

HORMONE EXCRETION IN PRECOCIOUS PUBERTY IN GIRLS

BY

R. D. BULBROOK, F. C. GREENWOOD and A. H. SNAITH

From the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields and The Hospital for Sick Children, Great Ormond Street, London

(RECEIVED FOR PUBLICATION JANUARY 29, 1958)

At puberty in girls the sex hormones are secreted in increasing quantities and the clinical signs of puberty follow. In precocious puberty it is generally accepted that the excretion of these hormones will reach adolescent or adult levels but will not be abnormally high as it may be in tumour or hyperplasia of the gonad or the adrenal cortex. Published reports vary depending on the laboratory investigations carried out, and also from case to case (Fischer, 1940; Novak, 1944; Hain, 1947; Seckel, Scott and Benditt, 1949; Lloyd, Lobotsky and Morley, 1950; Lowrey and Brown, 1951; Mossberg, 1951; Paschkis, 1952; Lloyd, 1955). In general, the 17-ketosteroids tend to be a little higher than normal for the chronological age but they rarely reach more than the lower limits of adult levels and may be quite normal. Gonadotrophin determinations are usually positive, but were negative in the cases described by Gross (1940) and by Bronstein, Luhan and Mavrelis (1942). Oestrogen excretion has been measured by biological methods. The results are difficult to evaluate but on the whole oestrogen excretion tends to be low.

The chemical method recently developed by Brown (1955a) has made possible the separate estimation of oestrone, oestradiol-17 β and oestriol in human urine. This study was therefore undertaken to assess the clinical value of simultaneous estimations of urinary oestrogens, 17-ketosteroids and gonadotrophins in 11 cases of early or precocious puberty. Vaginal smears were also examined. Hormone estimations were carried out on a control series of 15 normal girls in the age range 3 to 10 years.

Methods

Oestrogens. Brown's method (1955a) was used to determine the amount of oestrone, oestradiol-17 β and oestriol in urine. The accuracy, precision, sensitivity and specificity of this method have been described (Brown, Bulbrook and Greenwood, 1957).

The lower limit for the method has been approximated to be 1.0 μ g./24 hours for oestrone and oestradiol and 1.5 μ g./24 hours for oestriol. Amounts below this have been recorded as zero. It must be emphasized that oestrogen may be present in such urines but in amounts below the calculated limit of sensitivity for the chemical method. For example, in urine from a normal 6-year-old girl the chemical method gave a value for oestrone below the limit of sensitivity and therefore recorded as zero. However, there was good agreement between parallel chemical and biological assays on this urine using the experimental design of Bulbrook, Greenwood and Williams (1957).

Urinary Gonadotrophins. The method of Dekanski (1949) was used to estimate gonadotrophins. The hormone was eluted from kaolin by 20 ml. of N/10 sodium hydroxide in the initial estimations but subsequently elution was carried out at pH 11.0 to 11.2 (Loraine and Brown, 1956). The biological test was done on female mice, 21 days old and weighing 10 g. A positive response was an increase in uterine weight of 100% above controls. Three mice were injected at each dose level and doses were adjusted so that a positive response indicated the presence of 5 mouse units or more.

17-ketosteroids. A method similar to that of Holtorff and Koch (1940) was employed to estimate 17-ketosteroids in urine. This method uses aqueous potassium hydroxide and a control reading, and gives higher results than methods using alcoholic potassium hydroxide and the Talbot, Berman and MacLachlan correction (1942).

Vaginal Smears. Vaginal smears were fixed in ether/alcohol and stained with Harris's haematoxylin and Shorr's trichrome stain. They were graded into negative or positive according to the presence of leucocytes and of keratinization of the epithelial cells.

Case Material

Four cases under the age of 3 years are described, none of whom had regular menstrual cycles. A further seven cases, ranging in age from 6 to nearly 11 years, were children who had signs of early puberty but who could not be classified as being definitely outside the normal range for the onset of puberty (Tanner, 1955). Of 39 children attending The Hospital for Sick Children in recent years because of precocious puberty, 29 fell into the latter group. None of the 10 cases in the former group had regular menstrual cycles.

Where possible, urine for oestrogen determination was collected from normal children in the same age groups as the patients described above.

Results

In 15 normal girls in the age range 3 to 10 years no oestrogen was detected in the urine, with one exception, a 7-year-old girl who excreted 2.9 µg./24 hours. In children over the age of 11 years who had breast development and pubic hair, but who had not menstruated, measurable amounts of oestrogen were found (2.9 to 7.1 µg./24 hours).

Oestrogen excretion, 17-ketosteroid excretion, gonadotrophin excretion and the grading of the

vaginal smears of the children with early puberty are shown in Table 1.

In contrast to the control group, nine of the cases in this series were excreting amounts of oestrogen above 1.0 µg./24 hours. In seven of the cases, positive vaginal smears were correlated with the finding of oestrogen in the urine, and in three cases negative smears were correlated with the absence of oestrogen. In Case 1 a negative smear was obtained in conjunction with 1.8 µg. of urinary oestriol, while in Case 5 a positive vaginal smear was obtained but no oestrogen was detected in the urine.

Although the number of cases is too small for statistical analysis there appears to be a good association between the vaginal cytology and oestrogen output, although the latter is very low (2.0 to 6.8 µg. total oestrogen/24 hours). Similar results obtained on a large series of women (Young, Bulbrook and Greenwood, 1957) suggest that a very small output of urinary oestrogen, relative to the amounts in menstruating women, cannot be regarded as too low to be of physiological significance.

Case Histories

Case 1. Aged 7 months when first seen, this baby had a blood-stained vaginal discharge at 4 months. This lasted for five days and was followed by episodes three weeks

TABLE 1
OESTROGEN, 17-KETOSTEROID, GONADOTROPHIN EXCRETION AND VAGINAL SMEAR FINDINGS IN GIRLS WITH PRECOCIOUS OR EARLY PUBERTY

Case No.	Age (years)	Oestrone	Oestradiol	Oestriol	Total (µg./24 hr.)	Vaginal Smear	17-ketosteroids (mg./24 hr.)	Gonadotrophins (m.u./24 hr.)
1	7/12	0.0 0.0	0.0 0.0	0.0 1.8	0.0 1.8	Negative Negative	0.7 (0.47±0.16)	Negative Negative
2	1 10/12	0.0 1.3	0.0 0.0	2.2 4.2	2.2 5.5	Positive Positive	2.5 (1.38±0.66)	Negative Negative
3	2	1.8	0.0	3.4	5.2	Positive	1.6	—
4	2 6/12	0.0	0.0	0.0	0.0	Negative	0.9	Negative
5	6	0.0	0.0	0.0	0.0	Positive	2.1 (2.3±0.85)	Negative
6	8 6/12	0.0 1.2	0.0 0.0	0.0 0.0	0.0 1.2	Negative Negative	4.2	Negative
7	10	3.8	0.0	3.0	6.8	Positive	3.7	Positive
8	8	1.9 2.0 1.3	2.1 0.0 0.0	1.7 2.1 4.2	5.7 4.1 5.5	Positive	4.4	Negative Negative Negative
9	10	0.0 1.4 0.0 0.0 0.0	0.0 1.0 0.0 0.0 0.0	0.0 2.2 1.9 2.5 2.1	0.0 4.6 1.9 2.5 2.1	Positive	4.1	Positive 5-20 5-20
10	10	See Fig. 1			11-38	Positive	5.1	Negative 5-20 10-20
11	10 10/12	See Fig. 1			2.6-29	Positive	7.1	10-20

(The figures in brackets give the normal 17-ketosteroid excretion in children of the age groups: 0-1; 1-5; 5-10).

later and again after a further four weeks. Immediately after admission a profuse menstruation lasting eight days began. Development of the breasts and external genitalia beyond the normal was very slight. The nipples were slightly pigmented. There was a single small patch of pigmentation on the abdomen. Subsequently the periods were very irregular with intervals of several weeks or several months; at the present time no loss has been seen for a year. The vaginal smear and a specimen of urine for oestrogen estimation were taken on the second day after the end of the period which occurred in hospital. The vaginal smear was negative and no oestrogen was detected in the urine.

It was not possible to keep the child in hospital but six months later she was re-admitted for 48 hours for urine collection. The vaginal smear was still negative but 1.8 μg . of oestriol were found in the urine. The 17-ketosteroid excretion was at the upper limit of the normal range.

Case 2. This child was admitted to hospital during the first menstrual loss, which persisted for five days, at the age of 1 year and 10 months. On examination, the breasts were moderately developed and a few pubic hairs were present. The height, weight and bone age were advanced by about a year. Subsequent periods occurred after intervals of two, four, and one month, and lasted from one to three days. A second admission was arranged before the next expected period and specimens taken. The vaginal smear was positive and oestrogen was detected in the urine on both occasions. The gonadotrophin estimation was negative.

Case 3. The child was admitted to hospital at the age of 2 years because of vaginal bleeding lasting for one month. The examinations were carried out during the first one or two days following the cessation of the loss. There was no advance in growth, bone age, or breast development, and no pubic hair. It was thought that she was probably not a case of precocious puberty. Examination under anaesthesia revealed no abnormality which could account for the bleeding, however, and the vaginal smear was positive and oestrogen was present in the urine. The 17-ketosteroids were normal. No specimen was obtained for gonadotrophin estimation.

Case 4. This child was admitted to hospital because of congenital dislocation of the hip. At the age of 2½ years she was observed to have pubic hair and minimal breast development. Her growth and bone age was not advanced. The vaginal smear was negative, no oestrogen or gonadotrophin could be detected in the urine. The 17-ketosteroids were normal. This case demonstrates that clinical signs of precocity may be present in the absence of any confirmatory laboratory tests.

Case 5. At the age of 6 years this child had the weight, height and bone age of a child of 8, 10 and 11 years respectively. The breasts were very well-developed. There was no pigmentation of the nipples, no pubic or axillary hair, and the vulva was not developed. On rectal examination, the cervix and uterus were not palpable. There had

been no menstrual loss. The vaginal smear was positive in this case although no oestrogen could be detected in the urine.

Case 6. On examination at the age of 8½ years, some breast development had taken place; there was much pubic hair and some axillary hair. No menstruation had occurred. The 17-ketosteroid excretion was just above the upper limit of normal (mean $+2 \times \text{S.D.}$). Oestrogen was detected in one of two specimens of urine examined.

Case 7. On examination at the age of 10, the height, weight and bone age were in advance of the average by about two years. The breasts were developed and pubic hair was present but menstruation had not occurred. The first period, lasting one day, began five weeks after this examination. Hormone excretion was indicative of puberty; oestrogen and gonadotrophin were present in the urine and the vaginal smears were positive.

Case 8. This child was seen at the age of 8½ years. A few pubic hairs and slight development of the breasts had been noticed for about six months and on one occasion there had been a slight loss of blood for one day. Her height, weight and bone age were all advanced by three or four years. During the next two years she had slight shows of blood, never lasting for more than one day and occurring at intervals of one to three months. Oestrogen was present in three specimens of urine; the vaginal smear was positive and the 17-ketosteroid excretion was just above the normal range for the age. Though gonadotrophin was persistently absent from the urine, it was eventually demonstrated to be present when the patient reached the age of 10 years and regular periods had commenced.

Case 9. When first seen, at the age of 10 years, there had been irregular menstrual loss for a year. The breasts were developed and there was scanty pubic hair. The height was advanced by two years but not the bone age; she was very obese. Weekly specimens of urine were obtained in an attempt to demonstrate cyclical ovarian activity, but the number of specimens available were too few for this. Of five urine specimens, four contained oestrogen. Gonadotrophin was present in the urine.

Case 10. First seen two weeks after the first period, at the age of 10 years; her breasts were developed and pubic hair was present. The height and weight were advanced about four years. Periods have continued at fairly regular monthly intervals. Urine specimens were collected at intervals throughout the cycle and the details of oestrogen excretion are shown in Fig. 1.

Case 11. Breast development was first noticed at the age of 8½ years. Menstruation commenced at 10½ years and continued at regular monthly intervals. When seen at the age of 10 years and 10 months, the height, age, and bone age were those of a girl of 15 years. The cycle of oestrogen excretion is illustrated in Fig. 1.

Certain miscellaneous findings are of interest. In a case of polyostotic fibrous dysplasia, aged 7 years, with early signs of precocious puberty but no menstrual loss,

oestriol excretion was 1.6 $\mu\text{g.}/24$ hours; the 17-ketosteroids 1.4 mg., and the gonadotrophins negative. The vaginal smear was negative. In a boy, aged 8, with gynaecomastia of one breast, the oestrogen excretion totalled 4.5 $\mu\text{g.}/24$ hours on one occasion and zero on two others. In one case of idiopathic breast development the vaginal smear was negative, no oestrogen could be detected in the urine, and the 17-ketosteroids were 0.6 mg./24 hours at the age of 15 months; the bone age was advanced six months. Material from a second patient in another hospital was also examined. The vaginal smear was positive; the total oestrogen excretion was 2.7 $\mu\text{g.}/24$ hours, and the 17-ketosteroids were 1.2 mg./24 hours at the age of 3½ years. Gonadotrophin was less than 5 m.u./24 hours. There was apparently no clinical evidence of precocity. Upon enquiry it was learned that breast development regressed after 12 months. The presence of oestrogen in this case suggests that a true precocity was present. In a case of Klinefelter's syndrome in a boy aged 15 with gynaecomastia, gonadotrophin more than 72 m.u. and female genetic sex, the total oestrogens were 4.8 $\mu\text{g.}/24$ hours.

Three normal boys and a girl, in hospital for tonsillectomy, had urines collected on the day of the operation, on the day before and the day following. Although the 17-ketogenetic steroids rose four-fold on the post-operative day, the level of oestrogen excretion was less than 1.0 $\mu\text{g.}$ in all four cases on each of the three days.

This result contrasts with similar studies in adult men in whom a rise in oestrogens accompanies the rise of other adrenal steroids following surgical stress.

Discussion

Biological assay results for urinary oestrogen excretion in normal children show considerable variation. For example, Nathanson, Towne and Aub (1941) found levels of up to 5 I.U. of total oestrogen in children of both sexes up to the age of 7 to 8 years, rising to 20 I.U. at the age of 11, and 240 to 382 I.U. in girls between the ages of 13 to 15 years. Dorfman, Greulich and Solomon (1937) found levels of 0 to 25 I.U. in five girls of ages varying from 7 to 12 years, while Pedersen-Bjergaard and Tønnesen (1948) found levels of up to 3 m.u. between the ages of 3 to 12 years. In one of the five cases of precocious puberty described by Hain (1947), the urinary oestrogens were 200 I.U./24 hours, and in the case reported by Gross (1940) they fluctuated between 405 and 12 I.U.; but this is exceptional and in the majority of reported cases oestrogens were absent or less than 10 I.U./24 hours. In his series of nine cases, Novak (1944) reported laboratory results as essentially negative in five; oestrogen levels were 4.4, 4.0,

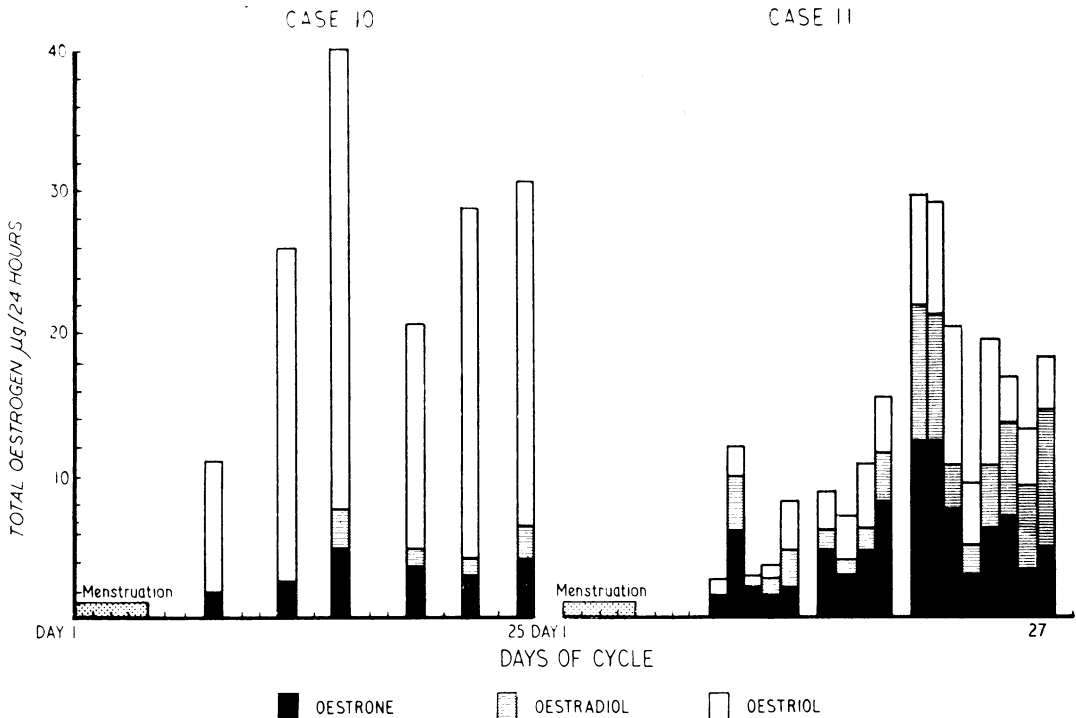


FIG. 1.—Oestrogen excretion during the menstrual cycle. Case 10, aged 10 years; Case 11, aged 10 years 10 months.

and 25.0 I.U. in three, and slightly lower than the normal pubertal level in one.

Although this study is concerned with only a small number of cases of precocious puberty, certain conclusions may be drawn. First, children who have developed certain of the secondary sexual characteristics, advanced growth, breast development and pubic hair, but who have not established regular menstrual cycles, excrete much less oestrogen than adult women (Brown, 1955*b*). It is not known whether the oestrogen excreted by children with precocious puberty is of adrenal and/or ovarian origin. Secondly, oestrogen excretion is nevertheless almost certainly greater in this group than in normal children without any of the signs of approaching puberty. Thirdly, a dramatic rise in oestrogen excretion occurs with the appearance of regular menstrual cycles undoubtedly of ovarian origin. This is illustrated by the two girls, both under 11 years of age, who had regular cycles and who achieved adult levels of excretion. It is probable that there is no essential difference between normal and precocious girls in these respects. It is not possible to say whether an *infant* with regular cycles would show this dramatic rise in excretion since none of the cases described here had the fully developed syndrome. This type of case appears to be uncommon since in Jolly's series of 31 cases only six had had regular cycles before the age of 8 years (Jolly, 1955).

Nathanson *et al.* (1941) reported that about a year and a half before the menarche, oestrogen excretion becomes cyclic in girls, and the intensity of these cycles gradually increases; it is possible that there is a rise and fall in oestrogen and in gonadotrophin excretion in precocious puberty. If this is so, estimations on single 24-hour specimens are necessarily of limited clinical value. Higher values may have existed in our cases and been missed, notably in the three infants who presented with bleeding.

Gonadotrophin was present in the urine of Cases 7, 9, 10 and 11, but absent in Cases 1, 2, 3, 4, 5, 6 and 8. In their extensive study of gonadotrophin excretion by the pre-menarche child, Catchpole and Greulich (1943) estimated the hormone in 24-hour urines every day for at least a month in 16 normal girls. With the approach of the menarche the number of days in which they were able to detect FSH in the urine approximated to 100% while the level of excretion also rose. The results in each case varied with the proximity of the first period. The average time elapsing between the date of the estimations and the date of the menarche in their cases was seven months, while the average percentage of days on which hormones could be detected at a level of 2 m.u. or more was only 43%. In adult women,

Levin (1941) found that the daily excretion of gonadotrophin was less than 7.5 m.u. per day during the greater part of the cycle, but peak periods lasting for two days occurred at the mid-cycle when excretion rose to 15 m.u./24 hours in one case and 35 m.u./24 hours in another. It seems probable, therefore, that the absence of gonadotrophin from the urine of some of these cases may be due either to the insensitivity of the test, or to the irregular excretion of the hormone.

Vulval bleeding from local causes may mimic menstrual bleeding if the cause is not obvious on examination. In this event the hormone determinations will be normal but this is insufficient ground for excluding a diagnosis of precocity. If, however, the vaginal smear remains persistently negative, even though further bleeding occurs, it is probable that oestrogen excretion is not raised.

It may be concluded from these results that oestrogen, 17-ketosteroid and gonadotrophin determinations carried out on single 24-hour specimens are not diagnostic of precocious puberty, since completely normal values are not incompatible with the diagnosis. Nevertheless, the results demonstrate quite clearly that oestrogen excretion is usually raised above control levels, associated with a positive vaginal smear. With the present chemical method the error of the determinations at the low oestrogen levels encountered in urine from cases of precocious puberty is very large (Brown, Bulbrook and Greenwood, 1957). It may well be that a more sensitive method which could differentiate with certainty between control levels and the small amounts of oestrogen generally found in precocious puberty might be of diagnostic use. Even with the present method of Brown (1955*a*) the results are useful, especially in conjunction with the 17-ketosteroid values, for, if a gross pathological condition in the gonads or adrenal cortices exists, the figures will probably be high, i.e. well within the adult range (Snaith, 1958). This is unlikely to be the case in precocious puberty, except when the periods are well established and not infrequent.

Summary

Urinary oestrogen, 17-ketosteroid and gonadotrophin determinations have been carried out and vaginal smears examined in 11 girls with precocious or early puberty. While the 17-ketosteroid levels are at the upper range of normal values and the oestrogen levels are above control levels, the results of these estimations are not diagnostic of the condition but are of value in differentiating true precocious puberty from precocity due to pathological conditions involving the gonad or the adrenal, and from

non-hormonal vulval bleeding. The results from a control series of 15 normal girls, in the age range 3 to 10 years, are also summarized.

We wish to thank the physicians and surgeons of The Hospital for Sick Children, Great Ormond Street, for permission to study their cases.

REFERENCES

- Bronstein, I. P., Luhan, J. A. and Mavrelis, W. B. (1942). *Amer. J. Dis. Child.*, **64**, 211.
- Brown, J. B. (1955a). *Biochem. J.*, **60**, 185.
- (1955b). *Lancet*, **1**, 320.
- , Bulbrook, R. D. and Greenwood, F. C. (1957). *J. Endocr.*, **16**, 41.
- Bulbrook, R. D., Greenwood, F. C. and Williams, P. C. (1957). *J. Endocr.*, **15**, 206.
- Catchpole, H. R. and Greulich, W. W. (1943). *J. clin. Invest.*, **22**, 799.
- Dekanski, J. (1949). *Brit. J. exp. Path.*, **30**, 272.
- Dorfman, R. I. (1948). In *The Hormones*, Vol. 1, edited by G. Pincus and K. V. Thimann. New York.
- , Greulich, W. W. and Solomon, C. I. (1937). *Endocrinology*, **21**, 741.
- Fischer, H. S. (1940). *Amer. J. Obstet. Gynec.*, **39**, 525.
- Gross, R. E. (1940). *Amer. J. Dis. Child.*, **59**, 579.
- Hahn, A. M. (1947). *J. clin. Endocr.*, **7**, 171.
- Holtorf, A. F. and Koch, F. C. (1940). *J. biol. Chem.*, **135**, 377.
- Jolly, H. (1955). *Sexual Precocity*. Springfield, Illinois.
- Levin, L. (1941). *Endocrinology*, **28**, 378.
- Lloyd, C. W. (1955). *J. clin. Endocr.*, **15**, 1518.
- , Lobotsky, J. and Morley, M. (1950). *Pediatrics*, **6**, 343.
- Loraine, J. A. and Brown, J. B. (1956). *J. clin. Endocr.*, **16**, 1180.
- Lowrey, G. H. and Brown, T. G. (1951). *J. Pediat.*, **38**, 325.
- Mossberg, H. O. (1951). *Acta paediat. (Uppsala)*, **40**, 261.
- Nathanson, I. T., Towne, L. E. and Aub, J. C. (1941). *Endocrinology*, **28**, 851.
- Novak, E. (1944). *Amer. J. Obstet. Gynec.*, **47**, 20.
- Paschkis, K. E. (1952). *Med. Clin. N. Amer.*, **36**, 1711.
- Pedersen-Bjergaard, K. and Tønnesen, M. (1948). *Acta endocr. (Kbh.)*, **1**, 38.
- Seckel, H. P. G., Scott, W. W. and Benditt, E. P. (1949). *Amer. J. Dis. Child.*, **78**, 484.
- Snaith, A. H. (1958). *J. Clin. Endocr.*, **18**, 318.
- Talbot, N. B., Berman, R. A. and MacLachlan, E. A. (1942). *J. biol. Chem.*, **143**, 211.
- Tanner, J. M. (1955). *Growth at Adolescence*. Oxford.
- Young, S., Bulbrook, R. D. and Greenwood, F. C. (1957). *Lancet*, **1**, 350.