Handbook of Endocrine Tests in Children

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with a foreword by
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WRIGHT
Bristol
1986

Published by
John Wright & Sons Ltd,
Techno House, Redcliffe Way, Bristol BS1 6NX

British Library Cataloguing in Publication Data

Hughes, I. A.
Handbook of endocrine tests in children.
1. Paediatric endocrinology 2. Endocrine glands—Diseases—Diagnosis
I. Title
618.92'40'75 RJ418

ISBN 0 7236 0719 2

Typeset by
Severntype Repro Services Ltd,
Market Street, Wotton-under-Edge, Glos.

Printed in Great Britain by
Billing and Sons Ltd, Worcester

PREFACE

This *Handbook* has one prime objective. The child investigated for a possible endocrine disorder should have the appropriate endocrine test to obtain the maximum information. The stimulus to prepare a *Handbook of Endocrine Tests* was prompted by requests from general paediatricians for information on the methods of investigation and the interpretation of hormone results. Children referred to a paediatric endocrine clinic all too often need re-investigation because inappropriate tests were performed initially. Even if further discomfort for the infant or child can be avoided, the *Handbook* will have served some useful purpose.

The user is well-advised to read carefully the contents of Chapter 1 on the General Principles of Endocrine Tests. Particular note should be taken of the need to collect the correct samples and ensure safe transport to the laboratory. The loss of valuable serial samples obtained during a technically difficult test performed in a distraught toddler must be avoided. The more commonly used endocrine tests in children are described. Many paediatric endocrine units will have their own detailed test protocols. Tests are classified according to major endocrine organs. Newer protocols are constantly described; this is especially the case in the assessment of growth hormone secretion. A series of case illustrations at the end of each chapter provides examples of how the author has interpreted the use of some of the *Handbook* tests to investigate patients referred to his clinic.

Most of the hormone measurements whose results are detailed in case illustrations, were performed in the Tenovus Institute SAS Steroid Laboratory and the SAS Polypeptide Laboratory of the Department of Medical Biochemistry, University of Wales. I am grateful to the laboratory staff for providing reliable results and for help with their interpretation. Some colleagues kindly provided material from suitable clinical cases for illustration. They are acknowledged in the appropriate chapters. I am deeply indebted to Miss Helen Powell for her secretarial assistance. She painstakingly typed the manuscript and more than 50 case illustrations. Finally, since the field of endocrinology is changing so rapidly, I invite readers to submit any comments or suggestions about other suitable endocrine tests not currently included in the *Handbook*.

I. A. Hughes 1986

LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone (vasopressin)
BS	blood sugar
Ca	calcium
CAH	congenital adrenal hyperplasia
cAMP	cyclic adenosine monophosphate
Ccr	creatinine clearance
Cp	phosphate clearance
CRF	corticotrophin-releasing factor
CT	computerized tomography
DDAVP	1-diamino-, 8-D-arginine-vasopressin (desmopressin)
1,25-DHCC	1,25-dihydroxyvitamin D
DHEA	dehydroepiandrosterone
DHT	dihydrotestosterone
dopa	3,4-dihydroxyphenylalanine
dopamine	3,4-dihydroxyphenylethylamine
E_2	oestradiol (estradiol)
FSH	follicle-stimulating hormone
FT_4	free thyroxine
FT_3	free tri-iodothyronine
FT_4I	free thyroxine index
FT_3I	free tri-iodothyronine index
GFR	glomerular filtration rate
GH	growth hormone
GHRH (GRF)	growth hormone releasing hormone (growth hormone releasing factor)
GnRH	gonadotrophin-releasing hormone
HbA_1	glycosylated haemoglobin
25-HCC	25-hydroxyvitamin D
HCG	human chorionic gonadotrophin
HLA	human leucocyte antigen
HVA	homovanillic acid
ITT	insulin tolerance test

17-KGS	17-ketogenic steroids
17-KS	17-ketosteroids
LATS	long-acting thyroid stimulator
LATS-P	long-acting thyroid-stimulating protector
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone (gonadotrophin-releasing hormone; gonadorelin)
17-OGS	17-oxogenic steroids
N	normal
OGTT	oral glucose tolerance test
17-OHCS	17-hydroxycorticosteroids
17-OHP	17-hydroxyprogesterone (17OH-progesterone)
17-OS	17-oxogenic steroids
PEI	phosphate excretion index
PIF	prolactin-inhibiting factor (dopamine)
PO_4	phosphate
PRA	plasma renin activity
PRL	prolactin
PTH	parathyroid hormone
RIA	radioimmunoassay
rT_3	reverse tri-iodothyronine
T	testosterone
T_4	thyroxine
T_3	tri-iodothyronine
TBG	thyroxine-binding globulin
Tg	thyroglobulin
TMCa	tubular maximum reabsorption of calcium
TMP	tubular maximum reabsorption of phosphate
TRH	thyrotrophin-releasing hormone (protirelin)
TRP	tubular reabsorption of phosphate
TSH	thyroid-stimulating hormone
TSI	thyroid-stimulating immunoglobulin
T_3U	T_3 resin uptake
VMA	vanillylmandelic acid

FOREWORD

Professor **R. Hall**
Professor of Medicine
University of Wales College of Medicine

I am very pleased to write an introduction to this first edition of a *Handbook of Endocrine Tests in Children.* Dr I. A. Hughes is well qualified to produce this book. He graduated at the Welsh National School of Medicine and furthered his endocrine training both in this country and in North America. He gained particular insight into the field of steroid biochemistry during his studies at the Tenovus Institute. He is an acknowledged expert in the field of neonatal endocrinology, particularly in the diagnosis and management of disorders of sexual differentiation.

The subject of endocrine tests is complex and, in some areas, controversial. As new hormones are identified new possibilities in testing emerge. For example, with the availability of growth hormone-releasing factor and corticotrophin-releasing factor we now have the ability to test GH and ACTH release directly. Most new tests are established in adults and their application to children needs close liaison between the paediatrician and the adult endocrinologists. In Cardiff, at the University of Wales College of Medicine, close links exist between the author and a large group of basic and clinical endocrinologists including Dr J. Picton Thomas (adrenal); Dr M. F. Scanlon (neuro-endocrinology), Professor R. Hall, Dr J. Lazarus and Dr A. McGregor (thyroid), Dr S. Woodhead (parathyroid and calcium), and Professor K. Griffiths (steroid biochemistry). It is from these frequent contacts that Dr Hughes' endocrine approach has developed and matured.

The introductory chapter on general principles of endocrine testing in children is invaluable, drawing attention to the precautions that must be taken at all stages. The chapters classified according to the major endocrine glands are a model of clarity, with useful advice on the interpretation of the tests. At the end of each chapter illustrative case reports are provided which highlight the application of the tests.

There is no doubt that this text will prove invaluable to all paediatricians called upon to investigate children with endocrine disorders. It will also be helpful to the adult endocrinologist who, in the absence of a paediatric endocrinologist, is frequently involved in the assessment of children. With the amount and rate of expansion of endocrinology, I am sure that a second edition of this excellent Handbook will not be long delayed.

CONTENTS

CHAPTER 1

General Principles of Endocrine Tests

Tests of endocrine function (*see Fig.* 1.1) in children usually require serial blood samples and/or 24-h urine collections following the administration of agents which stimulate or suppress hormone levels. The procedures can be unpleasant and may produce side-effects. Appropriate testing should be performed only after detailed history and physical examination has suggested the presence of a specific endocrine disorder. For example, a short child should be investigated for growth hormone (GH) deficiency only after the clinician has been satisfied that the cause is not due to or associated with low birth weight, parental size, adverse social factors, general systemic disease etc. In this particular example, an accurate record of decreased growth velocity determined preferably over one year should be available before arranging the tests.

RANDOM OR DYNAMIC TESTS

In general, random blood samples for hormone measurements in children are unhelpful. This is in contrast to some adult endocrine disorders, such as acromegaly, hyperprolactinaemia and hyperparathyroidism where random GH, prolactin (PRL) and PTH measurements, respectively, are often diagnostic. Since dynamic endocrine tests in infants and children can be technically difficult, it is imperative that no mistakes are made with patient preparation, sample times and volumes, labelling of tubes and request forms, and transporting samples to the appropriate laboratories. If not, the interpretation of results is made difficult and the child may be subjected unnecessarily to a further unpleasant procedure.

PREPARING THE CHILD

The child should be fasted for most endocrine tests. If the child lives locally, the test can be performed as an out-patient. The older child

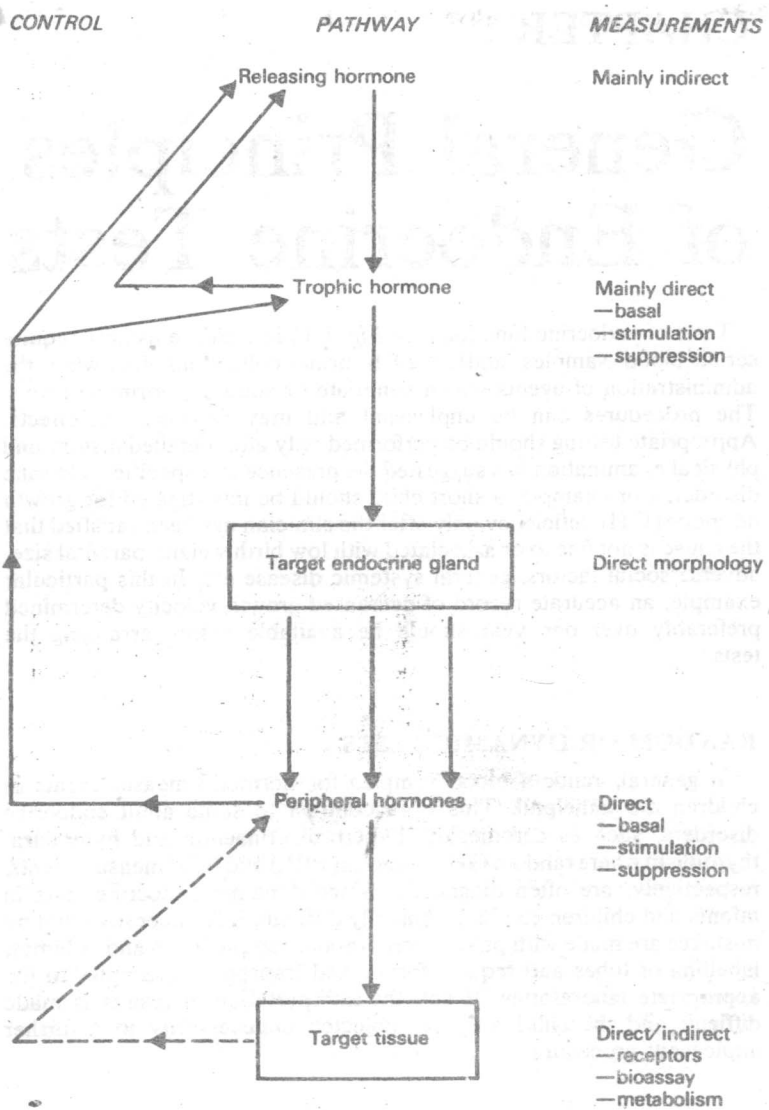

Fig. 1.1. Generalized scheme of hormone action and measurement.

should be fasted (nothing to eat or drink except water) from midnight; this assumes the test will start *no later than* 08:00–09:00. It is unkind, unnecessary and occasionally dangerous to fast a child longer than this. The duration of fasting for infants should not exceed 4–6 hours. If the child is unwell, or is only recently recovering from a systemic illness, the test should be postponed. Ensure that the child has not been receiving any medication or foodstuffs which may interfere with the interpretation of results. Examples include anticonvulsants and thyroid function, iodine-containing compounds or foods (e.g. fish) and radioactive iodine uptake, bananas and urine VMA etc.

COLLECTION OF BLOOD SAMPLES

A reliable intravenous line (i.v.) should be positioned in a large forearm or antecubital vein when sampling blood serially. A 21- or preferably 19-gauge butterfly intermittent infusion set with reseal injection site as an alternative to connecting the i.v. line to a 3-way stopcock allows greater mobility for the child. The infusion line must be maintained patent; this can be achieved using a 20 units/ml solution of heparin–saline (mix 0·2 ml of 1000 units/ml heparin solution with 20 ml saline). Another alternative often used is to connect the i.v. line to a slowly running infusion of *saline* (NB dextrose–saline solution will affect the hormone measurements). A small volume of blood is withdrawn and discarded before the required venous sample is collected. The infusion set should be adequately 'flushed' through after each sample withdrawal. Careful attention to these details should ensure that the i.v. line functions satisfactorily for the duration of the test.

Baseline samples must be collected before stimulation and suppression tests are started. Thus, this sample is labelled, $t = 0$ min; if possible an earlier sample $t = -30$ min should also be collected in an attempt to reduce the effect of stress on hormone levels, viz. GH and cortisol. Collect an adequate volume of blood (check with the laboratory) at the required times for each hormone to be measured. Plain (clotted) or heparinized tubes are used depending on whether serum or plasma is the matrix for hormone determination. More than one hormone measurement will usually be determined in each sample. It is advisable to liaise with the laboratory staff who normally prefer to aliquot the sample for the different hormone assays *after* the plasma/serum has been separated following centrifugation.

COLLECTION OF URINE SAMPLES

A 24-h urine sample is required for most urinary hormone measurements. Sometimes a fractionated collection, i.e. in 8-h or 12-h aliquots,

is required as in the assessment of urine glucose output in diabetic patients. Make certain that the parents or child (if old enough) and *the medical attendants* understand how to collect a 24-h urine sample. The bladder should be emptied and the *time recorded.* This is the *start* of the collection. Each urine voided thereafter for the next 24 hours is collected into a suitable container. At the end of the collection period, the time is recorded on the container. This time signals the start of the next 24-h collection when sequential samples are required. Most urinary hormone measurements do not require the sample to be collected on ice or with a preservative (boric acid or hydrochloric acid) in the container.

Accurate 24-h urine collections in infants and young children are difficult to achieve. Adhesive plastic bags applied around the external genitalia are most commonly used with varying success. Centres where 24-h urine collections are frequently performed may need to design a metabolic crib.[1–3] The creatinine concentration of the urine sample should be determined routinely in order to check the completeness of the 24-h collection.

COLLECTION OF SALIVA SAMPLES

This is currently not a standard procedure in clinical investigation. However some endocrine disorders can be investigated by measurement of steroid concentrations in saliva. The advantage of saliva collection over blood or urine sampling is the facility to collect an unlimited number of serial samples by a non-invasive method. The technique is particularly applicable to children. Before sample collection, the mouth should be rinsed with tapwater to remove any food debris. After waiting 1–2 min, whole saliva is collected by gently dribbling into a wide-neck plain tube. Saliva flow can be stimulated by using a drop of citric acid syrup on the tip of the tongue, although this is seldom required.

ABELLING AND PROCESSING OF SAMPLES

This is the time when most mistakes occur. The child's name, hospital umber, ward and date of test must be recorded on each sample tube. For namic tests (i.e. stimulation and suppression), the time must be *clearly* corded (i.e. $t = 0$, 15, 30, 60, 90, 120 min etc.). Laboratory request rms must be completed in full, again with the emphasis on name, date d times when samples were collected.

Most laboratories require a separate request form for each hormone termination, particularly if more than one laboratory is involved in the rmone assays. This may create a large amount of paperwork, so it is se to spend time completing the request forms before starting the test.

Pertinent clinical details to be recorded include the child's age, sex and likely diagnosis. The information can help the laboratory staff who may wish to modify the hormone assay in order to obtain maximum information of value to the clinician.

When the test is finished, samples must be transported to the appropriate laboratory without delay. If necessary the investigator should transport the samples personally. This is another link in the chain which can go wrong, particularly in large hospitals. Sometimes blood samples need to be collected on ice, and the plasma/serum prepared immediately using a refrigerated centrifuge, e.g. plasma renin, ACTH. Close liaison with the laboratory staff is essential to ensure satisfactory processing of samples. It is usually the responsibility of the laboratory staff to make arrangements to transport plasma/serum/urine samples to the laboratories in other centres if specialized assays are indicated.

The same precautions regarding labelling applies to 24-h urine samples. Either the total volume can be recorded on the ward (accurately) and an aliquot (50–100 ml) sent to the laboratory or the entire volume transported to the laboratory. The request form should contain details of the time and date for the start/finish of the collection, in addition to relevant clinical information.

HORMONE ASSAYS

It is beyond the scope of this Handbook to discuss the methods of hormone measurements in plasma and urine. Nevertheless, the clinician should have some understanding of the principles involved and limitations imposed on accuracy, reproducibility and speed of 'turn around' in producing results.

Most protein and steroid hormone concentrations, particularly in blood, are currently determined by immunoassay procedures. The procedure involves an analysis of the competition in binding between the hormone in plasma (unknown quantity) and a known quantity of enzyme or radiolabelled hormone, and an antibody specifically directed against the hormone in question. Using a known set of standard concentrations of the hormone, the unknown concentrations of hormone in plasma can be derived from a displacement curve. In some assays, the antibody is radiolabelled. There are several variables in the assay procedure, so that the precision does not always compare favourably with other routine analyses in clinical chemistry. Laboratories who regularly perform immunoassays for hormone measurements participate in both internal and external quality control schemes. If there is 15 per cent variance or more in a result of a sample analysed twice in two separate assays, then it becomes difficult to interpret the absolute value of a single estimation. For example if GH deficiency is defined as a peak serum GH level

< 20 mU/l following stimulation, does a value of 18·6, which when repeated is 21·4, constitute GH deficiency? Obviously not. The entire GH profile obtained during a stimulation test must be considered in addition to the clinical findings. In general, results of a laboratory test serve to confirm or refute a diagnosis suggested by clinical examination. Hormone assays are no exception.

NORMAL RANGES FOR HORMONE CONCENTRATIONS

Each laboratory should determine its own range of normal values for hormone concentrations. However, this either may not be available for, or not possible to obtain in, children and young infants. The Appendix contains some data of normal values obtained from the literature; each test protocol is followed by a note on the interpretation of results.

The contents of this chapter may appear pedantic to the reader. However, it cannot be overemphasized that attention must be paid to the following check list when performing endocrine tests in children.

PATIENT PREPARATION

Is there an indication to perform the appropriate endocrine test based on detailed clinical evaluation?

Is the child adequately prepared?—Fasting
—Clinically well
—Any interfering foodstuffs or medications.

Has the height and weight of the child been measured on the day of the test?

Has the dose of the stimulatory/suppressive agent been calculated correctly? (NB. INSULIN DOSE for insulin tolerance test.) IF IN DOUBT, *ASK*.

SAMPLE COLLECTION

Blood

Secure a reliable i.v. line for serial blood sampling
Collect basal samples ($t = -30$, 0 min)
Ensure correct sample tubes—Plain (clotted)
—Heparinized.

Urine

Make sure child/parent and *you* understand how to collect a 24-h urine sample. Is there a suitable container?
Does it require a preservative?

SAMPLE PROCESSING

Blood tubes/urine containers to be labelled with:
- Name of patient
- Ward/clinic
- Hospital number
- Date of sample
- Time of sample (start and finish for 24-h urine)
- Volume of 24-h urine sample.

Laboratory request forms:
- Write LEGIBLY
- Name of patient
- Age
- Sex
- Ward/clinic
- Hospital number
- Date of test
- Time of sample (start and finish of 24-h urine)
- Test required
- Clinical details of relevance.

Transporting samples:
- Ensure rapid transport to appropriate laboratory
- If necessary contact laboratory staff initially
- If in doubt, transport sample PERSONALLY.

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CHAPTER 2

The Pituitary

The anterior pituitary gland secretes several hormones—GH, TSH, ACTH, PRL, LH and FSH—whose synthesis and secretion are controlled by hypothalamic releasing or inhibiting factors. Some of these factors have been isolated, characterized and synthesised and are used in the evaluation of anterior pituitary function. They include TRH and LHRH. Indirect methods of stimulation are currently required to test the secretory reserve of GH and ACTH. However, releasing factors for these two hormones, GHRH and CRF, respectively, have recently been isolated and characterized. Their use in diagnostic tests is now being evaluation.

ADH is synthesized in the hypothalamus, transported along axons in the neurohypophyseal tract and stored in the posterior pituitary gland. Secretion from the posterior pituitary is regulated mainly by changes in osmolality of the extracellular fluid. ADH release is assessed by indirect methods.

GROWTH HORMONE

Tests of GH secretion are classified into physiological and pharmacological. The former takes advantage of some normal physiological variables such as exercise and sleep where GH secretion is enhanced.

STIMULATION

Physiological

Random

Occasionally a random blood sample will detect an elevated GH level, particularly if the child is stressed or is fasted.

Such a result would exclude GH deficiency. However, the test is so unreliable that it is not recommended.

Exercise

This test should be performed using a bicycle ergometer to generate a standard amount of work which will vary according to the age and size of the child.

One should aim for a physical exertion between 150 and 300 kilopond. m/min equivalent to about 50 per cent maximal working capacity.

- Child fasted
- Blood sample at $t = -30$ and 0 min
- Exercise and bicycle for 10 min ($t = 10$ min)
- Collect blood samples at $t = 10$ and 20 min.

If a bicycle ergometer is not available, the following exercise test is sometimes useful to perform:

- Child fasted
- Blood sample at $t = 0$ min
- Run up and down stairs for 20 min
- Child should be tired, but not exhausted; heart rate should not exceed 180/min
- Blood sample at end of exercise ($t = 20$ min)
- Rest for 20 min; repeat blood sample ($t = 40$ min).

Interpretation: A GH level > 15 mU/l excludes GH deficiency. Lower values do not necessarily indicate GH deficiency. If necessary proceed to pharmacological tests. About 20 per cent of exercise-induced tests give false positive results for GH deficiency. Some protocols suggest priming the child with propanolol orally 0·5 mg/kg (maximum dose 40 mg) 1 hour before the test to enhance the GH response. *This carries a risk of hypoglycaemia.*

Sleep

Ideally this test should be performed with EEG monitoring if available.

- Insert i.v. line before bedtime
- Collect 2 blood samples 20 min apart while child awake
- Collect 2 blood samples 20 min apart between stages III and IV
- If no EEG recording, collect 2 samples 20 min apart between 30 and 90 min after onset of sleep.

Interpretation: GH levels during waking hours usually low; the rise in nocturnal levels occurs during stage III and IV sleep equivalent to slow wave sleep. Values > 15 mU/l exclude GH deficiency.