Abnormal steroid excretion in gestational trophoblastic disease complicated by ovarian theca-lutein cysts

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SUMMARY Serum and urine steroids were examined in two subjects with trophoblastic disease accompanied by large ovarian theca-lutein cysts and compared with those from 10 patients with trophoblastic disease but without palpable cysts. In the patients without cysts normal values were obtained for serum oestradiol, progesterone, 17α -hydroxyprogesterone and androstenedione, and for urinary total oestrogens, pregnanediol, pregnanetriol, and 17-oxosteroids. Nineteen urinary steroid metabolites, quantified by capillary gas-liquid chromatography, were either within reference limits or marginally raised. In several cases relatively minor increases in serum testosterone and cortisol and urinary free cortisol were observed. In contrast, the subjects with cysts showed pronounced excesses of androgen metabolites, 17α -hydroxypregnanolone, pregnanediol, and pregnanetriol, and both exhibited a similar pattern of unusual additional metabolites.

The profiles superficially resembled those seen in 21-hydroxylase deficiency adrenogenital syndrome, but there were important discrepancies reflecting known differences in ovarian and adrenal steroid metabolism.

Chemotherapy led to decline of human chorionic gonadotrophin concentrations, regression of the cysts, and return to normal of the steroid profile. Excess steroids in the patients with cysts may have originated in the ovary rather than in the trophoblastic tissue.

Steroid excretion in subjects with trophoblastic disease has been little studied but has, in most cases, been reported as within normal limits. Urinary pregnanediol (Table 1) and oestrogens are seldom increased over non-pregnant concentrations,^{1,2} and trophoblastic tissue is limited in its capacity to synthesise steroids.³⁻⁵

During routine follow up of subjects with gestational trophoblastic disease two patients had raised concentrations of human chorionic gonadotrophin (hCG) and large theca-lutein cysts of the ovary. In contrast to the other subjects with trophoblastic disease in whom ovarian cysts were not a feature, plasma and urinary steroids in these two patients were increased. Indeed, some steroids were present in very high quantities.

To delineate further the nature and extent of the abnormality urinary steroid metabolites were examined by capillary gas-liquid chromatography and gas

chromatography-mass spectrometry. Ten further subjects with gestational trophoblastic disease but without palpable ovarian cysts were also studied to determine whether the subjects with cysts would show a discrete disorder or one extreme of a continuum. This also permitted an assessment of whether these unusual metabolites originated in the trophoblastic tissue or the ovary.

Case histories

CASE 1

A 23 year old primigravida had an evacuation of a hydatidiform mole at about 12 weeks' gestation. One month later she was admitted for chemotherapy in view of raised urinary hCG concentrations (9830 IU/24 hours), persistent irregular vaginal blood loss, dyspnoea, chest pain, and colicky abdominal pain. Clinically, she had a bulky uterus with a large separate mass arising from the pelvis. Ultrasonography showed this to be an ovarian cyst 22 cm

Table 1 Non-standard abbreviations and trivial names of steroid compounds

Trivial names:	Systematic names:
Testosterone	17 β -Hydroxy-4-androsten-3-one
Androsterone	3 α -Hydroxy-5 α -androstan-17-one
11 β -Hydroxyandrosterone	3 α , 11 β -Dihydroxy-5 α -androstan-17-one
Aetiocholanolone	3 α -Hydroxy-5 β -androstan-17-one
11 β -Hydroxyaetiocholanolone	3 α , 11 β -Dihydroxy-5 β -androstan-17-one
11-Oxo-aetiocholanolone	3 α -Hydroxy-5 β -androstan-11, 17-dione
Androstenedione	4-Androstene-3, 17-dione
Androstenediol	5-Androstene-3 β , 16 α , 17 β -triol
Dehydroepiandrosterone (DHA)	3 β -Hydroxy-5-androsten-17-one
16 α -Hydroxy DHA	3 β , 16 α -Dihydroxy-5-androsten-17-one
Pregnenolone	3 β -Hydroxy-5-pregnen-20-one
17 α -Hydroxypregnenolone	3 β , 17 α -Dihydroxy-5-pregnen-20-one
16 α -Hydroxypregnenolone	3 β , 16 α -Dihydroxy-5-pregnen-20-one
Progesterone	4-Pregnene-3, 20-dione
16 α -Hydroxypregesterone	16 α -Hydroxy-4-pregnene-3, 20-dione
Pregnanediol	5 β -Pregnane-3 α , 20 α -diol
Pregnanolone	3 α -Hydroxy-5 β -pregnan-20-one
17 α -Hydroxypregesterone	17 α -Hydroxy-4-pregnene-3, 20-dione
17 α -Hydroxypregnanolone	3 α , 17 α -Dihydroxy-5 β -pregnan-20-one
Pregnanetriol	5 β -Pregnane-3 α , 17 α , 20 α -triol
11-Deoxycortisol	17 α , 21-Dihydroxy-4-pregnene-3, 20-dione
Tetrahydro-11-deoxycortisol	3 α , 17 α , 21-Trihydroxy-5 β -pregnan-20-one
Cortisol	11 β , 17 α , 21-Trihydroxy-4-pregnene-3, 20-dione
Tetrahydrocortisol	3 α , 11 β , 17 α , 21-Tetrahydroxy-5 β -pregnan-20-one
allo-Tetrahydrocortisol	3 α , 11 β , 17 α , 21-Tetrahydroxy-5 α -pregnan-20-one
Tetrahydrocortisone	3 α , 17 α , 21-Trihydroxy-5 β -pregnane-11, 20-dione
Tetrahydro-11-dehydrocorticosterone	3 α , 21-Dihydroxy-5 β -pregnane-11, 20-dione
Tetrahydrocorticosterone	3 α , 11 β , 21-Trihydroxy-5 β -pregnan-20-one
allo-Tetrahydrocorticosterone	3 α , 11 β , 21-Trihydroxy-5 α -pregnan-20-one
α -Cortolone	3 α , 17 α , 20 α , 21-Tetrahydroxy-5 β -pregnan-11-one
β -Cortolone	3 α , 17 α , 20 β , 21-Tetrahydroxy-5 β -pregnan-11-one
α -Cortol	5 β -Pregnane-3 α , 11 β , 17 α , 20 α , 21-pentol
β -Cortol	5 β -Pregnane-3 α , 11 β , 17 α , 20 β , 21-pentol
Oestrone	3-Hydroxy-1, 3, 5(10)-Oestratriene-17-one
Oestradiol	1, 3, 5(10)-Oestratrien-3, 17 β -diol

in diameter. A chest radiograph showed multiple pulmonary metastases.

She received five courses of methotrexate with folinic acid rescue, two courses of second line treatment (actinomycin D, cyclophosphamide, and vincristine),

Fig. 1 Abdominal enlargement (case 1) due to ovarian tumour

and six courses of VP16 (etoposide) in accordance with a modification of the regimen used by Bagshawe.⁶ Urine and serum hCG concentrations fell to within normal limits, and pelvic examination yielded normal results. Eighteen months later recurrent trophoblastic tumour was diagnosed by rising urinary hCG concentrations. Chest radiography and ultrasonography yielded normal results and the steroid urinary excretion profile was also normal. The recurrence responded to two courses of methotrexate and five courses of VP16.

CASE 2

A gravida 2 para 1 patient had an evacuation of a 13 week gestation hydatidiform mole at the age of 25. Six weeks later she presented with a raised serum β hCG concentration of 21 030 IU/l, urinary hCG of 16 480 IU/24 hours, gross abdominal distension (Fig. 1), and amenorrhoea. Examination showed a cystic swelling arising from the pelvis, and ultrasonography showed this to be consistent with a large ovarian cyst (Fig. 2). Chest x-ray showed multiple pulmonary metastases. She received five courses of methotrexate and five courses of second line treatment after which a chest radiograph, ultrasonography, and hCG excretion all yielded normal results.

Fig. 2 Ultrasound scan of multicystic mass (case 1) arising from ovary

Material and methods

Samples of blood from an antecubital vein were taken without anticoagulant and the serum separated by centrifugation as soon as practicable. Twenty four hour urine samples were collected into containers with 10 ml of 1% boric acid as preservative. Unless assayed within 24 hours, the separated serum and aliquots of urine were kept at -20°C until analysed. All samples were obtained on admission of the patient to hospital for chemotherapy.

URINARY STEROID PROFILE ANALYSIS

Urinary steroids were extracted from 10 ml portions of urine using Sep-Pak C18 cartridges.⁷ This method provides recoveries of standard steroids of 87–103%.⁷ Conjugates were hydrolysed using 100 μl *Helix pomatia* digestive juice containing 10 000 Roy units of sulphatase and 10 000 Fishman units of β glucuronidase (Reactifs IBF, Villeneuve-La-Garenne, France) in 5 ml 0.5 M sodium acetate buffer, pH 4.6, and incubated for 48 hours at 37°C . Conditions were chosen to produce maximal yield of free steroids.

Glucuronides and 3-sulphates are efficiently hydrolysed, while 17-sulphates of C_{19} steroids and 21-sulphates of C_{21} steroids are relatively resistant to this procedure. Freed steroids were extracted on Sep-Pak cartridges as before. After addition of internal standards methyloxime-trimethylsilyl ether (MO-TMS) derivatives were made⁸ and analysed on a

For comparison purposes 10 other patients undergoing follow up for trophoblastic disease were also screened before chemotherapy was started. At the time of screening hCG excretions varied between 45 000 and 459 000 IU/24 hours, but theca-lutein cysts of the ovary, when present, were only barely palpable.

Table 2 Urinary steroid profiles

Steroid ($\mu\text{mol}/24$ hours)	Methylene unit Nos	Ten cases of choriocarcinoma without large theca-lutein cysts	Case Nos		Reference range
			1	2	
		Range			
1 Androsterone	25-16	0.3-10.2 (5.8) ¹	27.2	37.2	2.1-6.5
2 Aetiocholanolone	25-33	0.4-6.9	14.6	58.4	2.7-6.9
3 DHA	25-85	< 0.1-0.4	1.7	—	0.2-1.0
4 11-Oxo-aetiocholanolone	26-28	0.3-1.3	4.7	36.6	0.1-0.8
5 11 β -Hydroxyandrosterone	27-00	0.6-3.1	95.1*	140.0*	1.2-3.8
6 11 β -Hydroxyaetiocholanolone	27-22	< 0.1-1.7 (0.7) ²	2.4°	5.3°	0.3-0.9
7 16 α -Hydroxy DHA	27-37/27-41	< 0.1-1.3	—	11.1	0.6-1.3
8 Pregnanediol	27-64	0.9-4.4	18.7	39.1	0.4-4.8
9 Pregnanetriol	27-98	0.2-2.9	104.0	143.0	1.0-4.2
10 Androstenetriol	28-41	0.1-1.2	3.4	12.3	0.3-1.9
11 Tetrahydrocortisone	29-68	2.4-8.7 (6.7) ¹	3.9	12.1	3.9-7.7
12 Tetrahydro-11-dehydrocorticosterone	29-77	0.1-0.5	0.1	0.7	0.1-0.4
13 Tetrahydrocorticosterone	30-00	< 0.1-0.3	0.1	—	0.2-0.7
14 <i>allo</i> -Tetrahydrocorticosterone	30-10	< 0.1-0.5	0.1	1.1	0.4-0.8
15 Tetrahydrocortisol	30-23	1.1-5.6 (2.6) ²	1.6	12.5	1.3-2.7
16 <i>allo</i> -Tetrahydrocortisol	30-39	< 0.1-2.5 (2.1) ¹	2.0	4.8	0.9-2.3
17 α -Cortolone	30-52	0.7-4.8 (2.1) ²	1.4	5.0	0.7-1.9
18 β -Cortol: β -Cortolone	30-81	0.4-3.2	0.6	4.9	0.9-3.7
19 α -Cortol	31-20	0.1-1.2 (0.7) ¹	0.2	2.2	0.4-0.7

Values in parentheses = highest values when 1 or 2 results disregarded.

* On this profile 17 α -hydroxypregnanolone (20) and 11 β -hydroxyandrosterone (5) could not be separated (gas-chromatography-mass spectrometry showed that principal contributor to peak was 17 α -hydroxypregnanolone).

° Peak overlaid with 3 α -hydroxy-5 β -pregnane-20-one.

— Steroid not found.

Conversion to traditional units: g = mol $\times$ molecular weight.

Table 3 Additional steroids present in urinary profiles from both patients*

Steroid ($\mu\text{mol}/24$ hours)	Methylene unit Nos	Case Nos	
		1	2
20 17 α -Hydroxypregnanolone	27-00	95.1†	140†
21 X-Hydroxypregnanolone	28-15	9.0	21.1
22 5-Pregnene-3 β , 20 α -diol	28-24	1.0	7.4
23 5-Pregnene-3 β , 16 ξ , 20 ξ -triol	28-52	4.6	12.2
24 Tetrahydro-11-deoxycortisol	28-67	—	11.8
25 5-Pregnene-3 β , 16 α , 20 β -triol	28-77	3.8	8.6
26 5 ξ -Pregnane-3 ξ , 16 ξ , 20 ξ -triol	28-81	3.2	5.2
27 5 ξ -Pregnane-3 ξ , 16 ξ , 20 ξ -triol	28-90	2.2	2.4
28 X, 17 α -Dihydroxypregnanolone	29-31	3.3	2.0
29 5-Pregnene-3 β , 17 α , 20 α -triol	29-47	2.7	8.5
30 5 β -Pregnane-3 β , 16 α , 20 α -triol	29-72	1.6	3.0
31 5 β -Pregnane-3 α , 11 β , 17 α , 20 α -tetrol	29-92	4.4	12.0

* Usually present $<0.7 \mu\text{mol}/24$ hours.† This peak also contains a very minor contribution from 11 β -hydroxyandrosterone.

— Steroid not found.

Table 4 Urine and plasma steroids in trophoblastic disease

Steroid	Values in 10 cases without pronounced theca-lutein cysts	Case Nos		Reference range	
		1	2		
Urine sample (nmol/24 hours (a)) (μmol/24 hours (b))					
Total oestrogen	a	34–328	4560	—	28–338
Pregnanediol	b	0.8–25	139	137	0.4–27
Pregnanetriol	b	<0.2–4.4	208	41*	0.3–5.3
Free cortisol	a	71–620 (2)	343	—	97–331
Oxosteroids	b	12–45	84	—	18–53
Serum sample (nmol/l (oestradiol pmol/l))					
Oestradiol		<100–1773	5969	>3690	74–1840
Progesterone		6–26	210	40	1–64
17-Hydroxyprogesterone		2.1–17	21*	192	1–18
Cortisol		219–1267 (4)	949	821	166–660
Testosterone		0.5–3.5 (4)	25	36	0.5–2.5
Androstenedione		1.0–9.9	—	>32	4.0–10.2

No of cases with values above the upper limit of the normal range are shown in parentheses.

Reference range dependent on stage of menstrual cycle.

* Sample obtained after cytotoxic treatment had been started.

— Not done.

Packard 429 gas chromatograph (Packard Instrument, Caversham, England) containing a 25 m silica capillary column, 0.32 mm internal diameter, coated with CP Sil 5, thickness 0.13 μm (Chrompack UK). Conditions were: 100–180° at 20°C/minute followed by 180–275° at 2.5°C/minute, injector and detector at 250°C, carrier gas helium, 2 ml/minute.⁸

Mass spectra were obtained of all components using a Varian 112 instrument (Varian Associates, Walton-on-Thames, Surrey).⁸ A compound was considered to be fully identified when retention time and mass spectrum matched those of standard compounds (obtained from the Medical Research Council Steroid Reference Collection, Queen Mary College, London). Partial identifications were obtained when mass spectra were similar to those of standard compounds but did not have matching retention times. Tables 2 and 3 show methylene unit values as an aid to characterising the partially identified components.

OTHER STEROID ASSAY METHODS

Urinary total oestrogen concentrations were assayed by a fluorimetric semiautomated procedure based on the method of Lever *et al*⁹ with prior separation of oestrogens on columns of Sephadex G-10 (Pharmacia Limited, Milton Keynes, Bedfordshire, United Kingdom) according to GH Beastall (personal communication). Pregnanediol and pregnanetriol concentrations were quantified by gas chromatographic techniques based on the methods of Podmore¹⁰ and Curtius,¹¹ respectively, and 17-oxosteroids by the technique of Gray *et al*.¹²

Serum oestradiol, progesterone, cortisol (and urine free cortisol), and testosterone were assayed by kits obtained, respectively, from Steranti (Steranti Research Limited, St Albans, Herts, United Kingdom), Cambridge Medical Diagnostics (CMD Limited, Bournemouth, Hants, United Kingdom), Amerlex (Amersham International PLC, Amersham,

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Fig. 4 Major (Δ_5) and minor (Δ_4) pathways of cortisol synthesis shown by heavy and light continuous lines, respectively. Pathways leading to the formation of normal and additional steroids shown by dashed and dotted lines, respectively. Numbers in parentheses refer to steroids listed in Table 2. Compound 21 – X = 16 α ? Compound 28 – X = 3 α ?

The predominance of 17 α -hydroxypregnanolone and pregnanetriol is also a feature of steroid excretion in 21-hydroxylase deficiency. There were, however, few similarities between the other components of the profiles. There were several 16-hydroxylated metabolites present in the subjects, but these are almost absent in 21-hydroxylase deficiency (Fig. 4). Recently, the presence of several 15-hydroxylated metabolites has been shown in 21-hydroxylase deficiency (Taylor NF, unpublished observations). There was no trace of these in either of the patients studied.

Discussion

STEROIDOGENESIS IN TROPHOBLASTIC TISSUE
Several investigators have assessed the capacity of trophoblastic tissue for steroidogenesis. Huang *et al*³ cultured trophoblastic tissue and showed synthesis of progesterone. Bahn *et al*⁴ cultured choriocarcinoma cells and confirmed in incubation studies that the main steroid synthesised was progesterone. Lesser quantities of pregnenolone and minimal quantities of 17 α -hydroxyprogesterone and 17 α -hydroxypregnenolone were also detected. Addition of calf serum to the incubates resulted in only minor increments in synthesis of any of these steroids but, in contrast, facilitated a many fold increase in oestradiol production, probably by providing

androgen precursors. As expected, addition of androgens stimulated oestradiol synthesis. The authors concluded that trophoblastic tissue lacked 17–20 desmolase and 17 α -hydroxylase, but that 3 β -hydroxysteroid dehydrogenase, $\Delta_4 \rightarrow \Delta_5$ isomerase, 17 β -hydroxysteroid dehydrogenase, and aromatase were all active. Similar results were obtained by Maeyama *et al*⁵ in homogenates of choriocarcinoma tissue in that they too were unable to show side-chain cleavage.

Conflicting results, however, were reported by van Leusden,¹³ who obtained evidence for side chain cleavage when incubating tritiated pregnenolone with chorionepithelioma tissue and showed the formation of androsterone, testosterone, oestrone, and oestradiol. The reason for the discrepancies in this work and that of the Maeyama and Bahn groups is not clear but may be due to the nature of the tissue studied by the different authors. Van Leusden investigated uterine chorionepithelioma, a primary focus, whereas Maeyama's group used cerebral metastatic tissue, and Bahn *et al* cultured chorionepithelioma cells.

In most cases of trophoblastic disease plasma oestrogen and progesterone concentrations have been found to be low or within normal limits for non pregnant subjects,² and urinary pregnanediol and oestrogen excretions have also been found to be low compared with those found in normal pregnancy.¹

On the basis of plasma progesterone assays Teoh *et al*¹⁴ considered that choriocarcinoma tissue had only a limited capacity for steroidogenesis. Dawood,¹ however, asserted that plasma progesterone concentrations correlated with the degree of differentiation of the trophoblastic tissue—concentrations were low in avillous carcinoma but higher in cases of invasive mole and villous carcinoma. He also found a correlation between hCG concentrations and progesterone in choriocarcinoma and believed that the ovaries were the principal source of the progesterone under stimulation from hCG.

Sadoff *et al*¹⁵ reported that in a male patient with metastatic choriocarcinoma, pretreatment plasma concentrations of cortisol, testosterone, and oestradiol were all grossly increased. High hCG values in the cerebrospinal fluid showed that the patient had developed cerebral metastases. Cerebrospinal fluid steroid concentrations, however, were low, suggesting that it was unlikely that the metastatic tissue was the source of the raised plasma steroids, and, more probably, that they had originated from the testis or adrenal gland, or both.

STEROIDOGENESIS BY THECA-LUTEIN CYSTS

Ovarian follicles are known to possess the capacity for side chain cleavage of C₂₁ steroids and also 17 α -hydroxylase and 16 α -hydroxylase activities: they lack 11 β - and 21-hydroxylase activities.¹⁶ Steroid values in fluid from pre-ovulatory follicles and in theca-lutein cysts have been reported as being very similar.¹⁷ The principal progestins present, such as progesterone, 17 α -hydroxyprogesterone, 16 α -hydroxyprogesterone, and 20-dihydroprogesterone, are potential precursors of the additional compounds identified in cases 1 and 2 (Table 3 and Fig. 4). Likewise, the principal androgens reported, DHA sulphate and androsterone sulphate, might account for the high concentrations of the urinary androgen metabolites, androsterone, aetiocholanolone, and 16 α -hydroxy DHA. Oestradiol concentrations were relatively lower in cyst fluids than in normal follicles, and interestingly, in our patients with ovarian cysts oestrogen concentrations were proportionately less raised than were progesterone and 17 α -hydroxyprogesterone and their metabolites.

There have been comparatively few other assessments reported on the contribution of theca-lutein cysts to steroidogenesis, and most published reports have been concerned with steroid production in hydatidiform moles. Stitch *et al*¹⁸ reported a case of molar pregnancy associated with increased urinary pregnanetriol excretion. At the time of removal of the mole by abdominal hysterectomy the ovaries were seen to contain massive theca-lutein cysts, which were believed to have enlarged further immediately after

the operation: concomitantly, urinary pregnanetriol output became even higher than before and only returned to normal as the cysts regressed. The authors considered the cystic ovaries to be the most likely source of the pregnanetriol precursors.

Reporting on a similar case of hydatidiform mole with theca-lutein cysts and increased urinary pregnanetriol excretion, Coutts *et al*¹⁹ concluded that, although molar tissue was active in steroid metabolism, the excessive pregnanetriol originated from the polycystic ovaries. In support of these conclusions evidence was presented that whereas molar tissue contained pregnenolone, 17 α -hydroxypregnenolone, pregnanediol, pregnanetriol, and androstenedione, 17 α -hydroxyprogesterone was undetectable and progesterone present only in trace amounts. On the other hand, theca-lutein cyst fluid was found to contain all these steroids, progesterone and 17 α -hydroxyprogesterone being present in considerable quantities.

Dawood, Ratnam, and Teoh²⁰ and Dawood²¹ investigated molar pregnancies with and without theca-lutein cysts. They showed that in both "intact" moles—that is, before loss of vesicular tissue—and aborted moles the presence of ovarian cysts was accompanied by higher plasma oestradiol and progesterone concentrations. They suggested that molar tissue was an active steroidogenic tissue but that there was additional steroid synthesis from the ovary when theca-lutein cysts were present.

The only report concerning the effect of cystic ovaries is that of Samaan *et al*²² who reported raised plasma testosterone concentrations in women with trophoblastic disease and showed that the highest testosterone values were seen in those patients in whom the ovaries were grossly enlarged. Oophorectomy and chemotherapy were effective in reducing both hCG and testosterone values. Cyst fluid was obtained at laparotomy from the ovaries of a patient with metastatic trophoblastic disease and enlarged ovaries, and it was shown that the content of testosterone was much higher than in the circulation. It was suggested that the ovarian cysts might be a principal site of testosterone formation but, because oophorectomy was not required in this patient, they were unable to rule out other possible sites of testosterone production.

In the patients with trophoblastic disease complicated by the presence of ovarian theca-lutein cysts reported here the principal steroids present in excess in serum and urine were similar to those shown by Vanleuchene *et al*¹⁷ to be the normal constituents of lutein cyst fluid. They also comprised the steroids shown by Abraham²³ to be those that peak at mid cycle and are not diminished by dexamethasone suppression, indicating an ovarian origin.

The report of Teoh *et al*¹⁴ that choriocarcinoma

tissue has only a limited capacity for steroidogenesis, and the suggestion by Sadoff *et al*¹⁵ that metastases were unlikely to be the source of raised plasma steroids in metastatic choriocarcinoma support the concept that the most probable source of the abnormal and grossly raised steroids in our patients were the large ovarian cysts. The available evidence suggests that, similarly to ovarian tissue, theca-lutein cysts possess the enzymes required for progesterin, androgen and oestrogen synthesis. We suggest that the secretions result from the enormous proliferation of theca-lutein cells under the influence of excessive circulating hCG.

We also suggest that the additional steroids in the profiles of the cystic subjects arose from saturation of the normal metabolic excretion pathways, such that sufficient substrates were available for the activation of other pathways, present in all subjects but normally only minimally used, thus resulting in the observed bizarre excretion patterns. Fig 4 shows the possible metabolic pathways. These suggestions, however, can only be advanced with caution because of the incomplete characterisation of several of the steroids in the profile. In addition, a possible contribution from the trophoblastic tissue to steroid synthesis cannot be ignored.

To confirm the source of the "abnormal" steroids of the type described, which, at least superficially, mimic 21-hydroxylase deficiency, we suggest that simultaneous measurement of the steroids in both cyst fluid and the general circulation should be carried out in cases of trophoblastic disease with ovarian enlargement. It may also be of interest to measure the steroid content of the same two tissues in cases of hyperstimulation following follicle stimulating hormone treatment for infertility. The absence of trophoblastic tissue in infertility would certainly lead to a more accurate assessment of the likely source.

We thank Dr JW Honour for his help with the initial stages of the study, Mrs DK Patel for help with the steroid profiling, and Mrs TM Coe and Mr MJ Madigan for carrying out the mass spectrometry. The work of the technical staff of the endocrine laboratory, Jessop Hospital for Women, headed by Mr R Hall and Mrs CM Blair in carrying out the urinary and serum steroid assays is most gratefully acknowledged. Thanks are also due to Mrs A Walters and Mrs C Box, and in particular, to Mrs CJ Kennard and Mrs DS Kilner for their invaluable help in preparing the manuscript.

References

- ¹ Dawood MY. Serum progesterone and serum human chorionic gonadotrophin in gestational and nongestational choriocarcinoma. *Am J Obstet Gynecol* 1975;123:762-5.

Bevan, Savvas, Jenkins, Baker, Pennington, Taylor

- ² Li MC. Trophoblastic disease: natural history, diagnosis and treatment. *Ann Intern Med* 1971;74:102-12.
- ³ Huang WY, Patillo RA, Delfs E, Mattingley RF. Progesterone synthesis in the pure trophoblast of human choriocarcinoma. *Steroids* 1969;14:755-63.
- ⁴ Bahn RS, Worsha A, Speeg KV Jr, Ascoli M, Rabin D. Characterisation of steroid production in cultured human choriocarcinoma cells. *J Clin Endocrinol Metab* 1981;52:447-50.
- ⁵ Maeyama M, Ifuku M, Nakahara K. Steroidogenesis in cerebral metastatic chorionepithelioma tissue in vitro. *J Endocrinol* 1975;65:425-8.
- ⁶ Bagshawe KD. *Choriocarcinoma: the clinical biology of the trophoblast and its tumours*. London: Edward Arnold, 1969:257-63.
- ⁷ Shackleton CHL, Whitney JO. Use of Sep-Pak cartridges for urinary steroid extraction: evaluation of the method for use prior to gas chromatographic analysis. *Clin Chim Acta* 1980;107:231-43.
- ⁸ Kwan TK, Taylor NF, Gower DB. The use of steroid profiling in the resolution of pregnenolone metabolites from porcine testicular preparations. *J Chromatogr* 1984;301:189-97.
- ⁹ Lever M, Powell JC, Peace SM. Improved estriol determination in a continuous flow system. *Biochem Med* 1973;8:188-98.
- ¹⁰ Podmore DA. Routine determination of urinary pregnanediol using gas chromatography with automatic sample application. *J Clin Pathol* 1966;19:619-21.
- ¹¹ Curtius HC. Gas chromatographische Bestimmung von pregnanediol, pregnanetriol und pregnanetriolon in urin. *Zeitschrift für Klinische Chemie und Klinische Biochemie* 1966;4:114-9.
- ¹² Gray CH, Baron DN, Brookes RV, James VHT. A critical appraisal of a method of estimating urinary 17-oxosteroids and total 17-oxogenic steroids. A memorandum prepared at the request of the Clinical Endocrinology Committee of the Medical Research Council. *Lancet* 1969;i:124-7.
- ¹³ Van Leusden HA. Hormonal changes in pathological pregnancy. *Vitamins and Hormones* 1972;30:281-361.
- ¹⁴ Teoh ES, Das NP, Dawood MY, Ratnam SS. Serum progesterone and serum chorionic gonadotrophin in hydatidiform mole and choriocarcinoma. *Acta Endocrinol* 1972;70:791-800.
- ¹⁵ Sadoff L, Riley S, Quan L, Rowe J. New endocrine observations in a male patient with choriocarcinoma. *Oncology* 1974;29:227-37.
- ¹⁶ Sano V, Okinaga S, Arai K. Effects of ovarian steroids on the 17 α -hydroxylase activity towards progesterone in a human ovarian follicle at the late follicular phase. *J Steroid Biochem* 1983;18:55-8.
- ¹⁷ Vanleuchene E, Vandekerckhove D, Jonckheere J, DeLeenheer A. Steroid profiles of body fluids other than urine, obtained by capillary gas chromatography. *J Chromatogr* 1983;279:573-80.
- ¹⁸ Stitch SR, Levell MJ, Oakley RE, Scott JS. Excretion of pregnanetriol in a case of hydatidiform mole associated with ovarian theca-lutein cysts. *Lancet* 1966;i:1344-7.
- ¹⁹ Coutts JRT, MacNaughton MC, Ross PE, Walker J. Steroidogenesis in a case of hydatidiform mole. *J Endocrinol* 1969;44:335-48.
- ²⁰ Dawood MY, Ratnam SS, Teoh ES. Serum oestradiol-17 β and serum human chorionic gonadotrophin in patients with hydatidiform moles. *Am J Obstet Gynecol* 1974;119:904-10.
- ²¹ Dawood MY. Progesterone concentrations in the sera of patients with intact and aborted hydatidiform moles. *Am J Obstet Gynecol* 1974;119:911-18.
- ²² Samaan NA, Smith JP, Rutledge FN, Barcellona JM. Plasma testosterone levels in trophoblastic disease and the effects of oophorectomy and chemotherapy. *J Clin Endocrinol Metab* 1972;34:558-61.
- ²³ Abraham GE. Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. *J Clin Endocrinol Metab* 1974;39:340-6.

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