The Adrenal Cortex in Childhood

Part 1: Physiological Aspects*

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I—Hormones of the Adrenal Cortex

(1) Biosynthesis and metabolism of adrenocortical steroids
(2) Control of adrenal hormone release
(3) Function of adrenocortical steroids

II—Evaluation of Adrenocortical Function

In this review we shall deal with those aspects of adrenal physiology and disease that are of most interest to the paediatrician mainly concerned, as he must be, with the disorders of the adrenal cortex in childhood which are important clinically, and with the tests used to recognize them. The reader may also be directed to the several admirable textbooks which devote much attention to diseases of the adrenal cortex in childhood (Wilkins, 1965; Soffer, Dorfman, and Gabriole, 1961; Prunty, 1964; Mills, 1964; Cope, 1965), while there are a number of review papers on different specialized subjects which will be referred to later.

I: Hormones of the Adrenal Cortex
(1) Biosynthesis and Metabolism of Adrenocortical Steroids

More than 30 different steroids have been isolated from adrenal extracts, but only a few of these are biologically important. These active hormones can be divided into 4 groups: (1) the glucocorticoids, (2) the mineralocorticoids, (3) the androgens, and (4) the oestrogens. Cortisol (hydrocortisone, compound F) is the most important glucocorticoid and is produced mainly by the zona fasciculata. Corticosterone has weaker glucocorticoid activity and is produced by the zona fasciculata and the zona glomerulosa. In some animals, e.g. the rat, this steroid is the dominant glucocorticoid hormone. Aldosterone is the chief mineralocorticoid and is produced mainly by the zona glomerulosa. Both corticosterone and cortisol have mineralocorticoid effects, corticosterone being more active than cortisol in this respect. The adrenal androgens consist of a group of hormones, including dehydroepiandrosterone (DHA), androstenedione, and possibly testosterone, differing considerably in biological potency. The role of the adrenal oestrogens is not clear.

Fig. 1 demonstrates the steroid nucleus, indicating the four rings and the numbering of carbon atoms in the rings or attached to them. The carbon atoms at 18 and 19 are normally present as \(-\text{CH}_2\). \(\text{C}_{21}\)-steroids are steroids with 21 carbon atoms, and \(\text{C}_{19}\)-steroids have 19 carbon atoms, without a side chain at \(\text{C}_{17}\). Double bonds between two carbon atoms in the rings are indicated by \(A\), the specific site designated by two numbers or one number only, e.g. \(A_{4,5}\) or \(A_4\) (double bond between \(\text{C}_4\) and \(\text{C}_5\)). Hydroxy- and keto- (or oxo-) are prefixes used to indicate \(-\text{OH}\) and \(=\text{O}\), respectively. \(-\text{ol}\) and \(-\text{one}\) are suffixes used to indicate \(-\text{OH}\) and \(=\text{O}\), respectively. \(\alpha\) and \(\beta\) are used to describe whether a univalent substituent (\(-\text{H}\) atom or \(-\text{OH}\) group) lies above the plane of the molecule (and the paper) or below the plane of the molecule. Hydrogen atoms or \(-\text{OH}\) groups attached to carbon atoms in \(\beta\) position are indicated by a solid line, and in \(\alpha\) position by a broken line.

For most steroids both trivial names and systematic names are in common use.

The steroid skeleton as seen in Fig. 1 with \(-\text{CH}_3\) at \(\text{C}_{21}\) and \(-\text{CH}_2\) at \(\text{C}_{20}\) is known under...
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The name pregnane. When a double bond is introduced and the steroid becomes unsaturated, the name is pregnene. Cortisol (trivial name) is 11β, 17α, 21-trihydroxy-Δ₄ pregnene-3, 20-dione (systematic name).

The steroid skeleton without a side chain at C₁₇ is known as androstane, with a double bond the name is androstene. Testosterone (trivial name) is 17β-hydroxy-Δ₄-androstene-3-one (systematic name).

Although the main pathways in the biosynthesis of adrenal corticosteroids have been elucidated (Hechter and Pincus, 1954), the exact sequences of reactions are not fully known. Fig. 2 and 3 show the most important enzyme steps in the biosynthesis of adrenocortical steroids. Acetate or cholesterol are precursors for all adrenocortical steroids. Cholesterol is converted to pregnenolone; 20, 22-dihydroxycholesterol being an intermediate. Two hydroxylases at C₂₀ and C₂₂ and a 20, 22-desmolase are probably involved in these steps. The next step consists in the shift of the double bond from Δ₅-₆ to Δ₄-₅ position and oxidation of -OH to =O at C₃; these changes, requiring probably the activity of an isomerase and a dehydrogenase, result in the formation of progesterone. This steroid is subsequently hydroxylated at the positions C₁₇, C₁₁, and C₂₁ to cortisol and at C₂₁ and C₁₁ to corticosterone. In the zona glomerulosa corticosterone is probably converted to aldosterone via 18-hydroxycorticosterone, requiring an 18-hydroxylase and subsequently a dehydrogenase capable of oxidizing the 18-hydroxyl group to an aldehyde.

An important synthetic pathway for adrenal C₁₉ steroids or androgens is via pregnenolone, progesterone, and 17-hydroxyprogesterone to androstenedione and 11-hydroxy-androstenedione. Dehydro-

![Fig. 1.—Steroid skeleton. Numbering of carbon atoms, lettering of rings.](image)

![Fig. 2.—Most important enzymic processes in the biosynthesis of adrenocortical steroids.](image)

epiandrosterone (DHA) is derived directly from cholesterol or from 17-hydroxypregnenolone by splitting off the side chain at C17, and is subsequently converted to androstenedione. In vitro studies of normal human adrenal glands have demonstrated biosynthesis of testosterone and androstenedione from progesterone (Ichii, Forchielli, Cassidy, Rosoff, and Dorfman, 1962; Kase and Kowal, 1962). These steps require isomerization of $A_5$ to $A_4$ and dehydrogenation at $C_5$. Evidence that DHA-sulphate is secreted by adrenal tumours and by the normal human adrenal has been presented in recent years (Baulieu, 1960; Siiteri, Vande Wiele, and Lieberman, 1963), and steroid-sulphates may serve
as biosynthetic intermediates. Direct measurements in adrenal blood have confirmed that androstenedione, DHA, and DHA-sulphate are secreted by the normal human adrenal, but secretion of testosterone has not been definitely established in this way (Short, 1960; Wieland, de Courcy, Levy, Zala, and Hirschmann, 1965).

Very little is known about the adrenocortical enzyme systems concerned with the steroidal transformations, and none of the enzymes has been completely purified. It is probable that at several steps more than one enzyme is involved and that multiple enzyme systems exist which are more or less substrate-specific. Differences in enzyme kinetics for different substrates in normal steroid biosynthesis have been reported for 21- and 11-hydroxylation (Sharma and Dorfman, 1963; Tomkins, Michael, and Curran, 1957).

Fig. 4 demonstrates the most important enzymic processes in the metabolism of adrenocortical steroids. Cortisone, though so widely used therapeutically, is not secreted by the adrenal, but is converted peripherally, mainly in the liver, into the active hormone cortisol. Cortisol is metabolized peripherally into cortisone by oxidation at C11.

Other steroids with —OH group at C11 are also easily oxidized at this position.

Other important metabolic changes of the steroid molecules are reduction of ring A, and subsequent reduction at C90 and removal of the side chain at C17. Steroid metabolites are rapidly conjugated with glucuronic acid and sulphuric acid after reduction of ring A. These conjugates are much more water soluble than unconjugated steroids, and are readily excreted in the urine.

The most important metabolites of cortisol are shown in Fig. 5. In the process of reduction of the 4 3-keto group in the A-ring, four hydrogen atoms are added to the molecule. The metabolites that are formed are called tetrahydro-metabolites (TH—). By subsequent reduction at C90 the cortols and cortolones are formed, and by removal of the side chain at C17 a number of 11-oxy-17-ketosteroids. The two important metabolites of 17-hydroxypregesterone and 11-deoxycortisol (Compound S) are pregnanetriol and tetrahydro-S. These metabolites are excreted in large amounts in patients with congenital adrenal hyperplasia with 21- and 11-hydroxylation defects, respectively. Mineralocorticoids are mainly metabolized to tetrahydro-metabolites and as they have not undergone...
Fig. 5.—Most important metabolites of cortisol, aldosterone, and their precursors. Numbers in [] refer to hydroxylation and dehydrogenation enzyme steps. TH = tetrahydro. Urinary metabolites appear outside the vertical lines.
hydroxylation at C₁₇, the side chain is not split off.

Fig. 6 illustrates the metabolism of adrenal androgens (Dorfman, 1954). The most important metabolites of DHA, androstenedione, and testosterone are androsterone and aetiocholanolone (11-deoxy-17-KS), which are formed by reduction of the A-ring. The 11-oxy-17-ketosteroids arise from the metabolism of 11-hydroxandrostenedione and adrenosterone. Conversion studies in adults have shown that these compounds are partly (80% and 100% respectively) metabolized to 11-oxy-17-KS in a ratio of 5β/5α of 0.2 and 0.25 (Savard, Burstein, Rosenkrantz, and Dorfman, 1953; Bradlow and Gallagher, 1957). On the other hand the ratio of 5β/5α 11-oxy-17-ketosteroids arising from cortisol is about 10 (Fukushima, Bradlow, Hellman, Zumoff, and Gallagher, 1960). Thus 11-hydroxyandrostenedione is mainly derived from adrenal androgens, 11-hydroxyaetiocholanolone, and 11-ketoaetiocholanolone mainly from cortisol. It is not known if these differences in steroid metabolism apply also to children. There is some evidence for decreased 5α-steroid-reducing activity before puberty (N. I. Gold, J. F. Crigler, W. Teller, and H. K. A. Visser, to be published; Guignard-de Maeyer, Crigler, and Gold, 1963).

Differences in the metabolism of cortisol between newborn infants and adults have been demonstrated by several investigators. A delayed disappearance of exogenous cortisol from the blood of the newborn can be attributed to undeveloped liver function: the reduction of the A-ring (Δ₄-3-ketone moiety) as well as the conjugation with glucuronic acid (Grumbach, Ducharme, and Morishima, 1959; Ulstrom, Colle, Burley, and Gunville, 1960a, b). The enzymatic deficiencies responsible for these impaired liver functions are relative, as is shown by Cranny, Kirschvink, and Kelley (1960) who found a normal disappearance of cortisol when given in small doses. Ulstrom and colleagues (1960a) have shown that newborns produce relatively more of the polar metabolites of cortisol, mainly 6β-hydroxycortisol, and excrete them largely in the unconjugated form. Cathro, Birchall, Mitchell, and Forsyth (1963) found several unknown neutral steroids in the urine of newborn infants. One of these compounds has
TABLE I
Production, Excretion, and Plasma Levels of Cortisol, Corticosterone, Aldosterone, DHA, and Testosterone in Normal Subjects (Older Children and Adults unless Otherwise Stated)

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Secretion Rate</th>
<th>Urinary Excretion</th>
<th>Plasma Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol .. . . .</td>
<td>Basal (all ages), 5-30 mg./24 hr.; 8-17.5 mg./m.²/24 hr.</td>
<td>Ratio—Secretion rate urinary excr. 17-OHCS</td>
<td>10-20 µg./100 ml.</td>
</tr>
<tr>
<td></td>
<td>Maximum, after ACTH, 1, 125-200 mg./24 hr.; 2.5 mg./kg./24 hr.</td>
<td>Older infants, children, and adults 2-4, newborns 5-60</td>
<td></td>
</tr>
<tr>
<td>Corticosterone . . .</td>
<td>1.3-4.0 mg./24 hr.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldosterone . . .</td>
<td>Sodium Intake (µg./24 hr.)</td>
<td>Adults, 1-15 µg./24 hr.</td>
<td>0.03-0.08 µg./100 ml.</td>
</tr>
<tr>
<td></td>
<td>Normal Low, Adults, 40-250 mg./24 hr.</td>
<td>Infants and children, 6-5 mg./kg./24 hr.</td>
<td>0.08-0.08 µg./100 ml.</td>
</tr>
<tr>
<td></td>
<td>Infants, 2-5 mg./kg./24 hr.</td>
<td>Up to 1,500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Children, 5-60 mg./24 hr.</td>
<td>Up to 600</td>
<td></td>
</tr>
<tr>
<td>Dehydroepiandrosterone</td>
<td>15-25 mg./24 hr. (adults)</td>
<td>Adults, M, 20-200 µg./24 hr.</td>
<td>Adults, M, 0.3-1.1 µg./100 ml.</td>
</tr>
<tr>
<td>Testosterone . . .</td>
<td>Adults, M, 4-12 mg./24 hr.</td>
<td>Infants before puberty, F, 0.5 mg./24 hr.</td>
<td>Adults, F, 0.5-3.1 µg./100 ml.</td>
</tr>
<tr>
<td></td>
<td>Children before puberty, M, 0-3 mg./24 hr.</td>
<td>Up to 1,500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F, 0.5-3.1 mg./24 hr.</td>
<td>Up to 600</td>
<td></td>
</tr>
</tbody>
</table>


*STRESS*

1. HYPOTHALAMUS
2. CORTICOTROPIN-RELEASING FACTOR (CRF)
3. ANTENOR PITUITARY
4. ACTH
5. UNKNOW FACTOR STARTS AT PUBERTY
6. CHOLESTEROL
7. PREGNENOLONE
8. ANDROGENS
9. PROGESTERONE
10. CORTICOSTERONE
11. CORTISOL
12. ALDOSTERONE
13. INTRAMUSCULAR VOLUME CONTROL
14. Na/K BALANCE
15. SYNTHETIC STEROIDS
16. METOPRONE INHIBITS 11-HYDROXYLATION

**FIG. 7.—Control of adrenal hormone release.**

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[Diagram of the hypothalamic-pituitary-adrenal axis with labeled hormones and pathways.]
been provisionally identified as 3β-21-dihydroxy-Δ4 4-pregnene-20-one, indicating a different way of metabolism of adrenocortical steroids in the newborn period. Although temporary differences in the metabolism of cortisol (and probably other adrenocortical hormones) exist in the newborn period, the production of cortisol in newborn infants is not different from that in older children and adults, at least under basal conditions (Bertrand, Lorand, Gilly, and Cautenet, 1963a; Kenny, Malvaux, and Migeon, 1963). Adrenocortical metabolism in the newborn infant has been reviewed extensively elsewhere (Gardner, 1956; Migeon, 1959; Ducharme, 1961; Bertrand et al., 1963a; Visser, Degenhart, Cost, and Croughs, 1964).

Quantitative data on the production and metabolism of cortisol, aldosterone, and testosterone (including testosterone derived from the gonads) exist for adults, but hardly at all for infants and children. Table I provides summarized data from published reports for normal individuals, and will be used when discussing adrenal diseases in Part 2 of this article.

(2) Control of Adrenal Hormone Release

The rate of secretion of cortisol by the adrenal cortex is controlled by the pituitary hormone ACTH (corticotrophin) (Fig. 7). There is a feedback mechanism between the plasma level of cortisol and the release of ACTH. ACTH secretion by the anterior pituitary gland is in turn controlled by a hormone secreted by the hypothalamus, the corticotrophin releasing factor (CRF). Experimental work indicates that the inhibitory effect of cortisol and synthetic glucocorticoids on the release of ACTH by the anterior pituitary gland is mediated by the hypothalamus. CRF itself seems to be under control of the central nervous system. Under conditions of stress, high plasma cortisol levels fail to inhibit ACTH release (Estep, Island, Ney, and Liddle, 1963).

The nature of CRF is not known, but it is very likely a small polypeptide-like vasopressin. ACTH is a polypeptide consisting of 39 amino acids. The first 24 amino acids are identical in different animals, while the other amino acids vary. A biologically active ACTH polypeptide consisting of 24 amino acids has been synthesized recently (Kappeler and Schwyzer, 1961; Schwyzer and Sieber, 1963). The metyrapone test on the release of ACTH by the anterior pituitary gland depends on the negative feedback mechanism between cortisol and ACTH. Metyrapone (metopirone) inhibits 11-hydroxylation of 11-deoxycortisol to cortisol (see II).

Control of secretion of aldosterone by the zona glomerulosa is the subject of much research. Different complicated nervous and humoral mechanisms are involved. ACTH stimulates the secretion of aldosterone to some extent, but beyond this there are subtle mechanisms of aldosterone control. Intravascular volume control is probably mediated through pressure changes somewhere in the arterial vascular tree. There is strong evidence that the renin-angiotensin system participates in the regulation of aldosterone production; the probable site of production of renin is the juxtaglomerular apparatus in the kidneys. Changes in the Na/K balance of the organism indirectly, and probably also directly, influence the secretion of aldosterone. Sodium restriction is a most powerful stimulus in increasing aldosterone secretion. Potassium loading stimulates, potassium restriction reduces secretion of aldosterone. There is evidence against a direct feedback mechanism of aldosterone secretion: secretion of aldosterone by the adrenal gland does not reduce directly its secretion (Blair-West, Coghlan, and Denton, 1962).

The controversial subject of control of aldosterone secretion has recently been discussed by Blair-West, Coghlan, Denton, Goding, Wintour, and Wright (1963) and by a number of distinguished investigators at a symposium (Baillieu and Robel, 1964). It has been estimated that about 80% of corticosterone production takes place in the zona fasciculata and reticularis and is under the control of ACTH. About 20% is produced in the zona glomerulosa and is under control of the aldosterone-regulating mechanism (S. Ulick, 1964, personal communication; Bledsoe, Island, Riondel, and Liddle, 1964).

The production of adrenal androgens depends upon the age of the individual. Immediately after birth the urinary excretion of 17-ketosteroids (17-oxosteroids, 17-KS) is relatively high. Some weeks after birth the 17-KS excretion is very low and this continues until puberty. ACTH stimulates the secretion of androgens, but there is evidence that the increase in secretion of adrenal androgens in response to ACTH is less in children than in adults, though full studies in children of different ages have not been made (Mills, 1964.) The response of cortisol secretion to ACTH seems to be similar in children and adults, when calculated with reference to surface area. The increased production of adrenal androgens as puberty approaches might be the result of stimulation by a specific pituitary hormone ('androgen stimulating hormone') which is synergistic with ACTH, but so far this substance has not been isolated. This subject will be discussed further in the section on 'premature adrenarche' in Part 2.
(3) Function of Adrenocortical Steroids

It is remarkable that in spite of extensive investigations over the past 30 years we are still so ignorant about the role of adrenocortical steroids under normal conditions and of their precise mode of action. The function of cortisol during stress, for instance, is not exactly known, though we know that the hormone is essential for life. There is increasing evidence now that most, if not all, steroids exert their action by influencing enzyme systems.

Aldosterone regulates Na and K excretion by the renal tubule. Its main effect is reabsorption of Na from the tubular fluid in the distal tubule, in exchange for K and H ions. Aldosterone exerts similar effects on the electrolytes in saliva and sweat (Conn, 1949; Prader, Gautier, Gautier, Naf, Semer, and Rothschild, 1955; Siegenthaler, de Haller, de Haller, Hampai, and Muller, 1964).

Cortisol and related compounds are generally named glucocorticoids, as these steroids have important effects on carbohydrate metabolism. They stimulate the conversion of protein to glucose, which leads to gluconeogenesis and protein katabolism. Cortisol also has the same effects on electrolyte metabolism as aldosterone, but to a lesser degree. Table II shows a comparison of the glucocorticoid and mineralocorticoid activities of a number of natural and synthetic steroids.

Corticosterone has considerable glucocorticoid activity and somewhat more mineralocorticoid activity than cortisol.

Cortisol, and synthetic analogues such as prednisone, suppress the local and systemic reactions to inflammatory agents by inhibiting the basic processes of the inflammatory reaction. They also suppress allergic reactions.

Cortisol often induces an increase of the neutrophilic granulocytes in the blood, together with a decrease in circulating eosinophils.

Cortisol given to patients with cortisol deficiency corrects the abnormal water excretion test. Administration to normal individuals is followed by increased excretion of calcium and phosphorus. Gastric secretion of free HCl is stimulated by cortisol. Several neurological and psychiatric effects of glucocorticoids have been described, euphoria being common in patients, including children, treated with these steroids.

Maintenance of normal blood pressure depends also upon the presence of cortisol, as cortisol potentiates the effects of norepinephrine.

The role of adrenal androgens is not clear. The development of pubic and axillary hair in both boys and girls is regulated to some extent by adrenal androgens. This can be seen in patients with Turner’s syndrome and in girls with premature adrenarche (see Part 2). Androgens in general, when given to children, stimulate growth and osseous development by promoting positive nitrogen balance. It is not known if adrenal androgens stimulate statural growth during childhood under normal conditions. However, these steroids may influence growth and skeletal maturation, as they do under pathological conditions in untreated children with congenital adrenal hyperplasia. The subject has been reviewed recently by Visser and Croughs (1965).

Table III illustrates the differences in androgenic potency of some adrenal androgens (Dorfman and Shipley, 1956).

II: Evaluation of Adrenocortical Function

The symptoms of adrenocortical insufficiency depend upon the degree of hormonal inadequacy, upon the age of the patient, and upon the rapidity with which adrenocortical failure occurs. In
infancy, adrenocortical insufficiency is characterized by the salt-losing syndrome; dehydration, vomiting, poor feeding, and intermittent fever; decreased serum Na and Cl, and increased K. Older children with adrenocortical insufficiency may have anorexia, nausea, vomiting, weakness, and weight loss with low blood pressure and dehydration. Pigmentation is a typical feature of the patient with chronic cortisol deficiency.

Clinical symptoms of adrenocortical hyperfunction depend upon which of the various hormones or groups of hormones are in excess. Glucocorticoid excess causes Cushing’s syndrome, with obesity, ‘moon face’, hypertension, and purple striae, together with a decrease in growth rate. Androgen excess causes virilization, advanced growth, and osseous development, as seen in congenital adrenal hyperplasia. Oestrogen excess causes feminization, as seen in oestrogen-producing adrenal tumours. Mineralocorticoid excess: the most important symptoms of primary hyperaldosteronism are hypertension and hypokalaemia.

Tests used in the diagnosis of adrenocortical hypofunction and hyperfunction include the following (apart from specific chemical determinations of adrenocortical steroids in blood or urine): the water-load test; eosinophil depression test; measurements of fasting glucose and glucose-tolerance; serum electrolytes; and Na/K ratio in urine, sweat, and saliva (particularly during salt deprivation). The use of these tests in children has been reviewed by Migeon and Stempfel (1957) and Aceto, Blizzard, and Migeon (1962).

The tests are now discussed in relation to the specific hormones to which they apply.

**Cortisol**

**Water excretion test.** Patients with cortisol deficiency cannot excrete a water load normally. Apparently the kidneys need the presence of cortisol in order to produce an efficient diuresis in response to ingested water, the precise mechanism of the effect being obscure. The patient should have no food or water from 12 midnight. A water load of 20 ml./kg. is given orally at 8 a.m. within half an hour; the patient should void just before the water is given and urine is further collected during each of the next 4-hour periods. The child must remain horizontally in bed during the test. The normal person excretes more than 70% of the ingested load within 4 hours, while patients with cortisol deficiency excrete the load much more slowly. If the water excretion test is abnormal, it should be repeated. Administration of 10-50 mg. cortisol orally 2 hours before the water is given will reverse the abnormality in patients with cortisol deficiency.

The test was introduced by Robinson, Power, and Kepler (1941) and has been used for many years; although many factors influence water excretion, the test is still of considerable value as a rapid and simple screening for adrenal insufficiency. A normal test makes a significant degree of adrenal insufficiency unlikely, while improvement of an abnormal water excretion by oral cortisol provides strong evidence for cortisol deficiency. The potential danger of water intoxication should be kept in mind when applying the test.

**Eosinophil depression test.** It has been known for a long time that the eosinophil cells of the blood tend to disappear under conditions of stress. Administration of ACTH or cortisol induces a decrease in circulating eosinophils. Thorn, Forsham, Prunty, and Hills introduced the test in 1948. After an overnight fast 25 units ACTH are given intramuscularly. Eosinophil counts are made just before and 4 hours after the injection. In normal subjects a 70% or more reduction in eosinophils occurs. Patients with Addison’s disease show little or no drop. The test has fallen into disuse, mainly as the result of inconsistent findings in larger series of patients and normal controls (Best, Muehrcke, and Kark, 1952). It may be more useful when ACTH is given intravenously, and Cope (1965), who discusses the test in detail, describes the intravenous ACTH test as ‘the most sensitive and reliable test for adrenal insufficiency that is at present widely available.’ The use of the eosinophil drop is a relatively simple way of observing the adrenal response. It is more useful and sensitive than are analyses of urinary steroid metabolites. In laboratories or clinics where reliable plasma cortisol estimations can be obtained, this is a preferable index to the eosinophil drop.’

**Blood glucose.** The fasting blood glucose level and the blood glucose levels at the fourth and fifth hour of a glucose tolerance test may be abnormally low in patients with cortisol deficiency. Patients with overproduction (or overdosage) of cortisol may have decreased glucose tolerance.

**Urinary 17-hydroxycorticosteroids (17-OHCS).** Essentially, four steps are always involved in chemical measurements of urinary steroids. Steroid conjugates are hydrolysed by enzymic or chemical hydrolysis, free steroids are then extracted with organic solvents, extracts are subsequently purified, e.g. by chromatography, and steroids finally are quantitatively estimated using more or less specific colour reactions.
Urinary 17-OHCS have four different side chains at C₁₇ (Fig. 8). Porter and Silber (1950) described a specific reaction of the dihydroxyacetone group with phenylhydrazine in diluted sulphuric acid. Steroids measured with this reaction are often referred to as Porter-Silber steroids (cortisol, cortisone, and their tetrahydroxymetabolites). As other substances in urine may also react with phenylhydrazine, urinary Porter-Silber measurements include total Porter-Silber ‘chromogens’. The technique of Glenn and Nelson (1953) has been widely used in the U.S.A. for the measurements of urinary 17-OHCS. Steroids are extracted with chloroform after enzymic hydrolysis; the extract is then washed with NaOH solution and purified by chromatography on a florisil column. The Porter-Silber reaction is then carried out on the residue.

Norymberski, Stubbs, and West (1953) introduced another method which has been used widely in Europe. 17-OHCS are transformed into 17-KS by sodium bismuthate oxidation, and these compounds are then quantitatively estimated by the Zimmermann reaction, a specific reaction on ketone groups with m-dinitrobenzene, which gives a purple colour. Three types of side chain are oxidized in this way: dihydroxyacetone-type, glycerol-type (with —OH on carbons 17, 20 and 21, as in cortols and cortolones, see Fig. 4, c), and the 17, 20-glycol type, as in pregnanetriol (see Fig. 5). These steroids are generally referred to as 17-ketogenic steroids (17-oxogenic steroids, 17-KGS). In the Norymberski method the 17-KS in the urine extract, are determined both before and after oxidation. Thus 17-KGS are determined by difference. Appleby, Gibson, Norymberski, and Stubbs (1955) modified the method by first reducing ketogroups with sodium borohydride. Pre-existing 17-KS are in this way eliminated from further reaction with the Zimmermann reagent. Steroids with 17, 20-ketol groups (Fig. 8) are reduced to the corresponding 20-hydroxy compounds and included in the final Zimmermann reaction after oxidation of the extract with sodium bismuthate.

Determinations of urinary 17-OHCS and 17-KS must be made on aliquots of 24-hour urine specimens, as there is a diurnal variation in the secretion of the steroids. Normal values scatter widely and differ from one laboratory to another. In the newborn period, urinary excretion of 17-OHCS is very low and less than 1·0 mg./24 hr. (Ulstrom et al., 1960a, b). Normal values in children and adults are 3·0 ± 1·0 mg./m² body surface (Aceto et al., 1962; Kenny et al., 1963). Clayton, Edwards, and Renwick (1963) give the following normal values: 0-2 yr. 1·1-6·8 mg./24 hr.; 2-10 yr. 0·6-10·9 mg./24 hr.; 10-14 yr. 4·1-14·2 mg./24 hr. An important recent study on 26 normal children of different age is that of Lelong, Jayle, Joseph, Canlorbe, Job, Scholler, Borniche, and Pascalin (1962).

**Intramuscular ACTH test.** 20-40 mg. (or I.U.) of ACTH-gel is administered intramuscularly every 12 hours for 6 doses to the infant or child. Urinary 17-OHCS are determined on the two days...
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before and on the days of ACTH administration. In infants, children, and adults, a three- to tenfold rise in the urinary 17-OHCS has been obtained (Aceto et al., 1962; Lelong et al., 1962; Clayton et al., 1963; H. K. A. Visser, 1964, unpublished observations). This test is very useful in differentiating between primary adrenocortical insufficiency and cortisol deficiency secondary to ACTH deficiency.

Metyrapone (SU-4885) test. In most studies children and adults are given 500 mg., infants 250 mg., metyrapone every 4 hours for 6 doses. Urinary 17-OHCS are determined on the two days before, on the day metyrapone is administered, and on the two days after administration. The author prefers to give metyrapone for 12 doses every 4 hours. A three- to fivefold increase has been found in infants, children, and adults (Klein, Taylor, Hays, and Masquelier, 1962; Steiker, Bongiovanni, Eberlein, and Leboeuf, 1961; Aceto et al., 1962; Lelong et al., 1962; Bertrand, Ollagnon, Forest, Saez, Cotte, and Cautenet, 1963b; Bertrand, Ollagnon, Saez, Forest, Roux, and Cautenet, 1963c; H. K. A. Visser, 1964, unpublished observations).

Metyrapone blocks the conversion of 11-deoxycortisol to cortisol (Fig. 7). The most important metabolite of 11-deoxycortisol (compound S) is tetrahydro-S (THS; Fig. 5). This substance is measured by the Porter-Silber method as well as by the Norymberski-Appleby method. Thus, after metyrapone administration total urinary 17-OHCS rise as a result of the stimulation of ACTH. A normal response depends upon both ACTH and cortisol production. Failure to respond to metyrapone is thus seen both in patients with primary adrenocortical insufficiency and with ACTH deficiency.

The more sensitive method of measuring the increase of urinary THS after metyrapone administration is by chromatography of this individual compound (Steiker et al., 1961).

Urinary excretion of individual C₂₁-corticosteroids has been estimated by employing paper chromatography and other separations. Urinary C₂₁-corticosteroid excretion of cortisol, cortisone, and their tetrahydrometabolites, 11-deoxycorticosterone, corticosterone, and 11-dehydrocorticosterone, and their tetrahydrometabolites in normal infants, children, and adults have been described by Visser and Cost (1964). Such investigations are useful in the study of defects in the biosynthesis of adrenocortical steroids (see III and IV of Part 2).

Plasma 17-hydroxycorticosteroids. These are not usually determined in routine laboratories, but measurements before and after ACTH are very useful in the diagnosis of adrenocortical insufficiency. van der Wal, Wiegman, Janssen, Delver, and de Wied (1965) studied the reactivity of the hypothalamo-pituitary-adrenal axis in normal children by measuring the free cortisol and corticosterone content of plasma, in response to ACTH and the ACTH-releaser lysine-vasopressin. Another test of pituitary ACTH release is measurement of plasma 17-OHCS during insulin hypoglycaemia. Bertrand et al. (1963c) and Kaplan (1963) compared this test with the metyrapone test and found discordance in a few cases.

Aldosterone

Salt deprivation test. The infant or child is given a diet with no more than 10 mEq Na per day during 5 days. Serum and urinary electrolytes are determined daily. The normal child can maintain normal serum electrolytes by reducing Na excretion to very low values. Na balance is usually achieved by the third day. This mechanism is regulated by increased aldosterone secretion and depends also upon normal function of the renal distal tubules.

Na/K ratios have been determined in sweat and saliva during salt deprivation and have been used as a function test for adrenocortical insufficiency (Conn, 1949; Prader et al., 1955; Siegenthaler et al., 1964). In our hands these tests are difficult to standardize and the results are inconsistent.

Urinary excretion of aldosterone. These measurements are difficult to perform. The method of Neher and Wettstein (1956) has been used widely. Only a few data are available for infants and children (Blizzard, Liddle, Migeon, and Wilkins, 1959; Visser and Cost, 1964). Normal values (on a moderate Na intake) are 1-5 μg./24 hr. for infants and children; 1-15 μg./24 hr. for adults.

Androgens

Urinary 17-ketosteroids (17-KS). Urinary 17-KS include 11-oxy- and 11-deoxy-17-KS (see Fig. 5 and 6) and are derived both from adrenal and gonadal androgens. During childhood 17-KS are mainly derived from adrenal androgens, while in the adult male probably about one-third of urinary 17-KS comes from testicular androgens. Detailed studies during childhood are not available.

The original method of Callow, Callow, and Emmens (1938) has been modified by many investigators. Free steroids are extracted from the urine after enzymic or acid hydrolysis. The extract is purified and the Zimmermann reaction is applied (see under urinary 17-hydroxycorticosteroids (17-OHCS)).
During the first weeks of life normal values are between 0.5–2.5 mg./24 hr. (Zeisel and Pressler, 1953), after which the values fall to below 1.0 mg./24 hr. During childhood values increase slowly to about 5 mg./24 hr. until puberty approaches (H. K. A. Visser, 1964, unpublished observations). From then excretion of 17-KS increases progressively and sex differences become measurable. Adult values are 8–25 mg. (males), 5–15 mg. (females) per 24 hr. Normal data for children are given by Prout and Snaith (1958), Lelong et al. (1962), Aceto et al. (1962), and Kádár, Feher, and Koref (1964).

Measurement of urinary excretion of 17-KS is not helpful in the diagnosis of adrenocortical hypofunction, but is extremely important in the diagnosis of congenital adrenal hyperplasia.

Individual C19-steroids have been measured in the urine of infants and children. Not many data are available (Paulsen and Sobel, 1960; Beas, Zurbrügg, Cara, and Gardner, 1962; Cathro et al., 1963; Visser, Crigler, and Gold, 1962; Kádár et al., 1964). These studies are only possible in research laboratories, but are essential in studying the metabolism of adrenal and gonadal androgens under varying conditions.

Testosterone

Techniques to measure small amounts of testosterone in urine have become available recently (Camacho and Migeon, 1963). Normal values for adults are 20–200 μg./24 hr. (males) and 5–10 μg./24 hr. (females) (Camacho and Migeon, 1963; Rosner, Conte, Briggs, Chao, Sudman, and Forsham, 1965). Children before puberty excrete less than 5 μg./24 hr. (Degenhart, Visser, Wilmink, and Frankena, 1965).

Pregnanetriol

Pregnanetriol is the most important metabolite of 17-hydroxyprogesterone (Fig. 5). Excretion of pregnanetriol is greatly raised in children with congenital adrenal hyperplasia (21-hydroxylation defect). The method of Fotherby and Love (1960) has been widely used during recent years. Normal values in adults are 0·1–3·0 mg./24 hr. In infants and children before puberty we found less than 0·1 mg./24 hr. (H. K. A. Visser, 1964, unpublished observations).

Determination of Secretion Rate of Hormones

During recent years techniques for the determination of the secretion rate of several steroid hormones have been developed, using isotope dilution methods. A known very small quantity of isotopically labelled hormone is injected and the specific activity (radioactivity per mg.) of a metabolite in the urine is measured. The specific activity of this metabolite would be the same as that of the injected hormone, were this metabolite not diluted with the metabolite of the endogenously produced hormone. Secretion rate then is ‘dilution multiplied by injected dose’. (For further details see Kliman and Peterson, 1960; Tait and Burstein, 1964.)

Normal values for secretion and production of several hormones are shown in Table I. Values under pathological conditions will be discussed in Part 2 of this article.

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