GRANULOSA-CELL TUMOURS CAUSING PRECOCIOUS PUBERTY OESTROGEN FRACTIONATIONS IN TWO PATIENTS

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A review of the literature in 1955 revealed 62 cases of precocious puberty associated with granulosa-cell tumour (Pedowitz *et al.*, 1955). Oestrogenic activity of the urine had been assayed biologically in some of these cases and found to be greatly raised.

We report three further cases of precocious puberty caused by granulosa-cell tumour, in two of which both urinary and tumour oestrogens were fractionated and assayed biochemically. So far as we are aware, this is the first report of quantitative biochemical oestrogen estimation in this condition.

Case 1

A coloured girl aged 2 years presented with a two-months history of progressive enlargement of the breasts and growth of pubic and axillary hair (Fig. 1).

FIG. 1.—Case 1. Demonstrating advanced breast development and large abdomen.

For two weeks before admission she had had intermittent vaginal bleeding, which varied in intensity. The breasts were lumpy—resembling fibroadenosis, though a biopsy was not done to confirm this.

The labia majora, labia minora, and clitoris were well developed and approached adult proportions. The mons veneris and labia majora were covered with a moderate growth of hair. There was a firm mobile abdominal mass arising from the left fornix. The uterus

and cervix were enlarged. Bone age was consistent with chronological age. An x-ray film of the skull was normal.

At operation a circumscribed ovarian tumour about 2 in. (5 cm.) in diameter was removed from the left side. The uterus measured 2 by 1½ by ½ in. (5 by 3.2 by 1.9 cm.).

Pathology.—The cut surface of the ovary consisted of yellow cellular tissue with several cysts, interspersed with fibrous bands (Fig. 2). Histological examination revealed masses of granulosa cells with little rosette arrangement, divided into islands by connective-tissue stroma. Mitotic figures were fairly numerous, and there were thin-walled vessels between the tumour cells, though without obvious vascular invasion. At one edge several follicular cysts were seen.

FIG. 2.—Case 1. Ovarian tumour, longitudinal section.

Intermittent vaginal bleeding persisted for eight days and was more severe than pre-operatively. Six months after the operation there had been no regression of the signs of puberty.

Case 2

An African girl aged 3 years had a history of progressive growth of breasts and pubic hair for eight months before admission. In addition there had been slight vaginal bleeding, of variable intensity, for the same period. The clinical picture was very similar to that of Case 1. There was a right-sided ovarian tumour about 2½ in. (6.4 cm.) in diameter, easily palpated abdominally. The perineum, however, was not as well developed as in Case 1, and was that of a prepubertal child. The clitoris was normal in size for the age of the child. The uterus was enlarged, but not to the same extent as in Case 1. The bone age was assessed at 4 years. X-ray examination of the skull revealed no abnormality.

FIG. 3.—Case 2.

Operation revealed a right-sided solid ovarian tumour, about 3 in. (7.5 cm.) in diameter, yellow in colour and with large areas of cystic change. The other ovary was quite normal. There was no evidence of spread. The uterus measured 1½ by 1 by ½ in. (3.8 by 2.5 by 1.9 cm.).

Pathology.—Macroscopically the ovary resembled that in Case 1. Microscopical sections showed a granulosa-cell tumour of the ovary in which there were fairly large areas of necrosis and areas that had undergone luteinization. The cell arrangement conformed to the diffuse type, without rosette formation. Very few mitotic figures were evident, and it was not possible to assess the degree of malignancy. A few cystic areas were seen.

On the third post-operative day the patient developed slight vaginal bleeding, which persisted for four days. Eight months after operation there was no change in the amount of pubic hair nor in the size of the breasts or uterus.

Case 3

An African girl aged 2 years 4 months presented with a history of growth of breasts and pubic hair and slight

FIG. 4.—Case 2. Appearance of tumour at operation (uterus indicated by arrow).

vaginal bleeding at regular intervals for several months. A right-sided ovarian tumour was easily palpated abdominally; it was about 2 in. (5 cm.) in diameter. The perineum was that of a child, with minimal development of the clitoris and labia minora.

At operation there was a localized solid yellow right-sided ovarian tumour about 1½ in. (3.2 cm.) in diameter.

The uterus was about the same size as in Case 2. No pathological report is available, the patient having been treated in a country hospital.

Slight vaginal bleeding was noted on the second and third post-operative days. There was no change in the features of precocious puberty after two months.

FIG. 5.—Case 3.

Endocrine Investigations

Methods.—The tumours were ground and the oestrogens extracted in ether and acetone. The quantitative biochemical estimation of individual oestrogen fractions, as described by Brown (1955), was applied to both the extract and the urine. In Case 1 stilboestrol was given in doses of 1 mg. three times daily for four days, and the urinary oestrogen estimated on the third and fourth days. Gonadotrophic hormone in the urine ("F.S.H.") was assessed by a rat ovarian

method, after extraction with kaolin (Albert, 1956). Urinary 17-ketosteroid and 17-hydroxycorticoid levels were assayed by the methods of Norymberski *et al.* (1953) and Appleby *et al.* (1954).

Results.—Twenty-four-hour urinary oestrogen estimations in Cases 1 and 2 are recorded in Table I, and compared with those of two control children. The total oestrogens in each case were much higher than in the normal children, but by far the greatest fraction was

TABLE I.—Urinary Oestrogen Excretions in $\mu\text{g. per 24 Hours}$

	Oestradiol	Oestrone	Oestriol	Total Oestrogen
Controls:				
1. Girl, aged 4½ years ..	1.7	2.1	4.0	7.8
2. " " 3½ " ..	2.4	2.0	1.4	5.8
Case 1:				
Pre-operative ..	2.3	3.7	15.1	21.1
After 9 mg. stilboestrol ..	2.6	3.5	15.3	21.4
12 " " " ..	1.8	2.9	13.1	17.8
11 days post-operatively ..	0.4	0.4	5.1	5.9
Case 2:				
Pre-operative ..	0.6	0.9	18.0	19.5
6 days post-operatively ..	0.6	0.0	8.4	9.0
14 " " " ..	0.3	2.4	0.7	3.4
6 months " " ..	0.3	3.1	3.4	6.5

The sensitivity of the method probably does not go below 2 $\mu\text{g.}$ of oestrogen.

made up of oestriol, while oestradiol was no higher (even lower in Case 2) than in the normals. Stilboestrol as administered produced no appreciable change in urinary oestrogen output. There was a gratifying post-operative fall in urinary oestriol in both cases, though the oestrone appeared to rise in Case 2. The oestrogen content of the tumour extracts (Table II) were

TABLE II.—Oestrogens Extracted From Tumours

Case No.	Amount of Tumour Extracted	Oestradiol	Oestrone	Oestriol	Total Oestrogen
1	11.4 g.	1.8 $\mu\text{g.}$	9.5 $\mu\text{g.}$	2.6 $\mu\text{g.}$	13.9 $\mu\text{g.}$
2	63 g.	0.9 "	0.6 "	0.6 "	2.1 "

TABLE III.—Urinary Gonadotrophins, 17-Ketosteroids, and Hydroxycorticoid Excretions

	F.S.H. (r.o.u.)	17-Ketosteroids (mg./24 hrs.)	17-OH-corticoids (mg./24 hrs.)
Normal for age ..	0 (Albert, 1956)		
Young adult normal ..	±14*		
Case 1:			
Pre-operative ..	14†	1.8	2.0
10 days post-operative ..	—	1.2	5.0
Case 2:			
Pre-operative ..	12	2.0	7.0
Post-operative ..	3‡	—	—

* This is a rough mean of Albert's normals, and tallies with our own experience in over 30 normal subjects.

† 100% increase in rat ovarian weight over control.

‡ No significant increase in rat ovarian weight.

remarkably low. The smaller tumour contained far more oestrogen. The urinary gonadotrophins (F.S.H., Table III) were raised to within the adult range in both cases, with a fall in the level after operation in Case 2. (They were not measured post-operatively in Case 1.) The urinary 17-ketosteroid and hydroxycorticoid (Table III) levels were not abnormal.

Discussion

There is a good correlation between the severity of the clinical findings and the level of total oestrogen secretion in Cases 1 and 2. Case 1, though younger, had more advanced pubertal changes than Case 2, and histology of the tumour also showed more active mitosis. The excretion of both oestradiol and oestrone—the two most potent oestrogen fractions—was much higher than in Case 2. Also there was apparently more oestrogen in the tumour of Case 1.

The level of the total urinary oestrogens and the individual fractions was no higher than we find in adult females around mid-cycle—in fact, not as high. In cases reported in the literature the oestrogen levels when assayed biologically have been found to be much higher in cases with granulosa-cell tumour than in adults with

normal ovulatory cycles (Pedowitz *et al.*, 1955). The comparatively low levels obtained with the chemical method in our cases suggest that the most active oestrogens produced by granulosa-cell tumours may not appear in the urine as oestradiol, oestrone, or oestriol. Furthermore, the low level of oestrogen found in the tumours could also be explained if they are producing a potent oestrogen, not measured by Brown's techniques. It is known that there are other oestrogens in normal urine and ovaries, though their importance is usually less than the three measurable fractions (Brown *et al.*, 1959).

Estimation of urinary oestrogens may be important both diagnostically and prognostically. After removal of the tumour urinary oestrogens should return to normal levels. In both these cases there was a marked decrease in the oestrogen levels in all three fractions by the second week. In Case 2 the oestrogen level had risen again six months post-operatively.* In any event, since there has been no clinical regression in signs in either child we feel that both should have regular clinical examinations and urinary oestrogen assays. A tendency of the latter to rise further would be an indication to re-examine the remaining ovary, since bilateral tumours, though uncommon, do occur, and the second tumour may be so small as to be missed at the first laparotomy. In most cases reported in the literature, a distinct regression in pubertal signs takes place fairly rapidly after removal of the tumour in younger patients (Anderson and Sheldon, 1937; Gross, 1940; Fuller and Smith, 1941; Haines and Jackson, 1950).

We were surprised to find elevated "F.S.H." levels, since granulosa-cell tumours are presumed to be autonomous. This finding has been reported in three other cases in the literature (according to Pedowitz *et al.*, 1955). In an attempt to see if any of the oestrogen secretion was under pituitary control, we gave stilboestrol for four days, hoping thereby to suppress any pituitary over-activity. If oestrogen production was in fact under pituitary control, this should also have been depressed. The oestrogen excretion, however, was not affected. The high "F.S.H." levels would appear to indicate either that the tumours are under some pituitary control (which the above-mentioned observation does not completely exclude) or that the tumours themselves produce a substance capable of increasing the size of rat ovaries.

Summary

Three cases of precocious puberty due to granulosa-cell tumours in infancy are described, together with biochemical estimation and fractionation of both urinary and tumour oestrogen content in two of them.

It is suggested that the tumours may produce a potent oestrogen which is not measured by the current chemical methods, and may also produce a gonadotrophic substance.

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*This rise is probably not significant, since the levels are not elevated above those found in our two controls.

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ST. ANTHONY'S FIRE REKINDLED: GANGRENE DUE TO THERAPEUTIC DOSE OF ERGOTAMINE

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In the tenth and eleventh centuries ergot-poisoning from rye infected with *Claviceps purpurea* was a common epidemic disease in France, and was known by a number of names, such as mal des ardents, ignis sacer, and feu sacré. The emphasis on fire is explained by the violent burning pain in the limbs; sufferers unable to bear the heat in their beds sought relief in the open air. With the onset of gangrene the blackened limbs were likened to charcoal. Several houses of the Order of St. Anthony were devoted to the care of sufferers from the holy fire, and the walls of their hospitals were painted a symbolic red or flame colour. It seems beyond reasonable doubt that St. Anthony's fire originally referred to gangrenous ergotism. However, the definition of St. Anthony's fire in many dictionaries and encyclopaedias mentions erysipelas. The patient whose case is described below presented with such a remarkably red face that we felt this might have accounted for the confusion with erysipelas and other infectious skin diseases.

The literature reveals a large number of cases of ergotism in both Great Britain and America, resulting from either liquid ergot or ergotamine tartrate, despite the fact that contraindications to the use of ergotamine are well documented. They were recently summarized by Greene (1959), and include peripheral vascular disease, hypertension, and coronary disease; pregnancy; thyrotoxicosis; gross sepsis; hepatic and renal disease; and anaemia. In the *British Medical Journal* (1959), under "Notes and Comments," the expert writes: "In those persons who are sensitive its [ergotamine] use has produced nothing less than disaster. Whenever a new case is reported it becomes a matter for astonishment that responsible manufacturers make it for medical use."

That serious ergotism can occur from normally accepted therapeutic doses of ergotamine in the absence of known contraindications was forcibly impressed on us recently.