steroid	Source	Reference	Source	Reference
20α-Hydroxypregn-4-en-3-one	Rat carcass	Wiest (1956)	Fibroblast culture	Sweat *et al.* (1958)
20β-Hydroxypregn-4-en-3-one	Human placentae	Zander, Forbes, Münstermann & Neher (1958)	Corpus luteum homogenate	Hayano, Lindberg, Wiener, Rosenkrantz & Dorfman (1954)
5α-Pregnane-3:20-dione	Human pregnancy urine	Lieberman, Dobriner, Hill, Fieser & Rhoads (1948)	Human-liver suspension	Atherden (1959)
5β-Pregnane-3:20-dione	Human urine	Lieberman *et al.* (1948)	Human-liver suspension	Atherden (1959)
3α-Hydroxy-5α-pregnan-20-one	Human urine	Dobriner & Lieberman (1950)	Human-liver suspension	Atherden (1959)
3β-Hydroxy-5α-pregnan-20-one	Human pregnancy urine	Pearlman, Pincus & Werthessen (1942)	Rabbit-liver homogenate	Taylor (1955)
3α-Hydroxy-5β-pregnan-20-one	Human urine	Dorfman, Ross & Shipley (1948)	Human-liver suspension	Atherden (1959)
3β-Hydroxy-5β-pregnan-20-one	—	—	Rat-liver homogenate	Ungar & Dorfman (1954)
5α-Pregnane-3α:20α-diol	Human pregnancy urine	Kyle & Marrian (1951)	—	—
5α-Pregnane-3α:20β-diol	—	—	—	—
5α-Pregnane-3β:20α-diol	Human pregnancy urine	Plotz & Davis (1956)	—	—
5α-Pregnane-3β:20β-diol	Pregnant mare urine	Brooks, Klyne, Miller & Paterson (1952)	—	—
5β-Pregnane-3α:20α-diol	Human urine	Dorfman *et al.* (1948)	Human-liver suspension	Atherden (1959)
5β-Pregnane-3α:20β-diol	Pregnant cow bile	Pearlman & Cerceo (1948)	Rat-liver homogenate	Ungar (1955)
5β-Pregnane-3β:20α-diol	Human urine(?)	Mason & Kepler (1945)	—	—
5β-Pregnane-3β:20β-diol	—	—	—	—

double-bond reduction of 20-hydroxypregn-4-en-3-ones (which have been isolated) could occur, unless steric hindrance between enzyme and steroid takes place.

SUMMARY

1. Progesterone has been incubated with a suspension of human liver with added nicotinamide, citrate and reduced triphosphopyridine nucleotide.

2. Six metabolites have been isolated: 5α- and 5β-pregnane-3:20-dione, 3α- and 3β-hydroxy-5α-pregnan-20-one, 3α-hydroxy-5β-pregnan-20-one and 5β-pregnane-3α:20α-diol.

3. The reductive metabolism of progesterone is discussed.

I wish to thank Dr W. Klyne for providing samples of 3α-hydroxy-5α-pregnan-20-one and its acetate from the Medical Research Council Steroid Reference Collection; also Ciba Laboratories Ltd. for a generous gift of progesterone. Thanks are also due to Dr W. Taylor, University of Durham, for gifts of 3β-hydroxy-5α-pregnan-20-one and 3α-hydroxy-5β-pregnan-20-one. The human liver was obtained with the willing co-operation of Dr J. F. Wilson of the Royal Infirmary, Sunderland, to whom I am most grateful.

REFERENCES

Atherden, L. M. (1956). Ph.D. Thesis: University of Edinburgh.

Atherden, L. M. (1959). *Biochem. J.* **71**, 411.

Atherden, L. M. & Grant, J. K. (1957). *J. Endocrin.* **14**, 371.

Brockmann, H. & Schodder, H. (1941). *Ber. dtsch. chem. Ges.* **74**, 73.

Brooks, R. V. & Klyne, W. (1957). *Biochem. J.* **65**, 663.

Brooks, R. V., Klyne, W., Miller, E. & Paterson, J. Y. F. (1952). *Biochem. J.* **51**, 694.

Butenandt, A. & Mamoli, L. (1935). *Ber. dtsch. chem. Ges.* **68**, 1847.

Butler, G. C. & Marrian, G. F. (1938). *J. biol. Chem.* **124**, 237.

Davis, M. E. & Plotz, E. J. (1956). *Acta endocr., Copenhagen*, **21**, 245.

Dobriner, K. & Lieberman, S. (1950). In *A Symposium on Steroid Hormones*, p. 46. Ed. by Gordon, E. S. Madison: University of Wisconsin Press.

Dobriner, K., Lieberman, S., Rhoads, C. P. & Taylor, H. C. jun. (1948). *Obstet. Surv. Baltim.* **3**, 677.

Dorfman, R. I., Ross, E. & Shipley, R. A. (1948). *Endocrinology*, **42**, 77.

Girard, A. & Sandulesco, G. (1936). *Helv. chim. acta*, **19**, 1095.

Haslam, R. M. & Klyne, W. (1953). *Biochem. J.* **55**, 340.

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

Hoffman, M. M. (1942). *Canad. med. Ass. J.* **47**, 424.

Klopper, A., Michie, E. A. & Brown, J. B. (1955). *J. Endocrin.* **12**, 209.

Kyle, T. I. & Marrian, G. F. (1951). *Biochem. J.* **49**, 162.

Lieberman, S., Dobriner, K., Hill, B. R., Fieser, L. F. & Rhoads, C. P. (1948). *J. biol. Chem.* **172**, 263.

Mason, H. L. & Kepler, E. J. (1945). *J. biol. Chem.* **161**, 235.

Pearlman, W. H. & Cerceo, E. (1948). *J. biol. Chem.* **176**, 847.

Pearlman, W. H., Pincus, G. & Werthessen, N. T. (1942). *J. biol. Chem.* **142**, 649.

Plotz, E. J. & Davis, M. E. (1956). *Acta endocr., Copenhagen*, **21**, 259.

Reaven, G. M. (1955). *Endocrinology*, **57**, 580.

Sweat, M. L., Grosser, B. I., Berliner, D. L., Swim, H. E., Nabors, C. J. jun. & Dougherty, T. F. (1958). *Biochim. biophys. Acta*, **28**, 591.

Talalay, P. (1957). *Physiol. Rev.* **37**, 362.

Talalay, P. & Dobson, M. M. (1953). *J. biol. Chem.* **205**, 823.

Taylor, W. (1954). *Biochem. J.* **56**, 463.

Taylor, W. (1955). *Biochem. J.* **60**, 380.

Taylor, W. (1956). *Biochem. J.* **62**, 332.

Tomkins, G. M. (1957). *J. biol. Chem.* **225**, 13.

Tomkins, G. M. & Isselbacher, K. J. (1954). *J. Amer. chem. Soc.* **76**, 3100.

Ungar, F. (1955). See Forchielli, E., Rosenkrantz, H. & Dorfman, R. I. (1955). *J. biol. Chem.* **215**, 720.

Ungar, F. & Dorfman, R. I. (1954). *Abstr. Amer. chem. Soc.*, 126th Meeting, pp. 28C–29C, New York.

Wiest, W. G. (1956). *J. biol. Chem.* **221**, 461.

Zander, J., Forbes, T. R., Münstermann, A. M. von & Neher, R. (1958). *J. clin. Endocrin. & Metab.* **18**, 337.