Diagnosis of Congenital Adrenal Hyperplasia by Measurement of Plasma 17-Hydroxyprogesterone

N. D. BARNES* and SHELIA M. AHERDEN

From The Hospital for Sick Children and the Institute of Child Health, London

Barnes, N. D., and Atherden, S. M. (1972). Archives of Disease in Childhood, 47, 62. Diagnosis of congenital adrenal hyperplasia by measurement of plasma 17-hydroxyprogesterone. Measurement of plasma 17-hydroxyprogesterone by a simple competitive protein-binding assay has proved of value in the diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Children with untreated adrenal hyperplasia had high resting levels from as early as the 6th day of life, and children treated with suppressive doses of cortisone showed a rise after adrenal stimulation with tetracosactrin. The method also promises to enhance the ease and precision with which the condition can be managed.

Congenital adrenal hyperplasia (CAH) is due, in approximately 95% of cases, to deficiency of 21-hydroxylase (New, 1968). This enzyme is necessary for the hydroxylation of 17α-hydroxyprogesterone (17-OHP) in the biosynthesis of cortisol. Deficiency results in the accumulation of cortisol precursors, chiefly 17-OHP, of which the plasma level may be 50 to 200 times greater than normal (Strott, Yoshimi, and Lipsett, 1969) and in the increased production of adrenal androgens. The diagnosis of CAH has hitherto depended on the demonstration of an excess of the metabolites of cortisol precursors and androgenic steroids in the urine. However, measurement of the plasma 17-OHP level seems likely to provide a more sensitive and convenient index of the condition. This paper reports determinations in children with CAH using a simple competitive protein-binding assay for the measurement of plasma 17-OHP, based on the work of Murphy (1967).

Subjects and Methods

Patients. Tests were performed on children undergoing investigations in The Hospital for Sick Children and, in 6 of the untreated CAH patients, on children referred to us by other hospitals.

Plasma 17-OHP. Plasma 17-OHP was determined by the competitive protein-binding method of Murphy (1967) with a number of modifications (see Appendix). Plasma (200 µl) was extracted with 2% ethanol in petroleum spirit and the extract used to displace tritium-labelled cortisol from corticosteroid-binding globulin. The displacement was compared with that caused by known quantities of 17-OHP.

Tetracosactrin (Synacthen) stimulation test. After breakfast at 6.00 a.m. the child was allowed only water until the test was complete. Between 8.30 and 9.30 a.m. a blood sample was collected by finger-prick or venepuncture into heparin. Tetracosactrin,* 0.25 mg per 70 kg body weight, was then given by intramuscular injection and a second blood sample collected 30 minutes later.

Other methods. Standard methods were used for the estimation of 17-oxosteroids (Norymberski, Stubbs, and West, 1953), pregnanetriol (Brooks and Prunty, 1960), and the 11-oxygenation index (Edwards, Makin, and Barratt, 1964).

Results

Normal children. Plasma obtained from the umbilical veins of 10 infants after normal deliveries gave falsely high values for '17-OHP', ranging from 5.1-22.2 µg/100 ml due to the high levels of maternal progesterone (van der Molen and Aakvaag, 1967). Plasma from 10 normal infants at the age of 6 days gave values less than 0.5 µg/100 ml.

Plasma from 20 children without evidence of endocrine disease, 12 boys and 8 girls, aged from 1 to 13 years, was obtained before and 30 minutes after tetracosactrin. All showed a normal rise in plasma cortisol. In each case the resting 17-OHP

Received 27 July 1971.

*Present address: Mayo Graduate School of Medicine, Rochester, Minnesota 55901, U.S.A.

*Synacthen, Ciba.
value was less than 0.5 \( \mu g/100 \) ml and showed no detectable rise after adrenal stimulation.

**Adults heterozygous for CAH.** Six parents of children with CAH, 3 male and 3 female (tested during the follicular phase of the menstrual cycle), showed resting values below 0.5 \( \mu g/100 \) ml and no detectable rise after tetracosactrin stimulation.

**Children with CAH.** Resting plasma 17-OHP levels between 8:00 and 10:00 a.m., and other relevant details of 7 children with untreated CAH are shown in the Table.

In one patient (Case 6, Table) a marked diurnal variation in plasma 17-OHP level was seen, from 0.7 \( \mu g/100 \) ml at midnight to 13.8 \( \mu g/100 \) ml at 9.30 a.m. the following morning. Rapid suppression of the plasma 17-OHP level was also noted in this child. Two hours after intravenous administration of 100 mg hydrocortisone the level had fallen from 13.8 \( \mu g/100 \) ml to 3.5 \( \mu g/100 \) ml and two hours later to 1.5 \( \mu g/100 \) ml.

Plasma from 8 children with CAH, who were on treatment with cortisone, was assayed before and 30 minutes after tetracosactrin stimulation. 2 were boys and 6 were girls, their ages ranged from 11 months to 12 years, and the duration of treatment from 10 months to 10 years. The 4 children with salt-losing CAH received salt supplements but no salt-retaining hormone. All showed a rise in plasma 17-OHP as illustrated in the Fig.

Random plasma 17-OHP levels were compared with the 17-oxosteroid excretion in 16 children with treated CAH. Thirteen children with a normal 17-oxosteroid excretion (allowing for the contribution of administered cortisone) all showed plasma 17-OHP levels of less than 1.5 \( \mu g/100 \) ml, whereas 3 children with raised 17-oxosteroid excretion showed levels of 4.8, 5.0, and 8.6 \( \mu g/100 \) ml.

**Discussion**

A method for the measurement of plasma 17-OHP, in which a competitive protein-binding assay was preceded by two chromatographic stages, was described by Strott and Lipsett (1968) who also

![Graph](http://adc.bmj.com/)

**FIG.**—Plasma 17-OHP before and 30 minutes after tetracosactrin stimulation in 8 children with CAH.

### Table

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>11-oxygenation index</th>
<th>Plasma 17-OHP (( \mu g/100 ) ml)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 dy</td>
<td>F</td>
<td>0.8</td>
<td>1.0</td>
<td>Salt loss, virilization, sib also affected</td>
</tr>
<tr>
<td>2</td>
<td>7 dy</td>
<td>F</td>
<td>0.8</td>
<td>0.7</td>
<td>Salt loss, virilization</td>
</tr>
<tr>
<td>3</td>
<td>1-0 yr</td>
<td>F</td>
<td>5.0</td>
<td>0.9</td>
<td>Slight virilization, rapid growth, advanced bone age</td>
</tr>
<tr>
<td>4*</td>
<td>1-2 yr</td>
<td>F</td>
<td>2.6</td>
<td>1.0</td>
<td>Slight virilization</td>
</tr>
<tr>
<td>5*</td>
<td>3-5 yr</td>
<td>F</td>
<td>10.6</td>
<td>1.7</td>
<td>Virilization, rapid growth, advanced bone age</td>
</tr>
<tr>
<td>6</td>
<td>4-5 yr</td>
<td>M</td>
<td>20.0</td>
<td>1.8</td>
<td>Precocious sexual development, rapid growth, advanced bone age</td>
</tr>
<tr>
<td>7</td>
<td>5-0 yr</td>
<td>F</td>
<td>11.1</td>
<td>1.4</td>
<td>Slight virilization, rapid growth, advanced bone age</td>
</tr>
</tbody>
</table>

**Normal values**

- 17-oxosteroids: 0-1 mth 2.0 mg/24 hr 1 mth-5 yr 1.2 mg/24 hr 0-6 yr <0.2 mg/24 hr
- Pregnanetriol: 0-6 yr <0.2 mg/24 hr 11-oxygenation index Older than 8 dy <0.7
- 17-OHP: Older than 4 dy <0.5 \( \mu g/100 \) ml

*Sib
demonstrated that the plasma 17-OHP level was greatly raised in 5 children with simple virilizing CAH when they were taken off treatment (Strott et al., 1969). This method is sensitive and specific but too complex and prolonged for diagnostic use. The method described here is simple, rapid, and has adequate sensitivity and specificity for the diagnosis of CAH, though failure to separate progesterone from 17-OHP limits its value in other situations. The plasma progesterone is raised in CAH but only to approximately 5% of the 17-OHP level (Strott et al., 1969), and since progesterone shows less than half the competition for corticosteroid-binding globulin (CBG), the error so caused is small. Moreover, since progesterone undergoes 21-hydroxylation in the biosynthesis of aldosterone, the raised level is a consequence of the primary biochemical defect. The slight overestimation of the 17-OHP level that results will, therefore, increase the ability of the assay to discriminate between subjects with CAH and normal subjects.

Our results show that untreated CAH due to 21-hydroxylase deficiency, in both simple virilizing and salt-losing forms, is associated with high levels of plasma 17-OHP from as early as the 6th day of life. The marked diurnal variation demonstrated in one patient was an unexpected finding, as it has been assumed that no variation would occur (Strott et al., 1969).

Present methods for the diagnosis of CAH have a number of limitations (Clayton, 1970), and measurement of plasma 17-OHP offers theoretical and practical advantages. The metabolic clearance rate of plasma 17-OHP in CAH is approximately the same as in normal subjects (Strott et al., 1969) so the plasma level is directly related to the production rate. High plasma levels which result from deficiency of 21-hydroxylation are unlikely to occur in other conditions. Collection of urine specimens, a considerable problem in infancy, is avoided. The diagnosis can be made within 1½ hours using only a small capillary blood sample; this is a major advantage when, in an infant seriously ill with salt loss, diagnosis is a matter of urgency. When treatment is started, suppression of the abnormal metabolites provides final confirmation of the diagnosis. Several days of treatment have been necessary to demonstrate suppression of the urinary metabolites, but the plasma 17-OHP falls within hours.

Since treatment may be started before CAH is confirmed biochemically, it is important to be able to substantiate the diagnosis in a child already treated with a suppressive dose of a glucocorticoid.

To avoid interrupting therapy, ACTH can be given and the appearance of abnormal urinary metabolites demonstrated. However, several days of ACTH stimulation may be necessary (Clayton, Edwards, and Makin, 1971). A rise in plasma 17-OHP occurred 30 minutes after tetracosactrin stimulation in children with CAH but not in normal children nor in adults presumed to be heterozygous for the condition.

One of the problems in the management of CAH is the adjustment of glucocorticoid dosage throughout childhood and adolescence to achieve adequate adrenal suppression and yet permit optimal growth. The preliminary results of plasma 17-OHP determinations in children on maintenance therapy show that poor control is associated with high levels. It seems probable that carefully timed plasma levels will provide a more sensitive and convenient index of the adequacy of treatment than the methods currently in use, and this aspect of the problem is being studied.

We are grateful to the paediatricians who referred patients to us; to Dr. G. H. Newsom for permission to study children under his care, and to Professor Barbara E. Clayton, Dr. R. W. H. Edwards, and Miss Jennifer Joseph for valuable advice and assistance. N.D.B. gratefully acknowledges financial support from the Wellcome Trust.

REFERENCES


Murphy, B. E. P. (1967). Some studies of the protein-binding of steroids and their application to the routine micro and ultra micro measurement of various steroids in body fluids by competitive protein-binding radioassay. Journal of Clinical Endocrinology and Metabolism, 27, 975.


Diagnosis of CAH by Measurement of Plasma 17-Hydroxyprogesterone

Appendix
Measurement of 17-hydroxyprogesterone

Procedure. Plasma (200 μl) was extracted with 10 volumes of 2% ethanol in petroleum spirit (British Drug Houses, Ltd., Analar, B.P. 80-100°). Triplicate aliquots of the extracts and standards from 0-5 ng 17-OHP (Koch-Light Laboratories, Ltd.) were evaporated to dryness and dissolved in 1 ml of a 1% solution of reconstituted dry plasma containing approximately 20,000 counts/min per ml tritiated cortisol. After incubation at 45 °C for 15 minutes and at less than 5° C in an ice-bath for 30 minutes, 40 mg Florisil (Hopkin and Williams, Ltd. 60-100 mesh) was added to each tube and the batch shaken mechanically for 1 minute. 0.5 ml supernatant was removed and the radioactivity determined. A standard curve was prepared and the 17-OHP content of the unknown samples calculated. This value was corrected for 80% extraction, and the absolute value calculated. Values falling below 0.5 μg/100 ml were reported as being below the limit of sensitivity and values greater than 10 μg/100 ml were redetermined using diluted plasma.

Evaluation. The method proved simple and rapid, up to 20 samples being determined in triplicate in 4 hours.

Recovery. Extraction of tritiated 17-OHP (Radio-chemical Centre, Amersham) over the range 0.1-20 μg/100 ml was 80-8% (range 77-84%).

Precision. The mean value obtained on control plasma containing 2.5 μg/100 ml 17-OHP included in each assay over a three-month period was 2.6 μg/100 ml and the coefficient of variation was 8%.

Accuracy. The mean values obtained in repeated assays on control plasma with 17-OHP added in concentrations of 0.5, 5.0, and 10 μg/100 ml were 0.6, 5.1, and 10.5, respectively, and the coefficient of variation in every case was less than 5%.

Specificity. Compared with 17-OHP, cortisol showed 100% and progesterone 40% competition for CBG in the conditions of the assay. However, extraction of labelled cortisol over the range 5-50 ng gave recoveries of less than 0.5%. Plasma progesterone is raised in CAH but only to approximately 5% of the 17-OHP level and, since progesterone shows less than half the competition for CBG, the error so caused will be small. Cortisone, dexamethazone, and prednisone did not compete for CBG but prednisolone and deoxycorticosterone showed significant competition.

Correspondence to Mrs. Shelia M. Atherden, The Hospital for Sick Children, Great Ormond Street, London WC1N 3JH.