# CHAPTER I

## INTRODUCTION

At present the pharmacologic dose of synthetic glucocorticoids are widely used in Thailand both correctly and incorrectly. This may result in pseudoadrenocortical hyperfunction during administration which is usually followed by adrenocortical insufficiency for certain period of time after discontinuation of the steroid hormones.

The greatest practical importance of pituitary-adrenocortical hypofunction in patients undergoing long term steroid therapy is that their responsiveness to various kinds of stress is disturbed. During surgery plasma cortisol levels were markedly increased. (Franksson et al., 1954) The same situations were seen in several stressful conditions such as high fever, infectious diseases, crush injury and psychic trauma. Many serious consequences of iatrogenic adrenocortical insufficiency have been reported secondarily to corticosteroid therapy, including death-following surgical interventions or other stressful situations. (Salassa et al., 1953; Allanby 1957; Slanley and Brooke, 1957)

The term "glucocorticoid" has been applied to steroids that have distinct effects on carbohydrate metabolism, for examples; gluconeogenesis, increased liver glycogen deposition, and elevation of blood glucose. Of the naturally synthesized steroids, only

cortisol, cortisone, corticosterone, and 11-deoxycorticosterone have glucocorticoid activity. Among these, cortisol, the major adrenocortical steroid hormone is the most important one.

A great number of synthetic corticosteroid analogues have been synthesized and widely used in different purposes. These include prednisolone, prednisone, dexamethasone, betamethasone, triamcinolone, paramethasone, and many more. Their therapeutic values and toxic effects are different from one to another, however, all can suppress pituitary-adrenocortical function.(Kupperman et al., 1955)

Prednisolone or $\Delta^1$- cortisol and prednisone or $\Delta^1$ cortisone, were first introduced by Herzog et al. (1955) Both have similar physiological activity. Their anti-inflammatory effect is approximately five times greater than cortisol but they have less tendency to cause sodium retention, or mineralocorticoid activity.(Bunim et al., 1955; Hart et al.,1955)

Dexamethasone was introduced by Arth et al., (1958) . Its anti-inflammatory effect is twenty-five times greater than that of cortisol and it has no sodium retaining activity.(Boland ,1958)

Betamethasone prepared by Taub et al. (1958), has the same potency and properties as its isomer, dexamethasone.(Glyn and Fox,1961)

Triamcinolone synthesized by Bernstein et al. (1956), has the same anti-inflammatory action as prednisone but no sodium retaining effect.(Black et al., 1957) Systemic use of this steroid is not

recommended because of its tendency to produce myopathy. (Freyberg et al., 1958)

Paramethasone introduced by Edwards et al (1960), has about twelve times the anti-inflammatory effect of cortisol but no effect on sodium metabolism. (Irwin et al., 1961)

Pharmacologic effects of glucocorticoid

The anti-inflammatory effect The mechanism by which glucocorticoid blocks the inflammatory action is unknown. When an inflammation occurs, the administration of large doses of glucocorticoid results in : decreased vascular permeability at the injured area, cellular exudate is reduced, less edema occur and less fibrin is deposited, reduced "sticking" of polymorphonuclear leucocytes to the inflammed area, healing is delayed.

Hematologic effect Glucocorticoids cause destruction of the lymphocyte in the blood circulation, lymphoid tissue and thymus. The red blood cells tend to increase with pharmacologic dose of glucocorticoid. Platelet counts are also increased. Excess glucocorticoids raise the circulatory neutrophils. This is largely due to their reduction from the blood stream into the sites of inflammation. Cortisol and cortisone depressed the circulating eosinophils by increasing the sequestration of eosinophils in the lungs and spleen as well as increased their destruction in the blood circulation but have no effect on the marrow eosinophils.

Effects on bone metabolism Glucocorticoid blocks the new bone formation at the level of the matrix. This leads to decreased Ca deposition. The osteoblasts are also reduced. Glucocorticoid antagonizes the action of vitamin D leading to decreased Ca absorption in the gut and increased urinary loss of Ca. It also inhibits the GH release. It enhances every phase of osteoporosis.

GI effects Glucocorticoid increases gastric acidity. Peptic ulcer, hemorrhage and perforation may occur after large doses of glucocorticoids.

Effects on CNS The threshold for electrical excitation of the brain is lowered by glucocorticoid. Psychiatric disturbances are common both with cortisol deficiency or excess. Cerebral edema is somewhat reduced by pharmacologic dose of glucocorticoid.

Effects on CVS Glucocorticoid sensitizes the arterioles to the pressor effect of norepinephrine and related drugs. Excess glucocorticoid causes a rise in circulating blood lipids and cholesterol. This may lead to a tendency of atherosclerosis. Excessive glucocorticoid may increase blood pressure to some extent. In absence of adrenocortical hormones, the circulating blood volume cannot be maintained.

Effects on striated muscle In the absence of cortisol muscle weakness occurs. In excess, glucocorticoid leads to depletion of muscle protein, edema and fibrosis, resulting in muscular weakness.

Effects on gluconeogenesis Glucocorticoid stimulates

protein and increase conversion of amino acids to glucose. Its excess leads to hyperglycemia and glycosuria. Muscle mass is reduced but liver glycogen is increased by the pharmacologic doses of glucocorticoid.

Immunologic effects Large doses of glucocorticoid reduce production of new circulating antibodies by lysing fixed plasma cells and lymphocytes. The level of circulating antibodies which has already been present in the circulating blood is not altered by glucocorticoid. It does not alter the ability of antibody to combine with antigen but it is able to prevent the inflammatory reaction caused by antigen-antibody interaction.

Pharmacologic doses of glucocorticoids had great benefits in the treatment of varieties of diseases. Among these, are nephrotic syndrome, rheumatic carditis, shock following gram negative sepsis, aplastic anemia, autoimmune diseases and many others. With different individual response a great varieties of doses, duration, types of preparation, technique of administration and individual medical judgement had been applied depending on the diseases. The occurrence and severity of adreno-cortical suppression are different in patients treated with pharmacologic doses of glucocorticoid. Many questions are still uncertain in the medical practice, such as the followings :

1. severity of complications if any
2. recovery period after cessation of steroid therapy.
3. other undue effects during or following therapy.

Is there any valid method to

1. determine whether the adrenocortical insufficiency existed ?
2. measure the severity of adreno-cortical suppression?

How to prevent or lessen the undue effects and predict the possible complications ? How long it takes for each patient to recover from adrenocortical suppression after cessation of steroid therapy ? Nephrotic syndrome, rheumatic heart disease and rheumatoid arthritis are the diseases which pharmacologic doses of glucocorticoid treatment have a great benefit. It is good example for the study to evaluate adrenocortical functions of these patients.

Metabolism of the adrenal steroids

The major site of corticosteroid metabolism is the liver. The steroid skeleton is not damaged during this process. A series of enzymes found in the liver which are capable of altering the steroid molecules make them both biologically inactive and water soluble. There are two main steps involved in this process, firstly, the reduction and the removal of the side chain of the steroid molecule, and secondly, conjugation of the molecule with glucuronic acid or sulfate to form water soluble glucuronide or sulfate. The conjugates are poorly bound to plasma proteins so they are excreted rapidly through the kidney by simple glomerular filtration.

# Metabolism of Cortisol

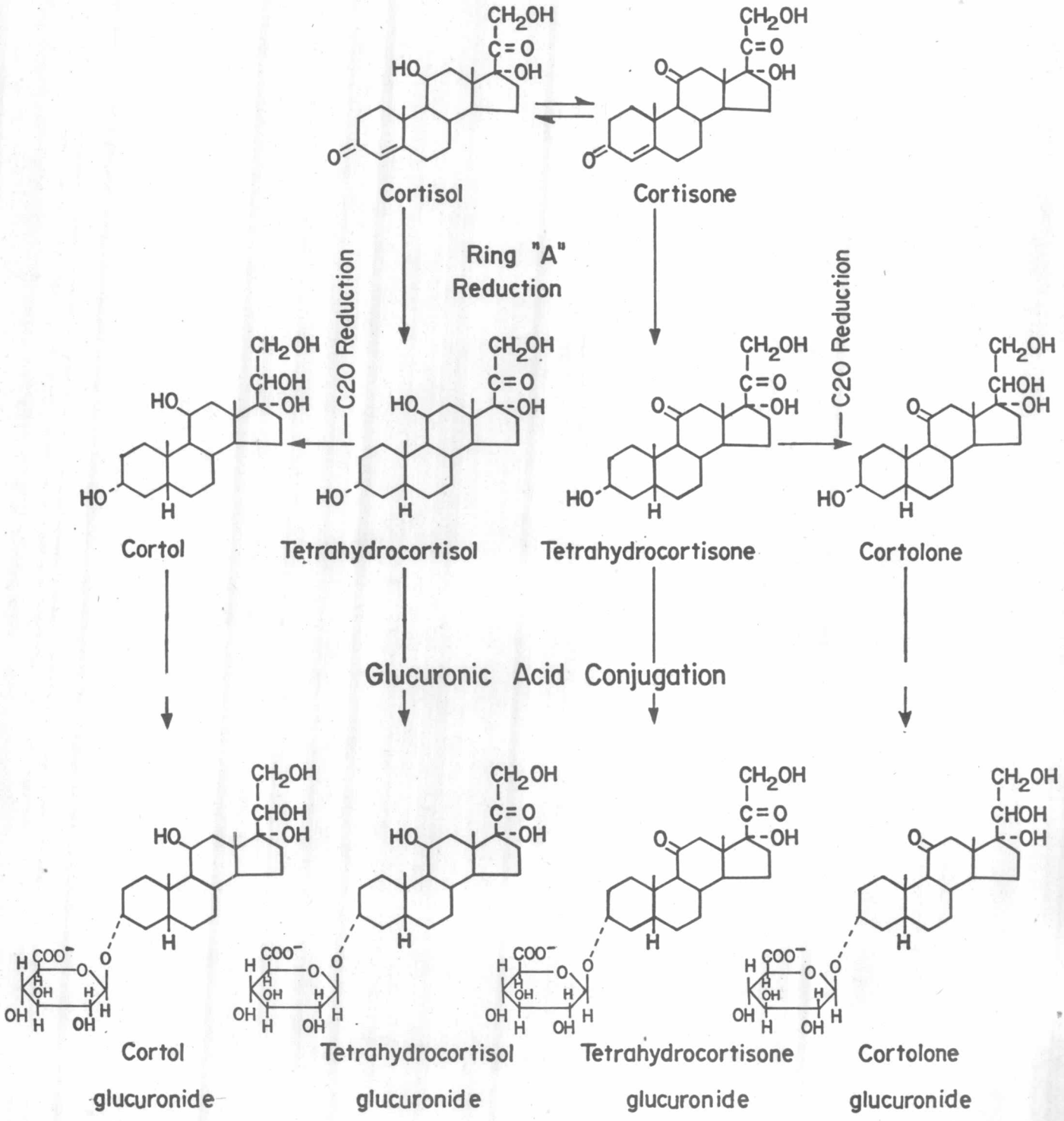



Fig. I Urinary Metabolites of Cortisol

This figure was taken from Khoprasert (1979)

The principal urinary metabolites of cortisol are tetrahydrocortisol glucuronide and tetrahydrocortisone glucuronide, appear in quantities equivalent to about 30 per cent of the cortisol secretory rate. These glucuronides are so water-soluble that they escape extraction with the organic solvents. Hydrolytic cleavage of the glucuronides with glucuronidase releases the free steroids, which can then be extracted with organic solvents and quantified as 17-OHCS by the Porter-Silber method. (Porter and Silber, 1950 ) Urinary 17-OHCS as determined in this way provide an extremely useful index of cortisol secretion.

# Metabolism of Adrenal Androgens

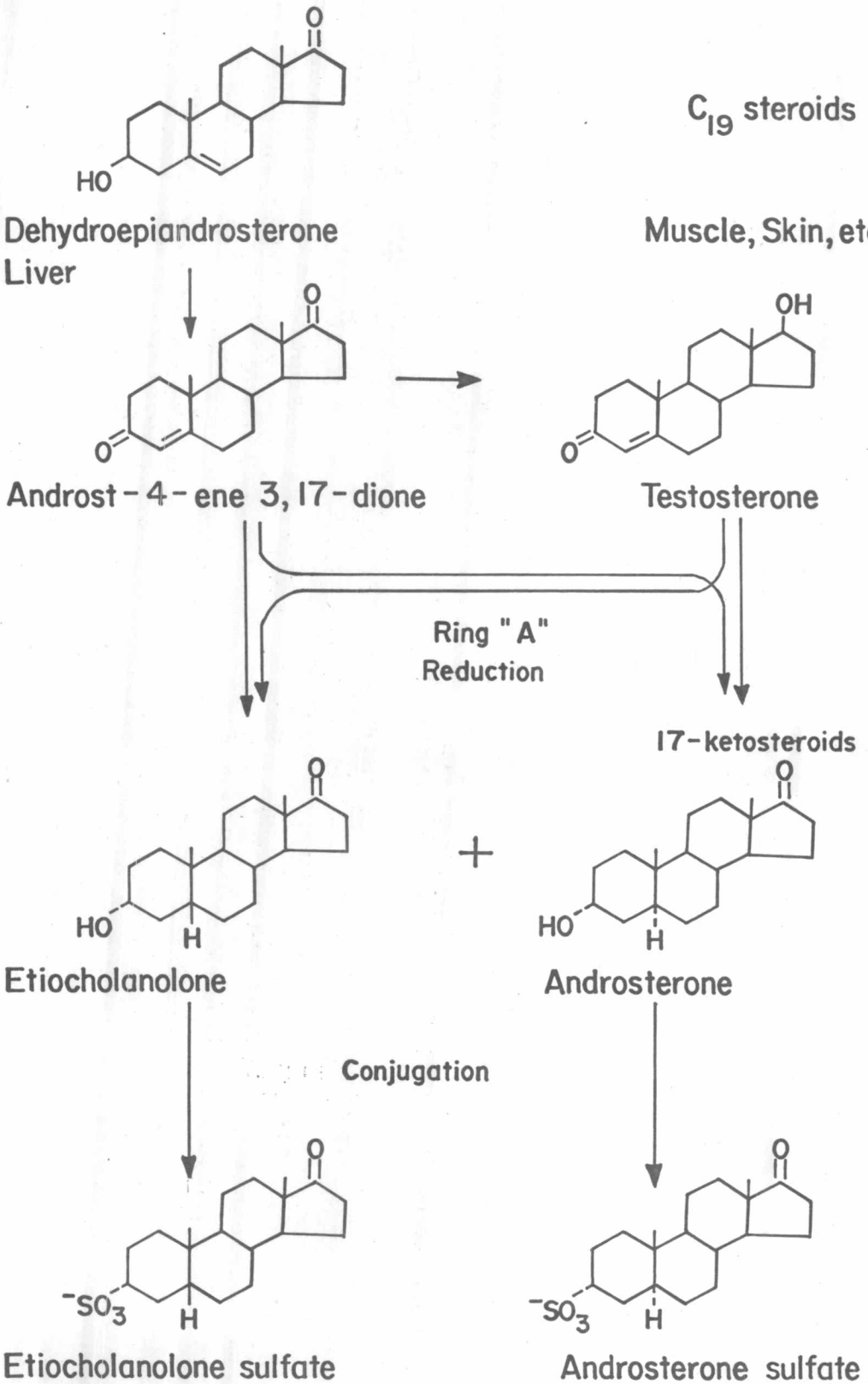



Fig. 2 Major Urinary Metabolites of Adrenal Androgens

This figure was taken from Khoprasert (1979)

The most abundant urinary 17-ketosteroids are androsterone and etiocholanolone, which appear in the urine principally as water-soluble conjugates (largely sulfates). The main precursor of the urinary 17-ketosteroids is dehydroepiandrosterone. The steroid sulfates are hydrolyzed by exposure to acid. The steroids can then extracted with an organic solvent such as chloroform and estimated colorimetrically by the metadinitrobenzene "Zimmermann" reaction. (Zimmermann, 1935)

Hypothalamo-pituitary-adrenal axis

The production of cortisol by the adrenal cortex is controlled by an elaborate mechanism. Synthesis and secretion of cortisol occur only when the adrenal is stimulated by ACTH from the anterior pituitary. ACTH is produced by the anterior pituitary under the stimulus of another hormone, corticotrophin-releasing factor (CRF) or corticoliberin from the hypothalamus. Hypothalamus, anterior pituitary and adrenal cortex thus form a hormonally linked functional unit known as the hypothalamo-pituitary-adrenal axis (HPA axis).

Control of adrenocortical secretion

The degree of activity of the hypothalamo-pituitary-adrenal axis has three main physiological determinants:

1. A circadian rhythm
2. A negative feed-back mechanism
3. Responsiveness to stress

1. Circadian rhythm The HPA axis shows a regular cycle of activity with a 24-hr periodicity. Such a rhythm is also known as

'nychthemeral'. The level of circulating cortisol reaches a peak between 6.00 a.m. and 8.00 a.m. and a trough at about midnight. The normal plasma cortisol at 8.00 a.m. is from 5 to 25 µg/dl and at midnight from 0 to 10 µg/dl. The concentration of cortisol in the plasma at any given moment is a function of its rate of secretion by the adrenal, the rate of its metabolic removal, and the level of corticosteroid-binding globulin (CBG). The rhythm is altered or lost in certain diseases, notably Cushing's syndrome and may occur in a number of pathological conditions, particularly those accompanied by severe stress, or by alterations in consciousness or affect. ( Ceresa et al., 1970; Fukushima et al., 1970; Krieger et al., 1971; Orth and Island,1968; Perkoff et al., 1959; Takahashi et al., 1968; Weitzman et al., 1971)

2. Negative feed-back mechanism The level of the plasma cortisol is believed to exert an effect on the secretion of ACTH in such a way that an increase in the former causes a decrease in secretion of the latter, and conversely a fall in plasma cortisol leads to increased secretion of ACTH. A mechanism such as this, whereby a change in the level of the hormone from a target gland produces an opposite change in the secretion of the corresponding trophic hormone from the anterior pituitary is a good example of a negative feed-back mechanism.

The hypothalamic centers controlling the feed-back mechanism are sensitive to exogenously administered glucocorticoids as well as to those secreted by the subject's own adrenal. Synthetic

corticosteroids, such as prednisolone, are many times more potent, weight for weight, in inhibiting HPA function than cortisol itself. The sensitivity of the negative feed-back mechanism to exogenously administered corticosteroids is subject to circadian variation.

3. Responsiveness to stress (Doar et al., 1970; Greenwood and Landon, 1966; Ontjes and Ney, 1972) One of the most striking attributes of the HPA axis is its ability to respond to stress by greatly increased activity. Stress is a term which is used to describe a very wide variety of stimuli. These include emotional states, such as excitement or fear, neurological stimuli such as pain or injury, and altered metabolic states, such as pyrexia or hypoglycemia. The brisk rise of plasma cortisol, sometimes to several times the basal level, which follows any of these stimuli is perhaps the most extensively documented of all aspects of HPA function. Nevertheless the stress response may be vital, and patients with adrenal hypofunction who are perfectly well under relatively basal conditions may suffer circulatory collapse if subjected to some intercurrent stress. Thus the HPA axis in its ability to respond to stress plays a life-supporting role, and the care of patients with HPA failure demands the provision of adequate substitution therapy during periods of severe stress.

The adrenal response to stress is mediated via the hypothalamus and anterior pituitary, but apparently the hypothalamic pathways controlling it are different from those controlling the circadian rhythm and feed-back mechanisms. The response to stress

will normally over-ride both of these, and the activity of the healthy HPA axis may increase at any time during the 24 hr even in the presence of high circulating levels of corticosteroids.

Effect of corticosteroids on the hypothalamo-pituitary adrenal axis

One of the principal physiological determinants of adrenocortical activity is the negative feed-back mechanism. As a consequence of this mechanism any significant rise in the level of circulating cortisol results in prompt cessation of ACTH secretion and synthetic glucocorticoids produce a similar effect. Administration of corticosteroids will thus inhibit ACTH secretion by the pituitary and this in turn results in cessation of synthesis and secretion of corticosteroids by the adrenal. This is an invariable response to corticosteroid administration in all subjects with a normal HPA axis. The hypothalamus and possibly the pituitary also, appear to be to sites at which corticosteroid-induced inhibition of HPA function occurs. (Yates et al., 1971) There appears to be no direct acute effect of corticosteroids on the sensitivity of the adrenal to ACTH.(Landon et al., 1965) The duration of the inhibition of HPA function depends on the steroid preparation used, the size of the administered dose, and the time of its administration.

The synthetic glucocorticoids most commonly used therapeutically are more potent inhibitors of HPA function than cortisol or cortisone. There is a direct correlation between the anti-inflammatory potency of a steroid preparation and the degree of

HPA inhibition which it produces. Thus steroids such as betamethasone and dexamethasone are the most powerful inhibitors, with prednisolone and prednisone intermediate between these and cortisol.

There is a marked variation throughout the 24 hr in the degree of HPA inhibition caused by a given dose of a corticosteroid. Administration in the morning, at about the time of the circadian peak of HPA activity, results in minimal inhibition, whereas maximum inhibition is seen when the corticosteroid is given at about midnight when HPA secretion is normally minimal. (Ceresa et al., 1970)

Assessment of hypothalamo-pituitary -adrenal function

Dynamic tests of HPA function

1. ACTH stimulation tests It is frequently of value to be able to assess the degree of responsiveness of adrenal cortex to ACTH. There are many other forms of ACTH stimulation test, involving intravenous infusion of ACTH for periods of from one to 48 hr, twice daily injections of ACTH gel, or a single injection of Zn-tetraco-sactrin. Plasma corticosteroids or urinary 17-OHCS may be determined for assessment of the response.

2. The metyrapone test Metyrapone is an amphenone derivative, related chemically to DDT which inhibits the 11 β-hydroxylation of 11-desoxycortisol (substance S) to cortisol. (Eberlein and Bongiovanni, 1956) Its administration therefore results in a fall in the plasma cortisol level and consequently promotes the secretion

of ACTH. This in turn causes increased production of steroids by the adrenal cortex, but the final step of cortisol synthesis is prevented by the blocked 11 β-hydroxylase. Substance S therefore accumulates as the secretion of ACTH rises still further. The test thus examines the ability of the pituitary to respond to a reduced plasma cortisol by increased output of ACTH. The response is usually measured indirectly by estimating the 17-OHCS in urine and is thus only a valid test of pituitary function when it has been demonstrated that the adrenal can respond to ACTH. The raised plasma level of substance S results in increased urinary tetrahydro-s; which is a 17-OHCS. This explains the apparently paradoxical situation of diminished plasma cortisol leading to increased 17-OHCS excretion. The situation is analogous to that occurring naturally in the less common form of congenital adrenal hyperplasia due to 11 β-hydroxylase deficiency. The metyrapone test is probably of considerable value in the differential diagnosis of Cushing's syndrome. (Bruno et al., 1971; Nattrass et al., 1972)

Measurement of hormone and their metabolites in plasma and urine

Because of the difficulty in measuring ACTH levels few direct studies of its concentration in the blood during corticosteroid treatment have been made. Therefore, in the assessment of hypothalamo-pituitary function reliance has been placed on tests which measure plasma or urinary corticosteroids as their end-point.

1. Measurement of plasma cortisol The corticoids are present in several forms in blood: they occur as unconjugated steroids or

as conjugated glucuronides and sulfates. The unconjugated corticoids, which are those usually measured, comprise a fraction bound to serum proteins (Corticosteroid-binding globulin (CBG, transcortin) and albumin) and an unbound.

1.1 Competitive protein-binding method. This type of analysis was first used in 1957 by Berson and Yalow for the measurement of insulin. The procedure was first applied to the determination of corticoids in 1963 (Murphy et al.) It consists of two parts: first, deproteinization of the plasma (to remove or destroy the CBG present), and secondly, quantitation of the corticosteroid in the deproteinized plasma by equilibrating it with a solution containing CBG just saturated with radioactive cortisol, then separating and counting the protein-bound fraction. The unlabeled cortisol competes with the tracer cortisol for the binding-sites on the CBG molecules, displacing some of them, so that the amount of radiocortisol in the protein-bound fraction is inversely proportional to the amount of unlabeled cortisol present in the sample.

1.2 Fluorometric method. This method measures only 11-hydroxylated corticoids and, thus, is more specific for cortisol and corticosterone. However, nonspecific interference by other compounds such as spironolactone, quinidine, quinine, tetracycline, heparin and bilirubin does occur.

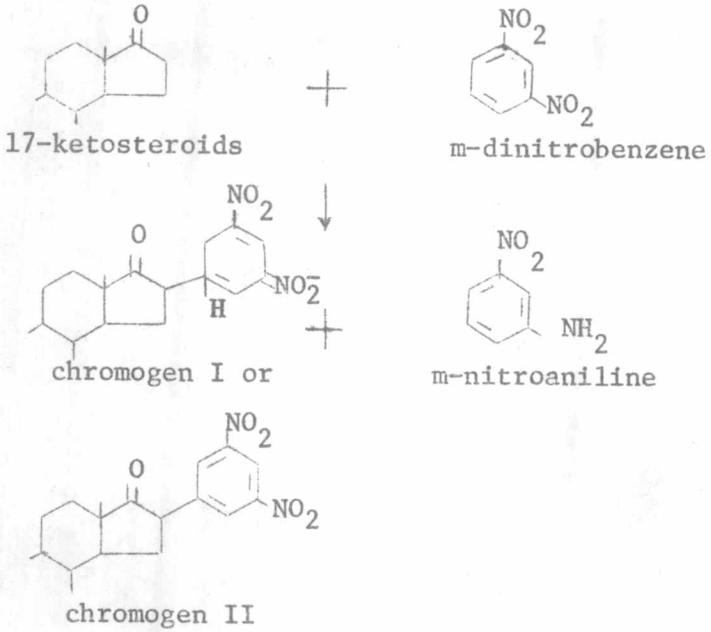
1.3 Cortisol by Radioimmunoassay.

2. Measurement of metabolites in urine

2.1 Urinary 17-hydroxycorticosteroids. Measurement of the 17-OHCS

(Porter-Silber chromogens) depends on the reaction of the "dihydroxyacetone" C-17 side chain from a urine extract with phenylhydrazine in sulfuric acid. This gives a distinctive yellow color reaction.

2.2 Urinary 17-ketosteroids. Zimmermann first described the reaction of metadinitrobenzene with steroids having a ketone group in position 17. The wavelength of maximum absorption of the chromogen is 520 nm. The Zimmermann color is known to be unstable. King and Newal (1962) have shown that protonation of the chromogen by acids afford a colorless compound from which the chromogen can be regenerated with alkali.



The purpose of the present study is to find out the long term effects of glucocorticoids, particularly prednisolone, on the levels of plasma cortisol and its urinary metabolites and evaluate adrenocortical functions of the patients with nephrotic syndrome , rheumatic heart disease and rheumatoid arthritis, who had been treated with pharmacologic dose of this steroid hormone for a length of time.