CONGENITAL ADRENAL HYPERPLASIA

The clinical problem, and the application of a new method of chromatography of the 17 ketogenic steroids to the diagnosis and the control of treatment of congenital adrenal hyperplasia.
## CONGENITAL ADRENAL HYPERPLASIA

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INTRODUCTION

Congenital adrenal hyperplasia is a genetically determined inborn error of metabolism. The fundamental lesion is now established as a partial enzymatic block in the biosynthesis of cortisol by the adrenal cortex. The inadequate synthesis of cortisol results in an increased secretion of corticotrophin and adrenocortical hypertrophy in an effort to overcome the deficiency. The hypertrophied adrenal produces an excess of androgens. The clinical picture is therefore one of a variable degree of adrenocortical insufficiency and virilization.

The deficiency of cortisol is readily corrected and the excess secretion of corticotrophin suppressed, by the administration of the synthetic corticoids. Congenital adrenal hyperplasia has become one of the important metabolic defects which are now amenable to treatment.

The ultimate aim of normal physical development however, depends primarily on early diagnosis, and in the young child the diagnosis can be difficult; and secondly on adequate suppressive therapy with corticoids.
The care of children with congenital adrenal hyperplasia covers a wide field including the treatment of the acute adrenal crises, the control of physical development, genetic and psychological counselling, and an understanding of the metabolism of the adrenal steroids.

My thesis deals with the clinical problems, the metabolic defect and with the application of a new method of chromatography of the $^{17}$ketogenic steroids to the diagnosis and the control of treatment of children with congenital adrenal hyperplasia.

**INCIDENCE OF CONGENITAL ADRENAL HYPERPLASIA**

The true incidence of congenital adrenal hyperplasia is not known. Most paediatric centres have some children with this metabolic defect under their care. From centres specialising in the care of these children, large numbers are reported. Bongiovanni in Philadelphia, has two hundred cases, Wilkins in Baltimore, over one hundred, and Russell in London eighty five. During my study of these children I have been involved in the clinical care or the biochemical
control of forty children, over the past four years. In the past eighteen months, during my study of the steroids excreted by children with congenital adrenal hyperplasia, fourteen new cases have been confirmed and urinary steroid estimations carried out on twenty-five others. Most of these children have come from the Midlands but a few from other parts of the country. It is of interest that in 1947, Allibone, Baar and Cant found only ten cases, in the records of the hospital draining the same area, in ten years. Zuelzer and Blum, in 1949, reported the clinical histories and the post mortem findings of four children with the "salt losing" type, in a series of 1069 autopsies in a period of six years. In this series there were 770 infants under one year, and as all the four children with congenital adrenal hyperplasia died before the age of one year, this gives an incidence of 0.52%. They suggested that the syndrome was not as uncommon as it was said to be. In the past all the "salt losers" died in adrenal crises; the cause of the deaths being ascribed to pyloric stenosis, gastroenteritis, pneumonia and possibly convulsions.
If some cases of congenital adrenal hyperplasia have missed detection, because of the severity of their disease, others have missed detection because of the mildness of their disease. Some of these patients are now being discovered in the sterility clinics and others are investigated chiefly because of the complaint of hirsutism. In this latter group however only a small proportion belong to the syndrome of congenital adrenal hyperplasia; the majority belong to a miscellaneous group which can be classified under the rather unsatisfactory general heading of the Adrenogenital syndrome.

THE THREE TYPES OF CONGENITAL ADRENAL HYPERPLASIA

Three types of the syndrome of congenital adrenal hyperplasia are now described. In the first type, the "Virilising" type, evidence of the metabolic defect is shown only by progressive virilization. This was the first type to be recognised and described. In the earlier works, Young in [1937] and Broster and Vines [1933], on the adrenal cortex, it was the only type recognised. Until recently nearly three-quarters of the patients were considered to belong to this type.
In the late 1930's a second type was being recorded. The children described all died in early life with signs of adrenal insufficiency, and in those on whom an autopsy was performed, adrenal hyperplasia was found. Not only were the adrenals enlarged, but the surface showed a peculiar convoluted appearance. The histology of these glands showed marked hyperplasia of the cortex. The zones of the cortex were poorly defined and cells resembling those seen in the foetal cortex were numerous and had in areas, apparently split the capsule, leading to the convoluted appearance of the surface. In some cases the absence of the zona glomerulosa was reported. Opinion is still divided as to the significance of the zones in the adrenal cortex however. The clinical course run by these children is well documented by these early writings, particularly by Butler, Ross and Talbot, Dijkhuizen and Behr, Wilkins, Fleischman and Howard, Thelander and Cholffin, Allibone, Baar and Cant, Darrow, and many others. The frequency with which this syndrome of adrenal insufficiency associated with adrenal hyperplasia is being recognised is still
increasing. In a review of 153 cases, Russell in 1954 found 68% of 38 male children and 39% of 115 female children to show manifestations of adrenal insufficiency. Eight of the fourteen new cases diagnosed during this study are of the "salt-losing" type. Recent work then suggests that the "salt-losing" type is the commonest type; the present facilities for diagnosis and treatment being responsible for this apparent increase.

Still more recently a third type, the "Hypertensive" type, has been fully investigated and described by Wilkins, Bongiovanni and Eberlein [1955]. This is still a type that is rarely found. None of this type has so far been encountered.

The metabolic defect in the first two types is considered to be of a similar nature. In the third type it is different.

The incidence of the types may well vary in different parts of the world since the defect in the three types depends on the presence of an abnormal genetic make up.
THE SEX DISTRIBUTION

In the past this syndrome was considered to be more common in girls. This is understandable, for the clinical signs are far more striking in the female. As shown in the incidence many young infants with the salt-losing syndrome must have died without the diagnosis being made. The female children in this group were often considered to be males because of the masculinization of the external genitalia. Figures now suggest that the sex incidence is equal. This is shown by the presence of seven males and seven females in the newly diagnosed fourteen children.

GENETIC ASPECTS

This inborn error of metabolism is believed to be due to a recessive gene. This explanation, first proposed by Childs and Grumbach [1954], is now fully supported, and means that on an average one in four children of affected families will develop the defect. A familial history has been noted from the days when congenital adrenal hyperplasia was first described. In this series there are five affected siblings living, and in two
other families siblings have died with a clinical course very suggestive of the "salt losing" syndrome. In a family of eight adults examined, three of them were shown to have an abnormal steroid pattern on chromatography of the 17 Ketogenic steroids showing that they were suffering from congenital adrenal hyperplasia.

There have been no cousin marriages in this group and the urines of some of the parents examined have shown no abnormality. However, others have investigated the urine, of parents of known cases, following stimulation of the adrenals with corticotrophin and have shown an excretion of pregnanetriol in excess of that found in the normal. The presence of one abnormal gene is only detectable under stress, the presence of two abnormal genes gives rise to the recognisable clinical syndrome.

The type of defect is shown to run true to form. If one member is of the "salt losing" type, then any other affected child will also show this tendency.
I have found it interesting that many of the children in this study are of Irish origin, and though the maiden name of one of the parents is the same as one of the other affected families they have not been able to trace a common ancestor.

THE NATURE OF THE METABOLIC DEFECT

From the early days of steroid chemistry the syndrome of congenital adrenal hyperplasia has aroused interest because the large quantities of steroids synthesised by these patients have facilitated the extraction and the identification of the steroids excreted in their urine. This work has lead to the now generally accepted theory, first postulated by Bartter, Albright, Forbes, Leaf, Dempsey and Carroll [1951], that the fundamental lesion in congenital adrenal hyperplasia is an inability of the adrenal cortex to synthesise cortisol.

The first major step in the solution of the defect followed the publication by Hechter, Zaffaroni, Jacobson, Levy, Jeanloz, Schenker and Pincus [1951] of a scheme for the biosynthesis of
the adrenocortical hormones. Though the original work was done using the bovine adrenal gland, it has since been shown to be applicable to the human gland. The sequence of events starts with cholesterol found in large quantities in the adrenal gland and shown to be depleted following stimulation of the gland. The side chain of eight carbon atoms attached at the C17 position is degraded to produce a C21 nucleus with a side chain of 2 carbon atoms, \( \Delta 5 \) pregnenolone or a C19 nucleus with no side chain, dehydroepiandrosterone. The former is then converted into an active steroid progesterone (\( \Delta 4 \)-pregnene-3,20-dione) by oxidation at the C3 position and a shift of the double bond to the 4-5 position. The latter is concerned with the C19 steroids.
The biosynthesis of cortisol now depends on the hydroxylation of the progesterone at three positions, C17, C11, C21. This is brought about by three enzymes 17 hydroxylase, 11β-hydroxylase and 21 hydroxylase.
Jailer, in 1953, suggested that the inability to synthesise cortisol was due to an enzymatic failure and in 1954, Dorfman, postulated that congenital adrenal hyperplasia was due to a failure of the enzyme 21 hydroxylase. Evidence has accumulated to prove this aetiology in the "virilising" and the "salt losing" types.

Evidence to show that the rarer "hypertensive" type is due to a lack of 11\(\beta\) hydroxylase has been put forward by Bongiovanni and Eberlein [1955].

The first step towards the proof of this came in 1937 when Marrion and Butler isolated from the urine of two women with congenital adrenal hyperplasia a large quantity of a 17 hydroxylated C21 steroid and showed this to be 5\(\beta\) pregnane-3\(\alpha\) 17\(\alpha\)20\(\alpha\)triol, (pregnanetriol). Since then this steroid, found only in small amounts in the urine of normal people, has been shown to be invariably raised in the urine of those with congenital adrenal hyperplasia, by many workers in this field notably Bongiovanni, Clayton, Eberlein. Stimulation of the adrenal cortex with corticotrophin in the normal person increases by a small amount
only, the excretion of pregnanetriol. In contrast, following stimulation of the adrenals of patients with congenital adrenal hyperplasia, there is a marked increase in the secretion.

Mason and Kepler in 1945 reached the conclusion that pregnanetriol was a metabolite of 17α-hydroxy progesterone. Proof of this has come from the work of Bongiovanni, Eberlein and Cara [1954], and Jailer, Gold, Vande Weile and Lieberman (1955), who administered 17α-hydroxy progesterone to patients with congenital adrenal hyperplasia, to those with a low excretion of steroids, and to normal subjects.

The intermediary metabolite between 17α-hydroxy progesterone and pregnanetriol, (Δ4 pregnene-17α-ol-3,20-dione) 17 hydroxypregnanolone, was first shown to be present in large amounts in the urine of a patient with congenital adrenal hyperplasia by Appleby and Norymbersky in [1955]. In a recent paper Bongiovanni, Eberlein, Smith and McPadden, have again demonstrated the presence of large quantities of 17α-hydroxypregnanolone in the urines of patients with congenital adrenal hyperplasia.
There is therefore ample evidence that hydroxylation readily occurs at the C17 position and also that there is an excess of these steroids suggesting a failure of conversion of these substances to cortisol along the normal pathway.

There is equally strong evidence that hydroxylation at the C11 position can take place in the majority of people with congenital adrenal hyperplasia. Firstly it has been demonstrated that amongst the C19 steroids found in excessive amounts are those oxygenated at the C11 position. Bongiovanni and Eberlein [1955] have shown that between 18% and 25% of the C19 steroids are oxygenated at the C11 position, whereas in the normal person the excretion of the C11 oxygenated steroids is only 10% of the total. Secondly, in 1953 Finkelstein, v Euw and Reichstein isolated and identified from the urine of those with congenital adrenal hyperplasia a new steroid hydroxylated at the C11 position, pregnane-3α17α20α-triol-II-one (11 ketopregnanetriol). Working with Cox he has studied this steroid further and
believes that it is a more specific finding in congenital adrenal hyperplasia than is pregnanetriol. Bergstrand, Birke and Plantin have also shown this substance to be present in large amounts, and in the younger children, in greater amounts than pregnanetriol. This 11 oxygenated steroid has been shown to be present in large amounts in the recent paper by Bongiovanni, Eberlein, Smith and McPadden [1959] on the study of the three 17 hydroxylated C21 methylated steroids in congenital adrenal hyperplasia.

There is therefore evidence that hydroxylation at the C11 position does take place readily in the majority of cases. However, in those showing a considerable degree of hypertension, no C11 oxygenated substances could be identified by Bongiovanni and Eberlein [1955]. There was, however, an excess amount of desoxycorticosterone and "desoxy" hydroxy corticosterone (compound S) excreted in the urine as tetrahydro S. This leads these workers to suggest that there was a failure of hydroxylation at the C11 position due to a lack of 11β hydroxylase in this type of congenital adrenal hyperplasia.
Evidence that hydroxylation at the C21 position did not occur was not substantiated. Steroids with hydroxylation at the C21 position were found in normal or only slightly reduced amounts in the patients with the virilising type of congenital adrenal hyperplasia. However, in those showing signs of electrolyte imbalance reduced amounts were found and furthermore, when corticotrophin was given to stimulate the glands to produce more cortisol, little or no response
was shown by those with congenital adrenal hyperplasia. But if there was evidence that cortisol was not excreted in the expected amounts following stimulation, there was evidence that the steroids, known to be the immediate precursors of cortisol, were excreted in very large amounts in the urine following this stimulation. These facts have modified the original hypothesis of a complete failure to form cortisol and lead to the much more acceptable view that there is a variable degree in the inability to form cortisol, and therefore to hydroxylate at the C21 position. The adrenal can produce some cortisol but in order to do this it is working at maximum capacity and in only some cases is there any reserve, while in those demonstrating signs of adrenal insufficiency it is unable to synthesise adequate amounts.

Proof of this comes from the knowledge that the adrenal glands are hypertrophied in all untreated cases of congenital adrenal hyperplasia. In 1953, Snydor, Kelley, Raile, Ely and Sayers showed that there was an increased amount of
circulating corticotrophin in the blood of patients with congenital adrenal hyperplasia. These facts suggest that only by hypertrophy and by increased stimulation can adequate cortisol be produced. The finding of quantities of C21 steroids with a methyl group at the C21 position, hydroxylated at the C17 or the C17 and the C11 position, prove that the metabolic defect lies in the hydroxylation at the C21 position.

The nature of the metabolic fault in the "salt losing" type with Addisonian like symptoms and electrolyte imbalance, has been the subject of much discussion and research. The first suggestion was that there was an inability to produce a salt retaining hormone. However, when aldosterone was isolated it was found to be present in normal or even in increased amounts in the urine of these children by Leutscher and Curtis, and Prader, Spahr, and Neher in 1955.

An increased excretion of a salt losing hormone was then postulated, but so far no such hormone has been isolated. Bergstrand, Birke and Plantin in their study of the excretory
patterns of steroids in children with congenital adrenal hyperplasia found in the "salt losers" a very much greater percentage of 11 keto pregnanetriol than pregnanetriol and this suggested the possibility of a different metabolic pathway in these children. However, it so happened that all their "salt losers" were young children and all their older children were non "salt losers". This suggested another explanation, that this difference was merely a function of age. This latter view has been substantiated by the recent work of Bongiovanni and by Finkelstein and Cox, and this is also supported by the present work.

There is then, at present, no proof that there is any difference between the virilizing group and those showing electrolyte imbalance other than one of degree, in the severity of the enzyme lack. The wide variance in the severity of the clinical picture lends support to this view. Some children show severe adrenocortical insufficiency by the end of the first week of life; others may only demonstrate a tendency to this, on stress. It is also known that these children lose, to a certain degree, their tendency
to electrolyte imbalance. There are, however, some points rather difficult to explain. The most important of these is the need for larger doses of corticoids than would be considered physiological, coupled with the administration of a salt retaining hormone and extra salt in order to keep these children in good health. The other point is the abnormal response that these children show to corticotrophin. Jailer demonstrated that a sodium loss rather than retention occurred in some of the children.

It is possible that the very large amounts of the intermediate products of cortisol known to be produced by these children and the further amount excreted following stimulation with corticotrophin, may play some part. It is still possible however that a salt losing hormone will be found. Research into this type of the syndrome was hampered in the past because all the children died at an early age. It is hampered now because of the need for early corticoid treatment if lives are to be saved. This latter point is supported by the number of sudden deaths reported during the investigation of these children.
THE CLINICAL PICTURE

It is convenient to describe the clinical picture as it presents in three different age groups.

(1) THE NEWBORN

In the newborn the problem presents as one of correct sex differentiation between the male child with hypospadias, and the female child with prenatal masculinisation of the external genitalia and the rarer true hermaphrodite. Since Barr [1953] described the morphological difference in the chromatin pattern of the female cell this problem resolves itself into determining the chromosomal sex on a buccal smear. However, should the smear show the infant to be chromatin positive, the differential diagnosis lies between androgenisation due to congenital adrenal hyperplasia and androgenisation due to the administration of progesterone or diethyl stilbestrol, Wilkins et al [1958], Jones [1957], Hayles et al [1958], or androgens, to the mother during the early months of her pregnancy, and, more rarely still, to a maternal arrhenblastoma (Bretnall [1945]. Javert and Finn [1941]). The differen-
tiation of these three groups depends on the estimation of the urinary steroid excretion.

The anatomical features found in the female infants are similar, despite the different aetiology. The degree of masculinisation is however variable and the more the degree of masculinisation the more impossible it becomes to ascribe the correct sex to the child by the appearances of the genitalia alone. In congenital adrenal hyperplasia the commonest lesion is an enlargement of the phallus and a fusion of the labia to form a common urogenital sinus opening at the base of the phallus. This occurred in 13 out of 17 in the present study. The second commonest type shows enlargement to a varying degree of the phallus without fusion of the labia and the presence of a separate vaginal orifice. This was present in 3 out of 17 females in the study. The rarest type of all is the presence of a complete phallyl urethra, and this is present in only one of the children under study.

(ii) CLINICAL PICTURE IN INFANCY

In this group the child presents because
of adrenocortical insufficiency. The commonest manifestation of this is repeated vomiting which may be projectile and lead to the diagnosis of hypertrophic pyloric stenosis. Diarrhoea may accompany the vomiting suggesting gastro-enteritis. In both, the child rapidly becomes dehydrated, but unlike children with either pyloric stenosis or gastro-enteritis, children with congenital adrenal hyperplasia continue to pass adequate or even excessive quantities of urine and routine ward testing of this shows chlorides to be present. The dehydration is often severe, but the signs of peripheral failure, coldness and cyanosis of the extremities, is out of proportion to the dehydration. Collapse with profuse sweating, pallor and tachycardia may follow vomiting. In other infants there is a gradually increasing lethargy, feeding initially slow, is followed by complete failure to suck. Signs of peripheral failure supervene and if not recognised these infants may die quite suddenly. Convulsions may occur, in some instances these are due to hypoglycaemia, White and Sutton [1951], but in others they
appear to be a manifestation of adrenal insufficiency and not to the hypoglycaemia it may cause, Butler, Ross and Talbot [1938]. Pigmentation, generalised of a brownish yellow colour, or of a darker bluish-brown, present only on the scrotum, penis, peri-anal region and nipples, is usually present in those showing adrenal insufficiency. Pigmentation of the buccal mucous membrane may be present. The external genitalia in the female infant should provide the answer. In the male infant, and most of the children diagnosed in infancy should be males, if the female cases are recognised at birth, no help apart from the pigmentation can be expected from the examination of the genitalia. In the young infant the penis is not diagnostically out of proportion to the testis.

Serum electrolyte changes, a lowered sodium chloride and alkali reserve and a raised potassium and urea are usually present. These changes should not be unduly stressed as similar changes may be found in infants with gastro-intestinal upsets, and they may not be grossly abnormal in children with congenital adrenal hyperplasia.
The earliest age of onset of severe adrenocortical insufficiency in the present group of infants was at seven days old, the oldest at three months.

(iii) CLINICAL PICTURE IN THE OLDER CHILD

If diagnosis has not been made in infancy then the child will be brought to notice because of precocious development, isosexual in the male and heterosexual in the female.

The boy is taller and more muscular than boys of a similar age. He excels in sports and though these children are usually of normal intelligence, they are often expected to behave and show the intelligence of children of a similar stature rather than of a similar age; consequently they are often said to disappoint in their school work. They are, however, very good on the sports field because of the advantage of their size. Fusion of the epiphysis is early, and takes place between the eighth and fifteenth year. Growth ceases at this early age and the children who have been tall boys are dwarfed adults. The secondary sexual manifes-
tations appear early. Pubic hair begins to grow from the first year onwards. The larynx enlarges and the voice deepens. Facial acne and hair develop early. On examination of the genitalia the penis is noted to be obviously enlarged in comparison to the rather small testis. Rarely the latter may be of normal size or even slightly enlarged. This excessive enlargement of the testis is considered to be due to the presence of adrenal rests. The prostate is enlarged.

In girls the precocious development is heterosexual. The consequences of delayed diagnosis and treatment are more severe, both from the physical and the psychological angle. These girls may be boyish in their nature, in that they are usually very good at games; however, they are on the whole feminine in their outlook and play with feminine toys. They may be over retiring because of the embarrassment caused by the effects of virilisation. The bodily configuration is male. The skin is coarse and greasy and facial acne is often troublesome. The hair is thick.
Secondary sexual characteristics develop along male lines. The larynx enlarges and the voice deepens. Pubic hair of a male distribution develops early, and later the growth of facial hair necessitates shaving. No breast development occurs. Menstruation does not usually take place but in the very mild forms of the syndrome, scanty and irregular menstruation, late in onset, may occur. The genitalia are masculinised. In this group where the diagnosis is delayed, no abnormality may have been noticed at birth but as the child grows, an enlargement of the clitoris is evident and this progresses until a penile like organ develops. On internal examination an infantile uterus can be palpated. As in the male, epiphyseal fusion takes place early and the height of the untreated or late treated child seldom exceeds five feet.

CONFIRMATION OF THE DIAGNOSIS

In the new born child the confirmation of the diagnosis depends firstly on the finding of
a female nuclear chromatin pattern in a child with abnormal genitalia. The steroid excretion should then be estimated in order to exclude the non-adrenal female pseudohermaphrodite. If a complete twenty-four collection can be readily obtained, then the 17 ketosteroids will be above 1 mg. in twenty-four hours and the 17 ketogenic steroids higher, chromatography of the 17 ketogenic steroids will show an abnormally high ratio (0.8 - 1) of the pregnanetriol fraction to the 11 oxygenated fraction. This latter estimation can be carried out on a single sample of urine and for this reason and because the 17 ketosteroids are sometimes high in normal new born infants, this laboratory test is probably the most reliable one at present available. The reservations in its use are discussed later. The estimation of the bone age in this group has not been sufficiently abnormal to be of real use in confirmation of the diagnosis. Adrenal insufficiency does not occur in the first few days of life so that serum electrolyte changes will not be present.
In the infant the diagnosis depends on a high suspicion index especially amongst male infants with vomiting or failure to thrive. The presence of a "hypospadias" in such infants should immediately call for further investigations. Suspicion will also be heightened by the presence of a lowered serum sodium and chloride and a raised potassium and urea and more so if the infant does not appear to be responding in a satisfactory manner to electrolyte therapy. The passing of large amounts of urine containing chlorides makes the diagnosis of adrenal insufficiency almost certain. Pigmentation is usually, but not always, present, and of course may mean adrenal hypoplasia, but again pigmentation of the genitalia is frequently seen in normal infants with dark hair. In the older infants the advancement in the bone age may be quite definite, but since these infants usually present in the first three months of life, advancement is seldom sufficient to be diagnostic. It is however in this group of children, where confirmation is most urgently needed by the clinician, that steroid estimation is quite
diagnostic. If time permits, then it is well to have a twenty-four hour collection to estimate the 17 ketosteroids, the 17 ketogenic steroids and the pregnanetriol fraction of the 17 ketogenic steroids. Chromatography of the 17 ketogenic steroids measures this fraction and when the child is in need of urgent treatment this method has the great advantage that a single sample of urine of five millilitres is all that is required. Treatment can then be given without any risk of complicating the results. The more severely ill the child, the more abnormal is the ratio of the pregnanetriol fraction to the 11 oxygenated fraction.

In the older child the finding of a greatly advanced bone age in a precociously developed child is confirmatory. Occasionally however an advanced bone age may be found in children without adrenal hyperplasia. The urinary steroid excretion should always be estimated. The 17 ketosteroids are raised but the 17 ketogenic steroids are raised to a greater extent. Chromatography of the 17 ketogenic steroids shows an abnormally high ratio of the pregnanetriol fraction to the 11 oxygenated 170H steroid fraction and is diagnostic of the condition. It is also important
in this age group to carry out a buccal smear examination.

The correct diagnosis of this condition remains a most important part of the work not only of the paediatrician but of the general practitioner, the obstetrician and the urological or plastic surgeon. Only by being fully aware of this condition can the tragedy of rearing in the wrong sex be avoided, and the "salt loser" treated before irreversible adrenal failure supervenes. Now that any child in extremis is liable to be treated with corticoids it is important that some test, readily applicable to the ill child, and the resources of the ordinary hospital, is available to prevent the needless continuation of corticoid therapy and, just as important, to avoid the stopping of corticoid therapy when life depends on its continuation.

In four of the children in the present study, laparotomy was carried out. All these children had normal internal female genital tracts. In the light of a better understanding of this syndrome and especially in view of the
possible precipitation of adrenal failure, laparotomy is no longer necessary as a confirmation of the diagnosis and may indeed be dangerous.

Genetic Counselling

All the parents have asked or have been told of the possibility of other children being affected, and they have usually considered it wise to restrict their families. However before the genetic implications were known to three of the parents they had second infants. Two of these children were unaffected, but the other was affected. These parents now have the worries involved in the upbringing of two children with the "salt losing" type of congenital adrenal hyperplasia.

AIMS OF TREATMENT

(1) SURVIVAL

Before cortisone became available for the treatment of this condition, few children with the "salt losing" type survived. Those who did were kept alive by the administration of salt and desoxycorticosterone acetate. The oldest
salt loser surviving in this group is now eleven years and may indeed be the oldest one alive today. He showed in childhood an instinctive craving for salt, and would consume as much as twenty grams of salt daily. Now that cortisone and more powerful salt retaining hormones are available these infants can be kept alive and will later show a lessening of the tendency to develop adrenal failure. The first two to three years of their lives, however, require careful supervision of the treatment if they are to survive. Parental knowledge and co-operation is of the utmost importance, and in this, the management of these children, is like that of the young diabetic.

The parents should be given sufficient knowledge of the defect for them to understand the needs of the child. As in the diabetic they can be profitably taught to increase the dose of corticoid when the child is unwell and advised to seek medical aid when response is not rapid. It is essential that they have ready access to hospital care when necessary, and are under medical supervision for even the most minor surgical procedures and all illnesses.
(11) PHYSICAL DEVELOPMENT

Normal physical development in the young infant with the salt losing tendency depends on the adequate provision of corticoids to prevent fluid and electrolyte loss, and to promote weight gain. Daily weighing is wise at this stage. In the older child normal physical development depends more on the adequate suppression of the excess androgen production, which causes rapid growth in height and more rapid skeletal maturation. During this period weight and height gains should be charted at one to three monthly intervals and bone age estimated at six monthly intervals. The aim is always to give the dose of corticoid that will maintain a normal weight, height and increase in bone age. In the young child with the "salt losing" syndrome the dose of cortisone adequate to prevent the occurrence of adrenal crises during any minor stress, has tended to retard growth and the normal advancement of the bone age, and produce a temporary cushingoid appearance. It is hoped that the use of the more powerful salt retaining hormones may avoid
this temporary cushingoid state. Since these children tend to show less salt loss as they grow older, the dose of cortisone required to maintain them is relatively smaller and advancement in bone age will proceed at a normal rate.

In the older child especially in those whose treatment is not started until there is a considerable advancement of the skeletal age over the chronological age, and of the height above the average for the child's age, the dose of cortisone needed to halt this abnormal physical development has caused a rapid gain in weight and even the development of striae. This has only been prevented, with difficulty, by the use of a strict diet.

The dose of cortisone in these two instances has to be adjusted to meet the overall needs of the child.

When treatment is started late, early fusion of the epiphysis and stunting seem inevitable. When treatment is commenced in infancy a normal height should be obtained by careful regulation of corticoid treatment.
(iii) SEXUAL DEVELOPMENT

In the male the excessive secretion of androgen causes the early appearance of secondary sexual changes. Pubic hair may appear in the second year, muscular development is excessive, the voice breaks early and facial hair develops in the early teens. The penis enlarges from an early age and by the end of the first year the large penis in contrast to the small testis is a diagnostic feature. The excess androgens are believed to cause disordered secretion of gonadotrophins and there is a resultant failure of the testis to mature. Sterility is the inevitable result of the untreated or inadequately treated condition. There is as yet no conclusive work on the gonadotrophins of children with this condition and indeed no large series on normal children for comparison. Much remains to be learnt in this particular field.

In the female the excessive androgen secretion is responsible for the masculinisation of the external genitalia of the foetus. Postnatally progressive virilization results. The
bodily configuration becomes masculine and muscular, breast development does not occur, the skin is coarse and facial acne often troublesome. The hair is thick and greasy. Hirsutism develops on the face in the young girl. There is also a disordered secretion of gonadotrophins, resulting initially in a failure of menstruation and ovulation and finally a complete failure of follicular production. Sterility, as in the male, is the inevitable outcome.

As in physical development the milestones passed depend, not on the chronological age, but on the bone age, so that the untreated child of eight may, on outward appearance, be a mature post pubertal male, and by the same age the girl may have a deep male voice and facial hair.

The aim of treatment in the male is to delay the onset of puberty to the normal chronological age. In the female to suppress the virilization and maintain a normal menstrual cycle. The results depend largely on the age at which treatment is started. If the bone age is well below the normal age at which puberty
occurs then, given adequate suppressive therapy, development can be slowed to a normal rate. If the bone age has already advanced to the age at which pubertal changes can be expected to occur, then the giving of cortisone will precipitate these changes. In the girl there is rapid breast development and the onset of menstruation. In the boy the development of facial hair and deepening of the voice. This precipitation of pubertal changes is believed to be due to the secretion of gonadotrophins following suppression of androgens.

In girls the facial hirsutism and the deep voice, once developed, do not respond adequately to treatment. The clitoris will not enlarge further on treatment, but surgical treatment is nearly always necessary as, even when suppressive therapy with corticoids is started in infancy, the clitoris remains abnormally large. In the older untreated girl, the great enlargement is sufficient to cause considerable psychological upset and no regression in the size of the organ can be expected on corticoid therapy.

The ultimate aim of procreation has been recorded in three women under treatment with
corticoids. Gans and Sen [1959] and Wilkins. Whether it is right to aim at the propagation of an abnormal gene is debatable, but it is certain that as normal physical development is achieved in more patients with congenital adrenal hyperplasia, more will be capable of procreation. Treated early and adequately, these patients can achieve normal physical development and release from all the mental trauma of the untreated sufferer. We cannot deny them this benefit.

**CONTROL OF TREATMENT**

In the young infant with the salt losing type, the control of treatment requires an almost hour to hour watch on the general condition, hydration, tone of muscles and circulation. A daily electrolyte estimation on the serum, and a daily estimation of the fluid loss in the urine. In failure, the amount of urine passed is always very large, a decrease in the amount passed is a good indication of progress in the control. Daily weighing is a further very good lead to adequate fluid and electrolyte replacement. At this stage daily steroid estimations are useful and are mentioned in detail later.
In the salt loser not in an adrenal crisis, weekly or monthly weighing and measuring and estimation of the steroid excretion, with an estimation of the bone age at six monthly intervals is used as a guide to adequate corticoid therapy. The well-being of these children is an excellent indication of adequate therapy. At this stage they are still very labile; listlessness, thirst, the excessive excretion of urine, and weight loss, call for more frequent supervision and adjustment of therapy. These minor manifestations of adrenal insufficiency are often precipitated by intercurrent infections. A rising steroid excretion with a rising ratio of the pregnanetriol fraction to the 11 oxygenated fraction has proved a particularly valuable laboratory guide to the inadequacy of the corticoid dosage in these instances.

In the older child and in the child who does not manifest cortical insufficiency the control of treatment rests mainly on watching the steroid excretion, the height and weight gain, at from monthly to three monthly intervals, and the
bone age at six monthly intervals. In the girl the evidence of virilization is an indication of inadequate treatment.

TREATMENT

The use of cortisone in the treatment of congenital adrenal hyperplasia was first reported by Wilkins [1950]. Cortisone, or its newer derivatives, suppress the production of corticotrophin by the pituitary, the adrenal overactivity diminishes and the hypertrophied adrenal cortex is reduced in size. Life depends chiefly on the administered cortisone and during periods of stress, illness or operations, larger doses of cortisone must be administered. For the convenience of the patient, especially the child, oral administration is to be preferred, but it has the disadvantage that the blood level is not evenly sustained over the twenty-four hour period and therefore larger doses are needed than are required when given by the intramuscular route (Wilkins, 1952). The dose required orally has been 15-100 mg. divided into three or four doses daily and no child in the present series
has been maintained on continuous intramuscular therapy. Intramuscular cortisone has been used, however, as the initial treatment of choice when there is a danger of vomiting. In these children the dose has ranged from 50-25 mg. once or twice daily, and represents an initial suppressive dose which is higher than the eventual maintenance dose. Of the newer corticoids, prednisolone has been used in the older children, in one fifth the dose of cortisone, but appears to have no advantage. The methylated corticoids have not been used as their tendency to promote salt loss, rather than retention, would appear to be a contra-indication to their use in this disease.

In the "salt loser", cortisone alone is not adequate to prevent adrenal crises without retarding growth excessively. In these children added salt is necessary, 2-5 g. daily, and in addition a salt retaining hormone. Desoxycorticosterone acetate was the drug of choice and can be administered as a daily intramuscular injection, as a monthly injection of the trimethyl acetate crystals, or as a subcutaneous implant.
The daily injection in doses 2-5 mg., and the monthly injection of the trimethyl crystals in doses of 25-250 mg., have been used in children during this study. Latterly 9 Fluorohydrocortisone with a salt retaining property some

50 times as great as cortisone has been found to be very successful in doses of 0.1-0.2 mg. daily in combination with cortisone. When given in larger doses it produced hypertension. In one infant oedema occurred with a dose of 0.5 mg. daily. The action of this corticoid is sustained for twenty-four hours and, used with care, it would seem to be the best drug for the purpose at present available in this country. It has the advantage that it is administered orally.

SPECIAL CARE - THE ACUTE ILLNESS

In children with congenital adrenal hyperplasia no illness should be dealt with lightly.
These children pass into severe adrenal failure with infections, especially if they are associated with the fluid and electrolyte loss associated with gastro intestinal disturbances. If they are vomiting, admission to hospital should not be delayed. Under these circumstances the corticoids are not being absorbed and a vicious circle is set up.

On admission to hospital their condition can be assessed. If the children are alert, the circulation and the blood pressure satisfactory, then treatment with oral fluids containing salt and intramuscular cortisone will be adequate. In the more severely ill child, intravenous infusion will be necessary. These children are usually lethargic, the muscle tone is poor, and the extremities are cold. Dehydration is always present.

The intravenous fluid requirements of these children are calculated to give the fluid requirements, to make up for the estimated loss due to dehydration and to provide the salt requirements and the deficiency of electrolytes due to the dehydration. The fluid requirements for maintenance are
150 ml/kg. or 75 ml/lb. for each twenty-four hours. The replacement fluid required to correct the dehydration is calculated from the degree of dehydration and the weight of the child. To allow for salt loss this fluid is given as a 4.3% solution of sodium chloride. The extra salt requirements of most of these children attending hospital will already be known and to this must be added the ordinary daily requirements of the normal child. Occasionally when there is a marked acidosis it may be wise to use a sodium lactate solution, and 5% dextrose is always added to the saline solution.

The fluid is best given into a scalp vein in the children under eighteen months. Cut down infusions should always be avoided in these children in order to preserve veins for future use.

An initial rapid infusion of 10 ml/lb. or 20 ml/kg. to which is added 10-25 mg. of cortisol is given and the remainder of the requirements given over twenty-four hours. There is usually
a dramatic response to the initial infusion. This response is a good indication of the severity of the child's condition, a poor response calls for larger doses of cortisol. It is wise to continue, in any case, the intravenous cortisol over the first twenty-four hours. The dose required over this period lies between 20-100 mg. This regime can be carried out satisfactorily without the help of serum electrolyte levels. They are however most useful in estimating the requirements of the ill child and are essential if intravenous therapy has to be continued for more than twenty-four hours. The sodium loss shown by these children is most striking. During the first few hours these children require very careful supervision.

During the second twenty-four hours, oral feeding may be given. If this is taken well and no vomiting occurs, the infusion can be discontinued. Parenteral corticoids should be given for a further day or two. Intramuscular cortisone is suitable, in a dose of 25-50 mg. daily, at this period.
The study of these children has demonstrated the need for energetic treatment rather than a wait and see policy. Two children died of adrenal insufficiency. The first child was admitted following a convulsion. Apart from a respiratory infection there was nothing of note. The blood pressure, the serum electrolytes and the blood sugar were normal. The child had been subject to convulsions with febrile illnesses and this was considered to be the diagnosis on this occasion. He was treated with intramuscular cortisone and antibiotics. Further convulsions occurred and he died rapidly. At postmortem the presence of a respiratory infection of a minor nature was confirmed. The adrenals were both small, well suppressed by corticoid therapy, but they showed the convoluted surface markings pathognomonic of congenital adrenal hyperplasia. More energetic treatment would have saved this child.

The second child, a five week old infant, was admitted to another hospital with the diagnosis of pyloric stenosis. His condition on
admission was so poor that he was treated with an intravenous infusion and corticoids. The serum electrolytes carried out on several occasions were not grossly abnormal. The child was at first considered to be a possible example of congenital adrenal hyperplasia, but the remarkable improvement of the child's condition and the normal electrolytes, did not support the diagnosis and the cortisone was reduced. A specimen of the child's urine was received for chromatography of the 17 ketogenic steroids. The result showed a ratio in the range of the young children with adrenal failure. Shortly after receiving the urine, the child collapsed and died. Postmortem revealed that this child was a female pseudohermaphrodite due to congenital adrenal hyperplasia.

SURGICAL TREATMENT OF THE MASCULINISED GENITALIA

Because of the tendency of these children, especially in the younger age groups, to develop severe adrenal insufficiency with stress, it is
wise to postpone any attempt at surgical correction of the masculinisation of the genitalia until the tendency to adrenal failure has diminished. This occurs by the fourth year in the majority of children. At this age the child is larger and the surgeon finds the operation technically easier. At the same time the child is not yet of school age. Provided the parents are assured that correction will be carried out at this age they are usually prepared to wait. Occasionally however the presence of the abnormal genitalia is distressing to the parents and then correction will have to be carried out at an earlier age.

The surgical procedure involves clitoroidec-tomy and also, in the majority, the division of the fused labia to expose the vaginal orifice (Jones and Jones, 1954). The operative procedure is not as difficult as the external anatomy would suggest and the children in this group subjected to it have good cosmetic results. The dose of cortisone should be increased over the operative period and it is wise to have an
intravenous infusion running during, and for the first twenty-four hours after the operation, to which hydrocortisone can be added if necessary.

INFECTIONOUS DISEASES

All the children in this study have been advised to have the routine immunological inoculations. Two specific exanthemata, chickenpox and measles, are considered to be of particular importance.

It is believed that treatment with corticoids at the time of infection with a virus can cause a sometimes fatal spread of the infection. Children on large doses of cortisone for rheumatic fever were reported to have died from chickenpox, (Haggerty and Eley, 1956). One of the children in this group developed chickenpox. His attack was mild and he developed no evidence of adrenal failure. Later however he was admitted to hospital with measles and showed moderately severe adrenal failure. A second child admitted with measles, modified by gamma globulin, showed mild evidence of adrenal failure. In 1957 Gardner and Wyatt reported the sudden and unexpected deaths of four children, with congenital adrenal hyperplasia, during the course of measles. None
of these children were considered to show the "salt losing" syndrome and only one was on cortisone. These writers suggest that measles must have an unusual potency for stimulating the adrenals. It is also of interest that one of the few "salt losers" to survive on salt and DOCA before the days of corticoid therapy died at the age of seven years during an attack of measles, Thelander [1946].

Protection with gamma globulin would appear to be part of the special care required by these children. The parents of all the children with congenital adrenal hyperplasia have been warned of the dangers of measles and advised to bring the children to the hospital for a protective dose of gamma globulin should they be suspected of being in contact with a case.

THE PROBLEM OF REARING IN THE WRONG SEX

In the group of children studied, six girls were ascribed to the wrong sex at birth. Now that cortisone is available as a specific treatment to prevent virilism, these children are a tragedy of misdiagnosis, which can be prevented
by the use of chromatin pattern as indication of the sex, and of the estimation of the steroid content of the urine as an indication of the source of the androgenising agent.

Once the wrong sex is ascribed to a child with congenital adrenal hyperplasia, the management becomes very difficult. In the child below the age of sexual understanding, the decision depends on the parents' attitude. They may be unwilling to change the sex because of the social difficulties involved. Unless they are able to move to a new neighbourhood these may seem unsurmountable. In some instances the child has been more readily accepted as a boy, despite the doubt at birth, because a boy was desired.

Despite these difficulties it is far better if the parents, in the full knowledge of the defect and the treatment available, can be persuaded to agree to a change of sex of their child. The social services of the hospital can do much to help the parents solve their social difficulties and support from all the doctors involved, the general practitioner, the public health doctor and the specialist, is a vital part of the care of
these particular children. The medical care of the child whose sex is changed in the first year of life does not differ from that of the correctly diagnosed case.

If change of sex is not accepted, then treatment must depend on the type of the syndrome. In those females, with the "salt losing" syndrome, reared as males, life depends on corticoid treatment. This results inevitably in the suppression of the virilising androgens and eventually the development of secondary female characteristics and the onset of menstruation further complicates the matter. In these children where corticoid treatment is necessary, the female internal organs must be removed before the appearance of the secondary sexual characteristics but not until the child is showing a lessened tendency to the electrolyte imbalance. In those children who show no signs of electrolyte imbalance, the only justification for treatment with corticoids is the prevention of early epiphysial fusion and the attainment of a normal height. Treatment however must stop before
feminine sexual development occurs or the female internal genital organs must be removed. In these children, the withholding of corticoid treatment may be justifiable on the grounds that, in this way, the child is made less aware of his abnormality. However stunting is not to be desired, particularly in the male. Once a reasonable height has been achieved in the treated child, corticoid treatment is withdrawn and virilisation allowed to proceed.

In these children, whether they be "salt losers" or otherwise, repair of the "hypospadias" will be necessary in all but the very rare type with the complete phallyl urethra.

Four children in the younger age group, in whom the diagnosis was initially missed, were seen in this study.

The first child in this group is the only one with a complete phallyl urethra and was a much wanted "boy" called John Calvin H. The father is strongly opposed to a change of sex, the reasons being the social difficulties and the strong desire for a boy. As this child is a severe salt loser, treatment with cortisone has
been necessary. The phallus has therefore remained small and there has been some difficulty over micturition. Before he reaches puberty it is proposed to admit him to hospital for removal of the internal genital organs and then to decrease, as far as possible, the dose of corticoid so that virilization can proceed. This decrease will, however, have to be regulated to prevent any adrenocortical deficiency. Though I feel certain that it would have been far better to have changed this child's sex, the wishes of the parents have had to be respected. Once the decision has been finally made, this must be supported so that no further misgivings are associated with the child's upbringing.

The second child has a history similar to this one, and in this case, mainly because of the difficulties associated with social life, the decision has been made not to change the sex.

The other two children were more fortunate in that the correct diagnosis of females with congenital adrenal hyperplasia was made - in one, at ten days, and in the other at two months. In both these children the sex has been changed and the social difficulties overcome.
In the older child the problem is even more complex. Money and Hampson [1954], writing on this problem, strongly advise that after the age of two years, the sex should not be changed, because of the psychological upset to the child. Bongiovanni, in a more recent discussion on this problem, however, said that they were now not so averse to changing the sex of the older children with congenital adrenal hyperplasia. This change of view has come about chiefly because of the good results of corticoid treatment. The solution of the problem may at times be helped by the female orientation of these children. However this is not always obvious and is often disputed by parents because of the stigma attached to femininity in the male. In these cases especially, much help can be obtained from the psychologist for the parents and the medical adviser. Should the decision be to change the sex of the child, then the whole family must be given the help of social and psychological services in order to make the adjustment as easy as possible. This may well mean the finding of new employment, new housing and new schools for the family.
This problem was seen in two of the older children in this group. The first child had a "hypospadias" repaired between the ages of two to five years. Excessive growth and the early development of pubic hair led to further investigations. The chromatin pattern was female, the bone age greatly in advance of the chronological age, and the excretion of steroids was very high with a pattern seen in cases of congenital adrenal hyperplasia. The child was said to be orientated as a male, and the parents were strongly opposed to any change of sex. Laparotomy was performed and the uterus and ovaries were removed. Cortisone treatment was then started to try and retard any further epiphysial fusion. However, there was no further growth in the next year despite very satisfactory suppression of the abnormal steroid excretion. Fusion of the epiphysis was almost complete, so corticoid treatment was slowly discontinued. During the period on corticoids, the child had shown certain female tendencies which would suggest, after all, that
had the sex been changed, female orientation would have been demonstrated.

The second child also had a "hypospadias" repaired between the ages of two and five years. Precocious development again led to further investigations and the correct diagnosis. Consultation with the psychologist showed that this child was not really happy in the role of a boy and was herself on the verge of enquiry as to her real sex. It was good fortune that at this time the father was about to take up a post in a different area and the family about to move. The child was admitted to one ward where splitting back of the fused labia and clitoroidectomy was performed without difficulty. She was, a little later, admitted to another ward for corticoid treatment. This girl chose as her first feminine clothes a red velvet skirt and red shoes, suggesting that she had long desired the bright clothes worn by other girls.

She was seen after an interval of two years and was a happy well orientated girl.
Her steroid pattern at this time showed incomplete suppression on a dose of cortisone sufficient to cause mild obesity. The parents, despite their desire for a boy and having another daughter only, are now pleased that the child's sex was changed.

It would appear from the frequency of wrong sex rearing, in even this small series of cases, that there is a need for more awareness of this condition and a satisfactory and readily available laboratory test to confirm the diagnosis of congenital adrenal hyperplasia.
URINARY STEROID EXCRETION IN

CONGENITAL ADRENAL HYPERPLASIA
The aim of this study was not to estimate the individual steroids excreted by patients with congenital adrenal hyperplasia, but rather to find a method, which could be carried out in the routine laboratory, for the diagnosis and control of children with congenital adrenal hyperplasia.

At the time these studies were started, Morris, at the department of clinical endocrinology, the United Birmingham hospitals, was perfecting a new method for the estimation of pregnanetriol [1959]. This method could be extended to include, not only pregnanetriol, but the 11 oxygenated 17-OH steroids could be estimated, by measuring a more polar fraction from the chromatographic column. This method was then explored.

In the pregnanetriol fraction, the second fraction, other 17 ketogenic steroids with no oxygen group at the C11 position were known to be excreted in the urine of patients with congenital adrenal hyperplasia. Quantitatively the most important
of these is 17 hydroxyprogesterone. The metabolite of 11 desoxycorticosterone is also measured in this fraction, and this is the important metabolite of the hypertensive type.

In the third fraction, the 11 oxygenated 170H steroid fraction, the metabolites of cortisol and cortisone are measured. In the urines of the patients with congenital adrenal hyperplasia however, is found an abnormal metabolite, 11 keto-pregnanetriol. This steroid would be eluted in the third fraction.

After using this method for some period, it was evident that there was not only a quantitative difference in the steroid excretion, but a qualitative difference, giving a different ratio of one fraction to the other, in those with congenital adrenal hyperplasia. This suggested, that using this ratio, a single specimen of urine would be adequate for the diagnosis of congenital adrenal hyperplasia. As a paediatrician, well aware of the difficulties of collecting complete twenty-four hour samples from young children, this seemed a major step in the finding of a satisfactory diagnostic test. This ratio was
also shown to correlate well with clinical assessment of the child's control. Single specimens could be collected at home. Periodic admissions to hospital for the sole purpose of collecting twenty-four hour urines would be unnecessary. The danger that the child might fall prey to some hospital infection during one of these stays would be avoided. The method and the results are fully discussed. The normal and the abnormal pathways of the synthesis of cortisol and their effect on the ratio obtained by chromatography of the 17 ketogenic steroids is illustrated in diagrams.

As well as this investigation, the 17 keto-steroids were estimated in all the children when a twenty-four hour urine collection was available. In the new cases the 17 ketogenic steroids (Norymberski) and in some the 17 hydroxypregnanolone were estimated.
DIAGRAM I

THE NORMAL SYNTHESIS OF CORTISOL AND THE RESULT OF CHROMATOGRAPHY OF THE 17 KETOGENIC STEROID IN NORMAL CHILDREN
NORMAL SYNTHESIS OF CORTISOL

CHROMATOGRAPHY OF THE KETOGENIC STEROIDS.

PATTERN OBTAINED IN NORMAL CHILDREN
DIAGRAM II

THE ABNORMAL PATTERN OF SYNTHESIS AND
THE RESULTS OF CHROMATOGRAPHY OF THE
17 KETOGENIC STEROIDS IN CHILDREN
WITH CONGENITAL ADRENAL HYPERPLASIA
SYNTHESIS OF CORTISOL, ABNORMAL PATHWAYS DUE TO PARTIAL BLOCK TO HYDROXYLATION AT C21.

CHROMATOGRAPHY OF THE KETOGENIC STEROIDS

1. THE OLDER
   CHILD

2. THE INFANT
   NOT IN ADRENAL
   FAILURE

3. THE INFANT
   IN ADRENAL
   FAILURE
   ■ FRACTION II
   ■ FRACTION III

PATTERNS OBTAINED IN CHILDREN WITH CONGENITAL
ADRENAL HYPERPLASIA.
1. 17 Ketosteroids

The estimation of the 17 ketosteroids in urine was the first routine laboratory test for diagnosis and control of treatment of congenital adrenal hyperplasia and is still the only test available to the clinician in many centres. The estimation of the 17 ketosteroids is simple and the result can be obtained in four hours but it has disadvantages. Firstly, a complete twenty-four hour urine collection is necessary. Secondly, in the young infant the results obtained may be greater than 1 mg. a day (Read et al 1950), and the level at which a diagnosis of congenital adrenal hyperplasia can be made with assurance, may not be reached in the first few days of life. Thirdly, the 17 ketosteroids in the urine increases when cortisone is administered, so that ketosteroids may not fall as expected when an adequate suppressive dose of cortisone is given.
Fourthly, they are not as specific as the 17 ketogenic steroids and therefore not so sensitive an index for diagnosis or control as the estimation of the 17 ketogenic steroids.

**Estimation of the 17 ketosteroids**

10 ml. of urine is pipetted into a 40 ml. stoppered tube and 1.5 ml. of concentrated HCL. is added. The mixture is placed in a boiling water bath for 10 mins. to hydrolyse the glucuronides and the sulphates to free steroids. These are then extracted by shaking with 15 ml. of ethylene dichloride for 5 mins.

The aqueous phase is allowed to separate and then removed by suction. The extract is washed with 2N NaOH and then water till neutral. Alcohol is added to assist removal of any traces of water that may remain and the extract evaporated to dryness.

The results are read on the Unicam S P 600 spectrophotometer. Dehydro isoandrosterone 100 μg. is used as a standard and a blank of reagents alone is set up. The reagents are those
of Zimmermann, 2% alcoholic solution of meta dinitro benzene and an 8N KOH in alcohol. The correction factor \( \frac{E_{520} - (E_{430} \times 0.6)}{0.73} \) is applied.

2. Total 17 ketogenic steroids

The estimation of the total 17 ketogenic steroids is carried out by the method of Appleby, Gibson, Norymberski and Stubbs [1955] and the following groups are estimated:

\[
\begin{align*}
&\text{CH}_2\text{OH} & \text{CH}_3 & \text{CH}_2\text{OH} & \text{CH}_3 \\
&C=O & C=O & \text{CHOH} & \text{CHOH} \\
&C--\text{OH} & C--\text{OH} & C--\text{OH} & C--\text{OH}
\end{align*}
\]

Four ml. of urine is pipetted into a 70 ml. stoppered tube. 20 mg. of sodium borohydride is added and the mixture allowed to stand overnight. The 17 ketosteroids are reduced to alcoholic steroids and are not estimated. The C21:17:20 ketols are reduced to C21:17:20 glycols. These glycols, combined with those already present in the urine, are then oxidised to 17 ketosteroids.
by shaking in the dark with 1 g. sodium bis-
muthate. The remaining bismuthate is removed
by the addition of 5 ml. 12% w/v sodium meta-
bisulphite. 4 ml. concentrated HCl is added
and the urine placed in a boiling water bath for
ten minutes to hydrolyse the ketosteroid sulphates.
The 17 ketosteroids are then extracted by shaking
with 15 ml. of ethylene dichloride for 5 minutes.
The aqueous phase is removed by suction and the
extract washed successively with 50% V/V HCl
water, 2N NaOH, and water till neutral. The
extract is then evaporated to dryness on a water
bath. The results are then read as for 17 keto-
steroids.

The estimation of 17 hydroxypregnanolone

In 1955 Appleby and Norymberski published
a method for the estimation of 17-Hydroxy-20-
oxosteroids unsubstituted at C21. 17-hydroxy-
pregnanolone is a member of this group. The
method consisted in the conversion of all 17-
hydroxy-cortic steroids, other than 21 deoxy-
ketols, into 17 ketones using sodium bismuthate.
The urine was then treated with sodium borohydride to reduce all the ketones to alcohols and at the same time to reduce the 21-deoxyketols to 17:20-glycols. These were then converted to 17 ketosteroids by oxidation with sodium bismuthate. The 17 ketosteroids thus measured are derived solely from the 21 deoxyketols.
The estimation of 17α-OH pregnanolone

Stage I | Stage II | Stage III
NaBio₃  | NaBH₄   | NaBio₃

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
\text{CHOH} & \quad \text{CHOH} & \quad \text{CHOH} \\
\text{C} \cdots \text{OH} & \quad \text{C} \cdots \text{OH} & \quad \text{C} \cdots \text{OH}
\end{align*}
\]

\[
\begin{align*}
\rightarrow & \quad \rightarrow & \quad \rightarrow \\
\text{CO} & \quad \text{OH} & \quad \text{OH}
\end{align*}
\]

Stage II

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{CH}_2\text{OH} \\
\text{CHOH} & \quad \text{CHOH} \\
\text{C} \cdots \text{OH} & \quad \text{C} \cdots \text{OH}
\end{align*}
\]

\[
\begin{align*}
\rightarrow & \quad \rightarrow & \quad \rightarrow \\
\text{CO} & \quad \text{OH} & \quad \text{OH}
\end{align*}
\]

Stage III

\[
\begin{align*}
\text{C} \cdots \text{OH} & \quad \text{C} \cdots \text{OH} \\
\end{align*}
\]

Zimmermann Chromogen

Blank estimation as control

NaBH₄  | NaBio₃  | NaBH₄  | NaBio₃

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 & \quad \text{CH}_3 \\
\text{C} \cdots \text{OH} & \quad \text{C} \cdots \text{OH} & \quad \text{C} \cdots \text{OH}
\end{align*}
\]

\[
\begin{align*}
\rightarrow & \quad \rightarrow & \quad \rightarrow \\
\text{CO} & \quad \text{OH} & \quad \text{OH}
\end{align*}
\]
Details of the method used for the estimation of 17-hydroxypregnanolone

10 ml. of urine containing no preservative was shaken in the dark for one hour with 2 g. of sodium bismuthate after it had been acidified by the addition of 10 ml. of glacial acetic acid. The sodium bismuthate was then reduced by the addition of 8 ml. of a fresh solution of 12% w/v sodium metabisulphite. 4 ml. of concentrated hydrochloric acid was added and the urine then shaken with 25 ml. of ethylene dichloride to extract the steroids. The extraction was then evaporated to dryness on a water bath. The steroids are then redissolved in \( \frac{1}{3} \) ml. absolute alcohol and \( 3\frac{1}{2} \) ml. distilled water. 20 mg. of sodium borohydride is then added and the extract allowed to stand overnight. After the addition of 5 ml. glacial acetic acid 1 G. of sodium bismuthate is added and the extract shaken in the dark for one hour. The remaining bismuthate is reduced by the addition of 5 ml. of the 12% w/v sodium metabisulphite. 4 ml. of concentrated hydrochloric acid is then added. The steroid is then extracted by shaking with 15 ml. of ethylene dichloride for five minutes. The extract is
evaporated to dryness on a boiling water bath. It is then applied to the column of silica gel in exactly the same way as described in the detailed method for the chromatography of the 17 ketogenic steroids.

The aetiocholanolone derived from the 17-hydroxy pregnanolone is measured in the second fraction. In order to be certain that the reagents are reacting properly at the same time one of the samples is treated with sodium borohydride before being estimated. This converts the Aetiocholanolone to an alcohol which, not being extracted, is not measurable. By the absence of any Zimmermann chromogens in the final reading, the effectiveness of the various steps are verified.
Diagram 6. The estimation of 17 hydroxy-pregnanolone. The demonstration of the quantity of this steroid measured by chromatography of the 17 ketogenic steroids in the pregnanetriol fraction and that estimated free from pregnanetriol.
Results

TABLE I AND DIAGRAM 6

<table>
<thead>
<tr>
<th></th>
<th>17 ketogenic steroids</th>
<th>21-deoxyketols (17 OH Pregnanolone)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ug/10ml.</td>
<td>Mg./24hr.</td>
</tr>
<tr>
<td>Pp.</td>
<td>79.5</td>
<td>4.7</td>
</tr>
<tr>
<td>Rf.</td>
<td>171.5</td>
<td>23.8</td>
</tr>
<tr>
<td>Cb</td>
<td>54.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Df</td>
<td>277.2</td>
<td>108.6</td>
</tr>
<tr>
<td>Sh</td>
<td>135</td>
<td>91</td>
</tr>
</tbody>
</table>

This method, though lengthy, is simple to carry out and has demonstrated a considerable amount of C21 deoxyketols chiefly of the type without an oxygen function at the C11 position, in the urines of children with congenital adrenal hyperplasia. The steroid present in the greatest quantity belonging to this group is 17-hydroxy-pregnanolone. Lieberman and Dobriner 1945, Mason and Stickler 1947, and Miller and Dorfman 1950.
In the third fraction the little steroid measured must be derived from C21 deoxyketols with an oxygen group at the C11 position. These have been found in the urine in small quantities, Burstein, Savard and Dorfman [1953].

**CHROMATOGRAPHY OF THE 17 KETOGENIC STEROIDS**

The adrenal steroids are largely excreted as water soluble glucuronides. In order to extract them from urine it is necessary to hydrolyse them to free steroids, which are soluble in water-immiscible solvents. The methods of Bongiovanni and Clayton [1954] and Sterne [1957], for the measurement of pregnanetriol make use of the enzyme β-Glucuronidase for this hydrolysis. This enzyme may be inhibited by substances in the urine. Preliminary extraction of the steroids from the urine overcomes this difficulty but lengthens the procedure, (Sterne, 1957). Both these methods for pregnanetriol estimation use solvents with a specific gravity less than water. Extraction
and washing has therefore to be carried out in a separating funnel.

The method of Morris [1959] overcomes both these disadvantages. No enzymes are used. The 17 ketogenic steroid glucuronides are oxidised to either free ketosteroids or their formates by the use of sodium bismuthate as described by Norymberski and Brooks [1953]. The solvent used, ethylene dichloride, has a higher specific gravity than water and the whole extraction is carried out in one tube.
THE CHEMISTRY OF THE METHOD

Urine contains 17 ketosteroids, the C19 steroids, and the ketogenic steroids, the C21 steroids. Borohydride is used first to remove the 17 ketosteroids by reducing them to alcohols which are not measured.

\[
\begin{align*}
\text{O} & \quad \rightarrow \quad \text{OH} \\
\wedge & \quad \wedge
\end{align*}
\]

At the same time the C21:17:20 ketols are reduced to glycols, as described by Appleby Gibson, Norymberski and Stubbs [1955].

\[
\begin{align*}
\text{CH}_3 & \quad \rightarrow \quad \text{CH}_3\text{OH} \\
\text{CO} & \quad \text{CH}_2\text{OH} \\
\wedge & \quad \wedge
\end{align*}
\]

2Deoxy-17:20 Ketol \quad 2Deoxy-17:20 glycol.

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \rightarrow \quad \text{CH}_2\text{OH} \\
\text{C}=\text{O} & \quad \text{CH}_2\text{OH} \\
\wedge & \quad \wedge
\end{align*}
\]

2Hydroxy-17:20 Ketol \quad 2Hydroxy-17:20 glycol.
The second reaction, oxidation with sodium bismuthate in the presence of acetic acid, results in the oxidation of the C21 glycols thus formed, and those already present in the urine, to 17 ketosteroids.

The 17 ketosteroids are then extracted with ethylene dichloride, washed and evaporated to dryness. The extract contains the 17 ketosteroi{d, Aetiocholanolone (3α-hydroxy-5γ-androstan-17-one) which is the oxidation product of pregnanetriol, and 17 ketosteroids with an oxygen grouping at the C11 position, (3α11γ di-hydroxy-5γ-androstan-17-one and 3α-hydroxy-5γ-androstrane 11,17-dione), the oxidation products of the 11 oxygenated C21, 17 OH steroids, the chief of which are tetrahydrocortisone E and F. In congenital adrenal hyperplasia the oxidation product of 11 keto-pregnanetriol will also be measured.
The former group is separated from the latter more polar substances by the use of column chromatography. Finally, the amount of steroid present is estimated by the colour reaction of Zimmermann [1935], whereby a purple colour is given by steroids with a (Ketol) group at the C17 position with Meta dinitro benzene and potassium hydroxide.

Reagents used

The sodium bismuthate (B.D.H. analar grade) was of coarse grain and yellow in colour. The fine grain, khaki coloured bismuthate was not found suitable.

The petroleum ether (B.D.H. analar grade b.p. 60-80) was re-distilled. The ethyl acetate (B.D.H. analar grade) was first washed with 10% w/v Na$_2$CO$_3$ and re-distilled. The solvent mixtures 25%, 35%, 65% ethyl acetate in petroleum ether were saturated with water at room temperature. A silica gel of consistent quality was obtained from Light and Co. Ltd., mesh size 100-200 Davison U.S.A. grade 923. Each fresh batch was tested with aetiocholanolone and an 11 oxygenated ketosteroid to ensure that the correct
elution pattern was obtained. The water content of the silica gel being adjusted as necessary. The results of plotting the amount of Zimmermann chromogen measured in 5 ml. fractions eluted from the column are shown in Diagrams 3, 4 and 5. The Zimmermann reagents 1% w/v m-dinitro benzene in re-distilled absolute alcohol and 8N KOH aqueous KOH to which is added 75% re-distilled ethanol in water, are described by Pearson and Giaccone [1948].

THE METHOD IN DETAIL

A complete twenty-four hour collection of urine or a single sample is obtained and no preservative is added. A 10 ml. fraction or, in children suspected of suffering from congenital adrenal hyperplasia, a 5 ml. fraction, is put in a glass stoppered tube of 70 ml. capacity, 50 mg. of sodium borohydride is added and the mixture allowed to stand overnight. Glacial acetic acid 10 ml. and 2 g. of sodium bismuthate are added, and the urine shaken in the dark for one hour. 8 ml. of a freshly prepared 12% w/v solution of sodium metabisulphite is added and the mixture
One month old $\delta$ congenital adrenal hyperplasia c.s.

Five day old $\Phi$ congenital adrenal hyperplasia c.B.

\[
\begin{align*}
\text{Fraction I} & \quad \text{20\,ug octiocholanolone} \\
\text{Fraction II} & \quad \text{10\,ug norexosterone}
\end{align*}
\]
DIAGRAM 4

S. H.

Girl infant congenital adrenal hyperplasia

mg/5 ml. Fraction

25% ethyl acetate I
35% ethyl acetate II
65% ethyl acetate III

D. D.

61/2 in adrenal failure

mg/5 ml. Fraction

I
II
III
Diagram 5

8 congenital adrenal hyperplasia
on 75 mg cortisone daily D.

μg/5 ml Fraction

25% ethyl acetate 35% ethyl acetate 65% ethyl acetate
is shaken till all the bismuthate is reduced, 5-30 mins. is the length of time necessary depending on the quality of the bismuthate used. To the resulting solution, 4 ml. of concentrated hydrochloric acid is added.

The steroids are then extracted with 30 ml. of ethylene dichloride by shaking for 5 mins. The aqueous phase is allowed to settle out by standing or, if an emulsion has formed, by centrifuge. The aqueous phase is then removed by suction and the extract washed successively with 50% V/V HCL, 2N NaOH, and distilled water till neutral. Three washes are found to be adequate. Absolute alcohol is added, till the solution is clear, to facilitate the removal of any remaining water and the extract is then evaporated to dryness on a water bath.

A chromatographic column 1 cm. in diameter is prepared by cleaning with alcohol, drying and then washing through with 25% ethyl acetate in petroleum ether. The column is then filled with this solution and 2 g. silica gel added. The silica gel is shaken and then allowed to settle under gravity so that no air bubbles are
present and it has a smooth ground glass appearance. The elution solution is run till it reaches the upper surface of the silica gel. 5 ml. of the 25% ethyl acetate/petroleum ether is added to the dried extract and this is applied to the chromatographic column. The extract is washed with further amounts of 25% ethyl acetate/petroleum ether until the first fraction of 20 ml. is collected and this is discarded. 35% ethyl acetate/petroleum is then passed through the column and the second 20 ml. Fraction (II) is collected. Finally 65% ethyl acetate/petroleum ether is passed through the column and a third 20 ml. Fraction (III) is collected.

Fraction II and III are then evaporated to dryness on a boiling water bath.

ZIMMERMANN REACTION

Two standards of 20μg. dehydro-iso-androsterone and a blank for reagents only, are set up. 0.4 ml. of 1% m-dinitro benzene in alcoholic solution and 0.3 ml. of an aqueous solution of 8 N KOH is added to each tube and the colour allowed to develop, in the dark, over 25 minutes. In the summer months it is found necessary to keep these reagents in a refrigerator
and allow the colour to develop while the tubes are kept in a cold water bath in order to avoid the development of a brown colour that interferes with the readings. 2 ml. of 75% aqueous alcohol solution is added to each tube and the optical densities read at 520, 440 and 600 μm on a Unicam S.P.600 spectrophotometer. A correction factor $E_{520} - \frac{1}{2} (E_{440} - E_{600})$ is necessary (Allen 1950). No correction is made for the different chromogenicity of the steroids measured and no correction has been made for the difference in the molecular weights of aetiocholanolone and pregnanetriol, because it is known that in congenital adrenal hyperplasia, and this study deals chiefly with this defect, that other steroids are also measured in Fraction II. The correction factor for Fraction III is not known and therefore it was felt unwise to correct either fraction for difference in the molecular weights between the 17 ketogenic steroids and the 17 ketosteroids.

The method has been found pleasant to use. It has the advantage of only requiring small
quantities of urine, indeed in some of the untreated patients with congenital adrenal hyperplasia only one ml. of urine was used. The results are obtainable within twenty-four hours, and it is possible to estimate four urines simultaneously. Satisfactory results were obtained on urines a week old, though in all cases it was felt wise to estimate the steroid content as soon as possible.

RESULTS OF CHROMATOGRAPHY OF THE 17 KETOGENIC STEROIDS

I. NORMAL CHILDREN

Estimations were carried out on the twenty-four hour collections of urine from thirty healthy children of colleagues or convalescent children in hospital. The results are shown in Table II and Diagram 7. These show that in normal children, the ratio of the pregnanetriol fraction II to the 11 oxygenated 170H steroids Fraction III lies between 0.06 and 0.57. The mean ratio is 0.24. The excretion of pregnanetriol increases with age but the ratio is independent of age.
Discussion

The estimations in normal children show that only a very small quantity of pregnanetriol like substances is measurable in the urine of normal children. At an age when the infant's fluid intake is high, that is before he is weaned on to solid food, he passes large quantities of dilute urine. The pregnanetriol content under these circumstances is not always measurable. However, there has always been a measurable quantity of the 11 oxygenated 170H steroids so that even in a single specimen of urine there has been no doubt that a ratio is normal and that the child does not suffer from congenital adrenal hyperplasia. The ratio of 0.24 is in keeping with the ratio of pregnanetriol to the 17 ketogenic steroids (Norymberski) of 1/3 quoted by Prunty [1958] in the normal adult.

The pregnanetriol excretion increased with age apart from this period in infancy when it could not always be estimated. The figure for normal children using this method is less than one mg./24hrs. The only two children with figures greater than 1 mg. in twenty-four hours were pubescent.
TABLE II  Results obtained from the urine of normal children.
Fraction II: - 17 ketosteroids derived chiefly from pregnanetriol.
Fraction III: - 17 ketosteroids derived from 11-oxygenated 17 ketogenic steroids.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Fraction No.</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>II ug/10 ml.</td>
<td>III mg/24 hr.</td>
</tr>
<tr>
<td>Female</td>
<td>8 days</td>
<td>3.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Female</td>
<td>12 days</td>
<td>2.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Male</td>
<td>4 weeks</td>
<td>4.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Male</td>
<td>6 weeks</td>
<td>7.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Male</td>
<td>6 weeks</td>
<td>0.45</td>
<td>0.14</td>
</tr>
<tr>
<td>Male</td>
<td>3 months</td>
<td>0.6</td>
<td>0.025</td>
</tr>
<tr>
<td>Female</td>
<td>4 months</td>
<td>4.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Male</td>
<td>5 months</td>
<td>2.0</td>
<td>0.05</td>
</tr>
<tr>
<td>Female</td>
<td>8 months</td>
<td>8.6</td>
<td>0.28</td>
</tr>
<tr>
<td>Male</td>
<td>8 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 months</td>
<td>4.3</td>
<td>0.18</td>
</tr>
<tr>
<td>Female</td>
<td>2 1/2 years</td>
<td>8.1</td>
<td>0.18</td>
</tr>
<tr>
<td>Male</td>
<td>2 1/2 years</td>
<td>1.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Male</td>
<td>2 1/2 years</td>
<td>7.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Male</td>
<td>3 years</td>
<td>8.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Male</td>
<td>4 years</td>
<td>6.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Male</td>
<td>5 1/2 years</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>Male</td>
<td>6 1/2 years</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>Male</td>
<td>6 1/12 yrs.</td>
<td>5.6</td>
<td>0.34</td>
</tr>
<tr>
<td>Female</td>
<td>7 years</td>
<td>14.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Male</td>
<td>7 years</td>
<td>7</td>
<td>0.35</td>
</tr>
<tr>
<td>Female</td>
<td>8 years</td>
<td>9.3</td>
<td>0.36</td>
</tr>
<tr>
<td>Female</td>
<td>8 1/2 years</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Female</td>
<td>10 years</td>
<td>2.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Male</td>
<td>11 years</td>
<td>4.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Male</td>
<td>11 years</td>
<td>4.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Male</td>
<td>11 years</td>
<td>6.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Male</td>
<td>12 years</td>
<td>7.5</td>
<td>0.46</td>
</tr>
<tr>
<td>Male</td>
<td>12 years</td>
<td>8.0</td>
<td>1.28</td>
</tr>
<tr>
<td>Male</td>
<td>12 years</td>
<td>7.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Female</td>
<td>12 years</td>
<td>20</td>
<td>1.6</td>
</tr>
</tbody>
</table>
DIAGRAM 7

THE RESULTS OF CHROMATOGRAPHY OF THE

17 KETOGENIC STEROIDS IN NORMAL CHILDREN
II. CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA

The results obtained from the estimation of the urinary 17 ketogenic steroids in fifteen children with congenital adrenal hyperplasia, proved on clinical grounds by the presence of adrenal insufficiency, electrolyte disturbances, abnormal genitalia, advanced bone age, and on biochemical grounds by the presence of a raised 17 ketosteroid level and a greatly increased 17 ketogenic steroid level, are shown in Table III.

These results show that the amount of steroid present in both fractions is higher than that found in the twenty-four hour estimation in normal children. In many cases this difference is so great that it is evident in the results of the 10 ml. fractions in spite of the varying concentrations of the urines. The most important difference, however, is that there is a disproportionate increase in Fraction II, the pregnane-triol fraction. The mean ratio of Fraction II/Fraction III in the cases of congenital adrenal hyperplasia is 2. Further analysis of these
### TABLE XII

**CHROMATOGRAPHY OF THE 17 KETOGENIC STEROIDS**

**RESULTS IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>24 hr. vol.ml.</th>
<th>Fraction No.</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ug/10 mns.</td>
<td>mg/24 hrs.</td>
</tr>
<tr>
<td>C.B.</td>
<td>2 days single</td>
<td>58</td>
<td>75.4</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>5 days 180</td>
<td>54.8</td>
<td>52.2</td>
<td>1</td>
</tr>
<tr>
<td>B.C.</td>
<td>8 days 290 started on I.V. drip with hydrocortisone during collection of urine.</td>
<td>51</td>
<td>21</td>
<td>1.5</td>
</tr>
<tr>
<td>S.H.</td>
<td>12 days single</td>
<td>50</td>
<td>56.5</td>
<td>-</td>
</tr>
<tr>
<td>F.P.</td>
<td>15 days single</td>
<td>317</td>
<td>105</td>
<td>-</td>
</tr>
<tr>
<td>S.B.</td>
<td>15 days 200</td>
<td>174</td>
<td>136.4</td>
<td>5</td>
</tr>
<tr>
<td>T.Q.</td>
<td>19 days single</td>
<td>90.6</td>
<td>58.6</td>
<td>-</td>
</tr>
<tr>
<td>C.J.</td>
<td>1 mth. single</td>
<td>180</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>P.C.</td>
<td>5 wks. On I.V. drip and cortisone</td>
<td>-</td>
<td>146.7</td>
<td>100.8</td>
</tr>
<tr>
<td>H.B.</td>
<td>2½ mths.</td>
<td>16.2</td>
<td>17.7</td>
<td>0.6</td>
</tr>
<tr>
<td>D.D.</td>
<td>3 mths. single</td>
<td>133.5</td>
<td>46.0</td>
<td>-</td>
</tr>
<tr>
<td>C.M.</td>
<td>15 mths.</td>
<td>261.6</td>
<td>68.6</td>
<td>11.4</td>
</tr>
<tr>
<td>L.M.</td>
<td>6 yrs. 430</td>
<td>200</td>
<td>51.5</td>
<td>8.6</td>
</tr>
<tr>
<td>C.T.</td>
<td>9 yrs. 1000</td>
<td>111</td>
<td>56</td>
<td>25.4</td>
</tr>
<tr>
<td>K.P.</td>
<td>10 yrs. 720</td>
<td>1190</td>
<td>362</td>
<td>85.7</td>
</tr>
<tr>
<td>R.T.</td>
<td>15 yrs. 1020</td>
<td>411</td>
<td>116</td>
<td>42</td>
</tr>
</tbody>
</table>

Adults
1. single 115 36
2. " 98 75
3. " 162 69.5

1.3
2.3
DIAGRAM 8

THE RESULTS OF CHROMATOGRAPHY OF THE
17 KETOGENIC STEROIDS IN CHILDREN WITH
CONGENITAL ADRENAL HYPERPLASIA
CHROMATOGRAPHY OF THE 17 KETOGENIC STEROIDS IN CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA.
results show that the ratio in the older children of (2-4) with a mean of 3.3, is higher than that found in the children under one year of age (0.8-3.4), with a mean ratio of 1.6. Another interesting difference is evident. The mean ratio of the three infants who showed no evidence of adrenal failure, at the time of their initial steroid estimations, have a mean ratio of 0.9, whereas those who were in adrenal failure at the time of the estimations have a mean ratio of 1.9. (See Table IV).
<table>
<thead>
<tr>
<th></th>
<th>A. Infants not in adrenal failure</th>
<th>B. Infants in adrenal failure</th>
<th>C. Older children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.8</td>
<td>1.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>0.9</td>
<td>1.2</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td></td>
<td>1.9</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Chromatography of the 17 ketogenic steroids. The ratios of Fraction II/III in children of different ages, in adrenal failure and not in adrenal failure.
THE DIFFERENCE IN THE RATIO OF THE PREGNANETRIOL FRACTION TO THE 11 OXYGENATED 17OH STEROID FRACTION SEEN IN DIFFERENT AGES AND IN DIFFERENT DEGREES OF ADRENAL DEFICIENCY, IN CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA
CHROMATOGRAPHY OF THE URINARY 17 HYDROXY STEROIDS IN CONGENITAL ADRENAL HYPERPLASIA
DISCUSSION

A. Infants showing no sign of adrenal failure at the time of estimation

In these children the mean ratio, of the pregnanetriol fraction II to the 11 oxygenated 17 hydroxy steroid, fraction III, is the lowest obtained in the known cases of congenital adrenal hyperplasia. This low ratio cannot be due to an increased excretion of the metabolites of cortisol for though these infants must be secreting sufficient cortisol for their needs, they cannot, by the very nature of the metabolic defect, secrete an excessive amount of cortisol. Since the amount of steroid measured in Fraction III is greater than that found in normal children of a similar age, there must be a qualitative difference, the presence of other steroids. One of these steroids is 11 ketopregnanetriol known to be excreted in large amounts in the urine of patients with congenital adrenal hyperplasia.

In a more detailed study of the groups of steroids excreted by children with congenital hyperplasia, Bergstrand, Birke and Plantin, in
1959, found that the excretion of pregnanetriol was less than the excretion of 11 ketopregnanetriol in three infants with the "salt losing" type of congenital adrenal hyperplasia. In three older children, none of whom showed a salt losing tendency, pregnanetriol was excreted in greater amounts than 11 ketopregnanetriol. The figures were given as percentages of the total excretion and groups of steroids, rather than single steroids, were measured, as in the present method. This difference in the two groups of children lead to the suggestion that there could be a fundamental difference between the "salt loser" and the non salt loser. However, they also recognised that this difference may be a function of age. The present work supports the latter view. Two of the three infants in this study, who on the initial examinations showed a high excretion of Fraction III, believed to be due to the presence of 11 keto pregnanetriol, later developed the classical signs of the "salt loser" and then showed an increase in the ratio.
Further evidence to show that this difference is due to age is found in the work of Finkelstein and Cox [1958]. These workers have separated 11-ketopregnanetriol from pregnanetriol by paper chromatography in six children with congenital adrenal hyperplasia. The method used enables these steroids to be measured in pure form, but the estimation is only partially quantitative. They are able to show a measurable amount of 11-ketopregnanetriol in the urine of all the children with congenital adrenal hyperplasia. On analysing their figures for the infants, I found that the ratio of 11-ketopregnanetriol over pregnanetriol was, mean of the three infants, 1.7. The same ratio in the older children, mean of three cases was 0.15. Finkelstein and Cox do not comment on this difference.

More recently, Bongiovanni, Eberlein, Smith and McPadden [1959], have found a greater amount of 11-ketopregnanetriol than pregnanetriol in the urine of infants. These were all of the "salt
losing" type. There was, however, an older child who had been a "salt loser" who showed a relatively greater amount of pregnanetriol. These workers have come to the conclusion that the relatively greater amount of 11 ketopregnanetriol in the urines of infants with congenital adrenal hyperplasia is a function of age and not of the type of the syndrome.

The presence of this relatively greater amount of 11 ketopregnanetriol in the infants, suggests that chromatography of the 17 ketogenic steroids would not give a diagnostic ratio of the pregnanetriol fraction to the 11 oxygenated fraction in infancy. This however is not the case, provided it is realised that the ratio II/III is not as high in infancy as it is in the older child. A diagnostic ratio is obtained in infants because a second C21:17 hydroxy methyl steroid is measured with pregnanetriol in fraction II. This steroid 17α-hydroxy pregnanolone is the immediate precursor to pregnanetriol. It is converted to pregnanetriol by the sodium borohydride used in
the estimation of the 17 ketogenic steroids. When the 17α-hydroxypregnanolone was measured separately, as already described, it was found to constitute some 20-50% of the total steroid measured in this fraction.

The amount of pregnanetriol and 17α-hydroxy-pregnanolone measured in Fraction II, in the infants, is just less than the amount of 11 keto-pregnanetriol and the metabolites of cortisol, measured in Fraction III. The ratio is therefore high compared to the normal child where Fraction III is three times as high as Fraction II.

These results are in keeping with the work of Bongiovanni et al [1959], who found that the sum of the quantity of pregnanetriol and 17α hydroxypregnanolone did not exceed the quantity of 11 ketopregnanetriol in the infants.

B. Infants showing clinical evidence of adrenal failure at the time of estimation

These infants have a higher ratio of pregnanetriol Fraction II/ the 11 oxygenated 17 OH steroid Fraction III. The mean ratio is 1.8. Since all these are young children, they must be
still excreting a relatively greater amount of 11 ketopregnanetriol than of pregnanetriol. By the known inability of these children to increase the production of cortisol, these children in adrenal failure must be producing inadequate cortisol. The excretory products of cortisol are 11 oxygenated and therefore are estimated in fraction III. There is an increase in the precursors of cortisol in the form of 17-OH-C21 methyl steroids, 17-OH-pregnanolone, pregnanetriol and 11 ketopregnanetriol, excreted in the urine of the children in failure. These are measured in both fractions. It would be expected then, that the lack of increase in the excretion of cortisol metabolites would cause an increase in the ratio of Fraction II to Fraction III.

This increase in ratio adds greatly to the value of Chromatography of the 17 ketogenic steroids in the diagnosis of congenital adrenal hyperplasia. These are the infants in need of urgent diagnosis and treatment. If on clinical grounds the diagnosis of adrenal insufficiency is entertained, then a single sample of urine can be obtained before cortisone is given or in the very
ill child during the first twenty-four hours, when the ratio will still be diagnostically abnormal.

C. Older children

In the older children, the ratio Fraction II/Fraction III has a mean value of 3.25. The reason for this high ratio, as already mentioned, is due to the higher percentage excretion of pregnanetriol to 11 ketopregnanetriol in the older age groups. There is usually little difficulty in obtaining a complete twenty-four hour collection of urine for estimation of the steroid content; however, a single specimen of urine obtained as an out patient can be used as a screening test in the child with precocious development.

**DIAGNOSTIC PROBLEMS**

The urines of twenty-six children in whom the diagnosis of congenital adrenal hyperplasia had been questioned were investigated by chromatography of the 17 ketogenic steroids (Table V, Diagrams 10, 11 and 12). In many of these, once
the method had been well tried, single specimens alone were investigated. No child with a normal ratio has subsequently been shown to have con­genital adrenal hyperplasia. The children can be grouped into those in whom the diagnosis was questioned because of abnormal external genitalia, those with vomiting, and those with precocious development.

There were twelve children with abnormal genitalia. Of these, four were chromatin negative and had normal excretion patterns. These were therefore male children with hypo­spadias. There were two children with chromatin positive buccal smears, and a normal excretion pattern. The mother of one of these had received progesterone during the early months of pregnancy. This child is a non adrenal female pseudohermaphrodite. The other child probably also belongs to this group, but there is no history of the administration to the mother of any substance likely to cause androgenization of the external genitalia. This child showed a normal response to A.C.T.H. and has a normal bone
Two older children were investigated because the clitoris was a little enlarged. Both these children had normal excretion patterns and one had a normal A.C.T.H. stimulation response. They are both considered to be normal girls.

There was a twelve year old "girl" who had a somewhat enlarged phallus at birth. At the age of 11½ years there had been a marked increase in the size of the phallus. The buccal smear showed a chromatin negative pattern, the 17 ketosteroids were rather high, 12 and 18 mg./24 hr. Chromatography of the 17 ketogenic steroids showed a normal ratio of Fraction II/Fraction III. She was eventually considered to be suffering from Gonadal dysgenesis with androgenization occurring at the time of puberty.

Finally, there were three females with congenital adrenal hyperplasia. All three showed the typically high ratio of Fraction II/III.

The second group consists of children who were vomiting or failing to gain weight. In some cases the incident was acute, the others were chronically ill with occasional periods of
diarrhoea and weight loss. The serum chemistry was found to be abnormal in some of the infants and in others scrotal pigmentation was considered to be excessive. Five children were admitted with a diagnosis of pyloric stenosis. Four of these had an abnormal ratio, a very high steroid output and were considered to be in adrenal failure from the "salt losing" type of congenital adrenal hyperplasia. The fifth was suffering from milk allergy; the ratio in this case was normal. Of the four children with mild vomiting and failure to thrive, all had normal ratios of Fraction II/III.

Four children's steroid excretion patterns were investigated because their development had been precocious. Three of these children had abnormal ratios and were shown to have high 17 ketosteroid excretion and an advanced bone age. One of these "boys" was shown to have a female chromatin pattern.

Chromatography of the 17 ketogenic steroids using either single samples or twenty-four hour collections has been a most satisfactory laboratory test in solving these many clinical problems.
Firstly it has differentiated the female pseudo-hermaphrodite of adrenal origin from those of non adrenal origin. It has confirmed the diagnosis in males of hypospadias by showing normal steroid ratios.

All but two of the children with signs suggestive of adrenal failure from the "salt losing" type of congenital adrenal hyperplasia had other confirmatory signs. In these children then, the test was more of a confirmatory nature. However, in two children the diagnosis was difficult from the clinical aspect. The first child showed no pigmentation despite his dehydration and abnormal serum electrolytes. His ratio was grossly raised, as were the total 17 ketogenic steroids and the 17 ketosteroids. He is a case of the "salt losing" syndrome. The second child, on the other hand, had definite and sufficiently marked scrotal pigmentation to suggest the diagnosis of congenital adrenal hyperplasia. Further support was given to this by a raised serum potassium, and a lowered serum sodium. The ratio of the ketogenic steroids was normal. He recovered from
his gastro-enteritis without the aid of corticoids and has remained well since. This test then has a negative as well as a positive diagnostic value.

The method is also shown to be useful in the differential diagnosis of the precociously developed boy. One of the cases on clinical examination alone was considered to be of the constitutional type. He had however an abnormal steroid ratio and raised 17 ketosteroids. An advanced bone age supported the abnormal ratio and the diagnosis of congenital adrenal hyperplasia was confirmed.
<table>
<thead>
<tr>
<th>AGE</th>
<th>FRACTION NO.</th>
<th>RATIO</th>
<th>DIAGNOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 days</td>
<td>75.4</td>
<td>0.8</td>
<td>Female pseudo hermaphrodite</td>
</tr>
<tr>
<td>3 days</td>
<td>23.4</td>
<td>0.46</td>
<td>Female congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>2 4 days</td>
<td></td>
<td>0.17</td>
<td>Male Hypospadia</td>
</tr>
<tr>
<td>5 days</td>
<td>8.8</td>
<td>0.3</td>
<td>Male Hypospadia</td>
</tr>
<tr>
<td>3 4 days</td>
<td>32</td>
<td>0.3</td>
<td>Normal child</td>
</tr>
<tr>
<td>4 12 days</td>
<td>56</td>
<td>0.9</td>
<td>Female congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>5 6 days</td>
<td>11.2</td>
<td>0.34</td>
<td>17KS</td>
</tr>
<tr>
<td>7 days</td>
<td>2.1</td>
<td>0.4</td>
<td>0.2 Male Hypospadia</td>
</tr>
<tr>
<td>8 days</td>
<td>16.3</td>
<td>0.4</td>
<td>0.22 Hypospadia</td>
</tr>
<tr>
<td>6 2 wks.</td>
<td>56.6</td>
<td>1.5</td>
<td>Male congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>7 15 days</td>
<td>166.2</td>
<td>1.8</td>
<td>Male congenital adrenal hyperplasia. Jaundiced</td>
</tr>
<tr>
<td>8 3 wks.</td>
<td>57.2</td>
<td>0.22</td>
<td>Feeding problem</td>
</tr>
<tr>
<td>9 3 wks.</td>
<td>2.1</td>
<td>0.14</td>
<td>Gastro-enteritis</td>
</tr>
<tr>
<td>6 wks.</td>
<td>8.1</td>
<td>0.05</td>
<td>Gastro-enteritis</td>
</tr>
<tr>
<td>10 3 wks.</td>
<td>21</td>
<td>0.3</td>
<td>Feeding problem</td>
</tr>
<tr>
<td>AGE</td>
<td>PROVISIONAL DIAGNOSIS</td>
<td>FRACTION NO.</td>
<td>FRACTION NO.</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>11</td>
<td>3 wks. Vomiting</td>
<td>11. 5. 23. 0.09 0.4 0.22</td>
<td>Milk allergy</td>
</tr>
<tr>
<td></td>
<td>Na. 135. mEq./l K. 5.9. mEq./l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1 mth. Male ?pyloric stenosis. High serum potassium low sodium. Treatment with D.O. C.A. started before collection of the sample and with cortisone before 24 hr. collection.</td>
<td>5. 105. 52.5 0.09 0.22</td>
<td>Male congenital adrenal hyperplasia</td>
</tr>
<tr>
<td></td>
<td>180. 80. single sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>5 wks. Male pyloric stenosis.</td>
<td>146.7. 100.8 3.4 2.3 1.5</td>
<td>Female congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>14</td>
<td>2½ mths. Hypospadias Congenital adrenal hyperplasia.</td>
<td>16.2. 17.7 0.6 0.65 1</td>
<td>Female congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>15</td>
<td>3 mths. Vomiting, weight loss. No pigmentation. Electrolyte changes.</td>
<td>133.5. 46. single sample</td>
<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>16</td>
<td>4 mths. Vomiting, failure to thrive. Congenital adrenal hyperplasia.</td>
<td>2.4. 10. 0.1 0.5 0.2</td>
<td>Gastrointestinal upset</td>
</tr>
<tr>
<td>17</td>
<td>7 mths. Enlarged phallus fused labia. Mother given progesterone during pregnancy.</td>
<td>0.03. 0.091. single sample</td>
<td>Non adrenal female pseudo hermaphrodite</td>
</tr>
<tr>
<td>18</td>
<td>15 mths. Precocious development Cong. adrenal hyperplasia.</td>
<td>264.6. 66.6 11.4 2.9 4</td>
<td>Male congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>19</td>
<td>2 yrs. Enlarged phallus Congenital adrenal hyperplasia.</td>
<td>1.6. 57.5 0.5 1.7 0.3</td>
<td>Female masculinised external genitalia</td>
</tr>
<tr>
<td></td>
<td>5.4. 32. 6.3 1.9 0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGE</td>
<td>PROVISIONAL DIAGNOSIS</td>
<td>FRACTION NO.</td>
<td>RATIO</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------</td>
<td>--------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II.</td>
<td>III.</td>
</tr>
<tr>
<td>20</td>
<td>3 yrs.</td>
<td>Hypospadias</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>?Congenital adrenal hyperplasia</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>5 yrs.</td>
<td>Hypospadias? female pseudo-hermaphrodite.</td>
<td>2.3</td>
</tr>
<tr>
<td>22</td>
<td>6 yrs.</td>
<td>Slightly enlarged phallus.</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>7 yrs.</td>
<td>Precocious development</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>?Congenital adrenal hyperplasia.</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>9 yrs.</td>
<td>Male precocious puberty.</td>
<td>111</td>
</tr>
<tr>
<td>25</td>
<td>10 yrs.</td>
<td>Repaired hypospadias. Precocious puberty. Female pseudo-hermaphrodite.</td>
<td>190</td>
</tr>
<tr>
<td>26</td>
<td>12 yrs.</td>
<td>Enlarged clitoris. Masculinisation.</td>
<td>12</td>
</tr>
</tbody>
</table>
CHROMATOGRAPHY OF THE URINARY 17KETOGENIC STEROIDS IN CHILDREN WITH ABNORMAL GENITALIA

Gender Distribution:
- Female (♀)
- Male (♂)

Age Distribution:
- 2 days
- 4 days
- 5 days
- 8 days
- 12 days
- 2 1/2
- 1/2
- 3 yrs
- 4 yrs
- 6 yrs

Legend:
- Congenital Adrenal Hyperplasia
- Others
CHROMATOGRAPHY OF THE 17KETOGENIC STEROIDS
IN CHILDREN WITH VOMITING

Ratio II/III

PREGNANETRIOL II
17-HYDROXYSTEROIDS III
CONGENITAL ADRENAL HYPERPLASIA.

Na128  Na124  Na128  mcg.
K83     K9.5   K7.5   mcg.

200  180  160  140  120  100  80  60  40  20

1/2  3/16  3/62  3/62  1/2  1/2  4/12
Diagnostic Problems.

♂ 10 yrs  
Precocious Puberty  
Bone age 16-18 yrs  
Ratio $\frac{III}{II} = 1.9$

♀ 10 yrs  
Repaired Hypospadias  
Bone age 18 yrs  
Ratio $\frac{IV}{III} = 3.4$

♂ 11 yrs  
? Cushing

After Treatment

Ratio $\frac{IV}{III} = 0.3$

- II Pregnanetriol
- III 11-oxygenated 170x
- IV 11x steroids

mg/24hrs
**EFFECT OF STIMULATION WITH ACTH. ON THE STEROID PATTERN**

In five children the effect of stimulation of the adrenal glands with ACTH was investigated (Diagrams 12-16, Table VI). Three of the children have congenital adrenal hyperplasia. One of these was receiving 25 mg. of cortisone a day at the same time. Of the other two, one is considered to be a non adrenal female pseudo-hermaphrodite, the other is a male with hypospadias who was said to have collapsed after the initial plastic repair.

In all the children there was an increase of the 17 ketosteroids and of the 17 ketogenic steroids. In the children with congenital adrenal hyperplasia, there was however a disproportionate increase in the pregnanetriol fraction so that the ratio Fraction II/III rose. In the other children there was no significant change in the ratio.

**Discussion of the results**

The increase in the ratio of Fraction II/III is, I believe, due to the fact that when there is stimulation of the adrenal glands of children
either by exogenous A.C.T.H. as in this group, or by endogenous A.C.T.H., as seen in the increased ratio of the infants in adrenal failure, there is an attempt to produce an increase in cortisol secretion. In children with congenital adrenal hyperplasia, there is a block to the production of cortisol. The precursors of cortisol are excreted in excess and these are measured in both Fraction II and III. There is not, however, the expected increase in the excretion products of cortisol which are measured in Fraction III. The ratio of II/III therefore rises.

In the normal child there is no significant change in the ratio. The minor increase seen may indeed be due to a temporary exhaustion of the hydroxylase enzymes brought about by the excessive stimulation by the corticotrophin.

This increase in ratio seen in the children with congenital adrenal hyperplasia was used to clarify the results before the diagnostic ranges of the ratio of Fraction II/Fraction III had been found out for the different age groups. The giving of corticotrophin to children with the "salt losing" syndrome may precipitate a crisis, as it did in one of these children. Now that further use of this method has clarified the results, corticotrophin is no longer used or recommended in any child unless there is no suggestion of a "salt losing" tendency and the diagnosis is in real doubt.
TABLE VI. THE EFFECT OF ACTH ON THE EXCRETION
PATTERNS OF 17 OH STEROIDS

<table>
<thead>
<tr>
<th>Day</th>
<th>24 hr. vol. ml.</th>
<th>FractiOn No. II ug/10 ml.</th>
<th>II FractiOn No. III mg/24 hrs.</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FractiOn No. III ug/10 ml.</td>
<td>FractiOn No. III mg/24 hrs.</td>
<td></td>
</tr>
<tr>
<td>I.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>FractiOn No. II 5 hr. No.</td>
<td>FractiOn No. III 5 hrs.</td>
<td>Ratio II/III</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>400 700 250 28 10</td>
<td>2.8</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>630 612.5 312.5 38.5 19.7</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>500 384 274 19.2 13.7</td>
<td>1.4</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>ACTH gel</td>
<td>10 units b.d.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1200 600 232 72 27.8</td>
<td>2.6</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>500 744 232 37.2 11.6</td>
<td>3.2</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1400 398 147 55.7 20.6</td>
<td>2.7</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1750 349 184 61.1 31.2</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>II.</td>
<td>3 yr. old chromatin negative child with scrotal hypospadias (Diagram 13).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>460 7 15.5 0.3 0.7</td>
<td>0.43</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>300 10 24.8 0.3 0.7</td>
<td>0.43</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>ACTH gel</td>
<td>10 units b.d.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>360 35 73.6 1.26 2.6</td>
<td>0.48</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>620 37.8 68 2.3 4.2</td>
<td>0.55</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>230 41.3 140 0.9 3.2</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>420 32 38.6 1.3 1.6</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>370 16.2 17.7 0.6 0.65</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>450 22 24.4 1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>III.</td>
<td>2½ months old female with congenital adrenal hyperplasia (Diagram 14).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>360 14 24.5 0.5 0.9</td>
<td>0.55</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>420 32 38.6 1.3 1.6</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>370 16.2 17.7 0.6 0.65</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>450 22 24.4 1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

continued...
### TABLE VI (Continued)

<table>
<thead>
<tr>
<th>Day</th>
<th>24 hr. vol. ml.</th>
<th>Fraction No. II ug/10 ml.</th>
<th>III ug/10 ml.</th>
<th>II mg/24 hrs.</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH gel 5 units b.d.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>490</td>
<td>23.9</td>
<td>19</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>470</td>
<td>55.5</td>
<td>27.7</td>
<td>2.6</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>575</td>
<td>27</td>
<td>29.5</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>625</td>
<td>37</td>
<td>19.4</td>
<td>2.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

IV. 4 yr. old chromatin positive child with masculinisation of the external genitalia. (Diagram 15).

| Control | 290 | 17 | 85 | 0.5 | 2.5 | 0.2 |
| 2 | 300 | 16 | 57.5 | 0.5 | 1.7 | 0.3 |
| 3 | 600 | 5.4 | 32 | 0.3 | 1.9 | 0.16 |
| 4 | 520 | 2 | 37.8 | 0.1 | 1.4 | 0.08 |

ACTH gel 10 units b.d.

| Control |  |  |  |  |  |  |
| 1 | 530 | 5 | 14.8 | 0.3 | 0.8 | 0.3 |
| 2 | 550 | 17 | 64 | 0.9 | 3.5 | 0.27 |
| 3 | 910 | 6 | 22.8 | 0.6 | 2 | 0.26 |
| 4 | 390 | 22.3 | 45.6 | 0.9 | 1.8 | 0.49 |

ACTH discontinued

| Control | 288 | 7.4 | 40.5 | 0.2 | 1.2 | 0.17 |

V. 3 weeks old child with congenital adrenal hyperplasia (Diagram 16).

| Control | Single specimen | 50 | 56 | 0.9 |

ACTH gel 5 units b.d.

| Control |  |  |  |  |  |  |
| 1 | 310 | 135 | 91 | 4.2 | 2.8 | 1.5 |
| 2 | 94 | 185 | 86 | 2.1 | 2.1 |
| 3 | 73 | 186 | 84 | 2.2 | 2.2 |

\[\gamma\] = Not complete 24 hr. specimens.
Congenital Adrenal Hyperplasia 4 yrs
Masculinization of external genitalia No 1
Ratio normal
Normal response to
Normal response to

ACTH
10 units B.D.

- PREGNANETRIOL FRACTION II
- 11 OXYGENATED 17 OH STEROIDS
  FRACTION III
- 17 KETO STEROIDS

mg/24 hrs
3yr Male with Hypospadias. No II

- Control period
- ACTH 10 units B.D.

Bars represent:
- Pregnanetriol Fraction II
- 11 Oxygenated 17α-H Steroid Fraction III
- 17-Ketosteroids

mg/24hrs.
Ratio abnormal with abnormal response to ACTH.

ACTH 5 units B.D.

- Degemtridol Fraction II
- 11-deoxycorticisol
- Fraction III
- 17 keto steroids.

mg/24 hrs.
Effect of Corticotrophin on a 4 yr old girl with congenital adrenal hyperplasia. No IV

Mean Normal

Ratio II/III

Fraction II

Fraction III

ACTH 20 units gel daily
CONGENITAL ADRENAL HYPERPLASIA RESPONSE TO A.C.T.H.

Graph showing the response to A.C.T.H. over 3 weeks with two different treatments: II pregnanetriol and III 11-oxygenated 17α-hydroxysteroids.

- Ratio
- ACTH
- Pregnanetriol
- 11-oxygenated 17α-hydroxysteroids

Legend:
- II PREGNANETRIOL
- III 11 OXYGENATED 17α-OH STEROIDS

Time:
- 0 days (baseline)
- 12 days
- 3 weeks
THE EFFECT OF CORTICOID TREATMENT ON THE STEROID EXCRETION PATTERN OF PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

The effect of treatment with corticoids has been followed in children with congenital adrenal hyperplasia. In all these children there is a gradual reversal of the ratio of the steroids measured in the two fractions until a normal ratio is achieved. The rate of this change depends on two factors; firstly the severity of the adrenocortical deficiency before treatment is started and secondly the route and dose of administered corticoid.

In the severe "salt loser" given oral or intramuscular cortisone an abnormal ratio of the two fractions persists for two to three days. Over this period there is, however, a marked reduction in the total quantity of the 17 ketogenic steroids excreted in the urine and a reduction of the ratio of the Pregnanetriol Fraction II/the 11 oxygenated 17-OH steroids Fraction III. In one severe "salt loser" given intravenous
Hydrocortisone, on the other hand, the ratio was reversed in the first twenty-four hours. In a second child, however, an abnormal ratio was still present thirty-six hours after the commencement of treatment of intravenous cortisol. It is therefore of importance that even after treatment has been given it may be possible to confirm the diagnosis of congenital adrenal hyperplasia on an abnormal steroid ratio, particularly in the "salt losers".

The effect of the use of the newer synthetic steroids is shown in the reduction in the amount of the 11 oxygenated 17-OH steroids measured in the third fraction. These steroids are given in very much smaller doses because their corticotrophin suppressive power is greater. Treatment with Prednisone or Prednisolone given in one-fifth of the dose of cortisone or hydrocortisone increases the ratio Fraction II/Fraction III by an amount that is not great enough to alter the usefulness of the ratio on estimations of single samples. However, if Dexamethasone is given in a dose of 1/75th to 1/100th of the dose of cortisone
then, as can be expected, the metabolites of this small amount of corticoid will not be measurable in the urine. When adequate suppression of the abnormal adrenal activity occurs, the ratio is still high, but very little steroid is measurable in either fraction. Until I have further experience with children on this corticoid I have felt it wiser to obtain a full twenty-four hour collection, so that the total pregnanetriol excretion can also be estimated. In one child whose urines are estimated regularly, treatment at one stage consisted of the powerful salt retaining hormone 9α-Fluorohydrocortisone alone. In this child, as in those of the former group, there was a high ratio despite adequate pregnanetriol suppression.

Children on this corticoid alone usually develop hypertension when adequate suppression of the adrenal is obtained. In small amounts, 9α-Fluorohydrocortisone has been used in conjunction with cortisone in the children with salt loss. Used in this way, there is no significant difference in the ratio as a control index than if cortisone
CHROMATOGRAPHY OF THE 17 KETOGENIC STEROIDS

DIAGRAM 17: THE EFFECT OF TREATMENT ON AN INFANT WITH CONGENITAL ADRENAL HYPERPLASIA OF THE "SALT LOSING" TYPE.

TABLE VII AND DIAGRAM 18: THE EFFECT OF CORTICOID TREATMENT ON A BOY OF FIFTEEN WITH THE VIRILIZING TYPE OF CONGENITAL ADRENAL HYPERPLASIA.

DIAGRAM 19: THE EFFECT OF STIMULATION WITH CORTICOTROPHIN FOLLOWED BY SUPPRESSIVE THERAPY WITH PREDNISOLONE ON AN ADULT WOMAN WITH CONGENITAL ADRENAL HYPERPLASIA.
CONGENITAL ADRENAL HYPERPLASIA

DOCA

CORTISONE

180
160
140
120
100
80
60
40
20
0

18 mg/10 ml
19
20
21
22
23
24
25
26
27
Aug

Pregnaneetriol
II
110xygenated
III

Normal
TABLE VII. EFFECT OF CORTICOIDS ON STEROID EXCRETION IN CONGENITAL ADRENAL HYPERPLASIA

<table>
<thead>
<tr>
<th>Day</th>
<th>24 hr. vol.</th>
<th>17-oxo-steroids mg/24 hr.</th>
<th>Fraction No.</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>II ug/10 ml.</td>
<td>III mg/24 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>1</td>
<td>750</td>
<td>33</td>
<td>246</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>1390</td>
<td>61.9</td>
<td>171.5</td>
<td>64.5</td>
</tr>
<tr>
<td>3</td>
<td>1070</td>
<td>41.5</td>
<td>325</td>
<td>71.5</td>
</tr>
<tr>
<td>4</td>
<td>Control period</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1350</td>
<td>37.5</td>
<td>61</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>1280</td>
<td>20.7</td>
<td>22</td>
<td>128</td>
</tr>
<tr>
<td>7</td>
<td>1620</td>
<td>23.8</td>
<td>34</td>
<td>44.5</td>
</tr>
<tr>
<td>8</td>
<td>1441</td>
<td>15</td>
<td>31</td>
<td>155</td>
</tr>
<tr>
<td>9</td>
<td>Cortisone 50 mg. 6 hourly.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1700</td>
<td>40</td>
<td>45</td>
<td>175</td>
</tr>
<tr>
<td>11</td>
<td>1280</td>
<td>56.8</td>
<td>48.5</td>
<td>232</td>
</tr>
<tr>
<td>12</td>
<td>1400</td>
<td>54.6</td>
<td>26.6</td>
<td>202</td>
</tr>
<tr>
<td>13</td>
<td>1280</td>
<td>23.6</td>
<td>27.5</td>
<td>110</td>
</tr>
</tbody>
</table>
THE EFFECT OF CORTICOSTERON ON THE HYDROXYSTEROID PATTERN IN A 15-YR-OLD BOY WITH CONGENITAL ADRENAL HYPERPLASIA.

- PREGNANETRIOL FRACTION II
- 11-OXYGENATED 17-OHSTEROIDS FRACTION III

Control Period

PREDNISONE 40mg daily

CORTISONE 200mg daily

Ratio

0 1 2 3 4

mg/24 hrs

DAYS
alone is used. The change in the ratio is shown in three different age groups in Diagrams 17, 18 and 19.

**CLINICAL CORRELATION**

Corticotrophin is shown to increase the ratio of the Pregnanetriol Fraction II/the 11 oxygenated 17-OH steroids Fraction III and corticoids are shown to decrease this ratio. The method should therefore be a good indication of adequate control of patients with congenital adrenal hyperplasia. Over the past eighteen months it has been used as a regular guide to treatment of the children whose case histories are given; it has also been used in isolated instances to estimate the adequacy of control in children being treated in other centres. Tables VIII and IX show the increase in ratio in two children with the "salt losing" type during periods when they were ill and the return to normal when they were clinically well. These high ratios were obtained despite the increased dose of cortisone given during the more severe illnesses.
TABLE VIII AND DIAGRAM 20

Clinical correlation - 4 year old "salt losing" male with congenital adrenal hyperplasia maintained on long acting D.O.C.A. crystals and cortisone. On reducing, and finally stopping, D.O.C.A., child lost weight and showed signs of adrenal insufficiency with mild infections. The cortisone dose was not altered. The pattern showed marked deterioration. 9αfluorohydrocortisone was added, 2 lbs. in weight was gained in a month and he remained well despite minor infections. Admitted with measles. During recovery, when urine was tested, pattern showed some deterioration but by discharge was normal.
<table>
<thead>
<tr>
<th>DATE</th>
<th>TREATMENT</th>
<th>CLINICAL ASSESSMENT</th>
<th>17-oxo steroids mg/24 hr.</th>
<th>FRACTION NO.</th>
<th>RATIO 11/111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 1958</td>
<td>Cortisone 10 mg. 8 hrly. Percorten crystals 50 mg. 4 wkly. Salt 3 G.</td>
<td>Well</td>
<td>1.8</td>
<td>11.5</td>
<td>81</td>
</tr>
<tr>
<td>Nov. 1958</td>
<td>Cortisone 30 mg. Percorten 25 mg. 4 wkly. Salt 3 G.</td>
<td>Well</td>
<td>3</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>Dec. 1958</td>
<td>Cortisone 30 mg. Salt 3 G.</td>
<td>Cough &amp; cold</td>
<td>6.7</td>
<td>54.6</td>
<td>90.5</td>
</tr>
<tr>
<td>Jan. 1959</td>
<td>do. do.</td>
<td>Cough, cold, listless, vomiting.</td>
<td>8.5</td>
<td>178.7</td>
<td>230</td>
</tr>
<tr>
<td>Feb. 1959</td>
<td>do. do. 9α-fluorohydrocortisone 0.25 mg.</td>
<td>Much better, gained 3 lbs.</td>
<td>2.2</td>
<td>14</td>
<td>218</td>
</tr>
<tr>
<td>March 1959</td>
<td>Cortisone 22.5 mg. Salt 3 G. 9α-fluorohydrocortisone 0.25 mg.</td>
<td>Well</td>
<td>1.7</td>
<td>6.4</td>
<td>80</td>
</tr>
<tr>
<td>April 11th 1959</td>
<td>Cortisone 30 mg.</td>
<td>Measles, recovering</td>
<td>2.2</td>
<td>17</td>
<td>67.5</td>
</tr>
<tr>
<td>12th 1959</td>
<td>9α-fluorohydrocortisone</td>
<td>Discharged</td>
<td>4</td>
<td>13.6</td>
<td>92</td>
</tr>
</tbody>
</table>
67 "Salt loser"
5 yrs

Demonstrating change of pattern on discontinuing Doca. and minor illness

Improvement on adding 9a Fluorohydrocortisone

Cortisone 30mg.
Urine brought up because child unwell. Bronchitis vomiting.

Cortisone 30mg
9a Fluoro Hydro C. 2.5mg.

Cortisone 30mg
Doca.

mg/24 hrs

8/10 10/11 23/12 12/1 20/2.
TABLE IX

Clinical correlation -

4 year old "salt loser". J.H.

October 1958, admitted for re-assessment as the child was having recurrent infections associated with signs of adrenal insufficiency and was not considered to be having adequate therapy. This appeared evident when he had an infection while in hospital. Improved on increased Cortisone, but better still when 9α-fluorohydrocortisone was added. Well until he developed measles. Patterns throughout correlate with clinical assessment of the child.
<table>
<thead>
<tr>
<th>DATE</th>
<th>TREATMENT</th>
<th>CLINICAL ASSESSMENT</th>
<th>17-oxo steroids mg/24 hr</th>
<th>FRACTION NO.</th>
<th>RATIO 11/111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 1958</td>
<td>Cortisone 5 mg. 8 hrly. Salt 3 g.</td>
<td>Admitted because of recurrent evidence of adrenal insufficiency with infection.</td>
<td>4.4</td>
<td>22</td>
<td>1.9</td>
</tr>
<tr>
<td>Age - 3 yrs. 7 months</td>
<td>Cortisone 7.5 mg. 8 hrly. Salt 3 g.</td>
<td>Developed respiratory infection &amp; vomiting in hospital.</td>
<td>6.3</td>
<td>267</td>
<td>47</td>
</tr>
<tr>
<td>Feb. 1959</td>
<td>do.</td>
<td>Well single sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18.7</td>
<td>55.7</td>
<td></td>
</tr>
<tr>
<td>March 1959</td>
<td>Cortisone 10 mg. 8 hrly. 9-fluorohydrocortisone 0.2 mg. daily</td>
<td>Admitted influenza 3rd day</td>
<td>76.25</td>
<td>67</td>
<td>6.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7th day</td>
<td>16.3</td>
<td>43.3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8th day</td>
<td>6.9</td>
<td>46.6</td>
<td>0.6</td>
</tr>
<tr>
<td>March 1959</td>
<td>do.</td>
<td>Well</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Very well</td>
<td>1.7</td>
<td>8</td>
<td>57.3</td>
</tr>
<tr>
<td>May 1959</td>
<td>Cortisone 25 mg. i.m. Cortisone 10 mg. 6 hrly 9-fluorohydrocortisone 0.2 mg. daily</td>
<td>Admitted with measles with clinical evidence of adrenal failure 3rd day</td>
<td>41</td>
<td>31</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4th day</td>
<td>52</td>
<td>29</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5th day</td>
<td>55</td>
<td>196</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Well</td>
<td>9</td>
<td>170</td>
<td>0.15</td>
</tr>
<tr>
<td>May 1959</td>
<td>do.</td>
<td>7th day</td>
<td>11.5!</td>
<td>50</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8th day</td>
<td>5.5</td>
<td>37.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9th day Discharged</td>
<td>8.4</td>
<td>45.5</td>
<td>0.4</td>
</tr>
<tr>
<td>DATE</td>
<td>TREATMENT</td>
<td>CLINICAL ASSESSMENT</td>
<td>17-oxo steroids mg/24 hr.</td>
<td>FRACTION NO.</td>
<td>RATIO</td>
</tr>
<tr>
<td>------------</td>
<td>-----------------------------------------------</td>
<td>---------------------</td>
<td>---------------------------</td>
<td>--------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>II</td>
<td>III</td>
<td>I</td>
</tr>
<tr>
<td>June 1959</td>
<td>Cortisone 10 mg. 8 hrly. 9α-fluoro-hydrocortisone 0.2 mg. daily.</td>
<td>Very well</td>
<td>2</td>
<td>7</td>
<td>62.6</td>
</tr>
<tr>
<td>July 1959</td>
<td>Cortisone 7.5 mg. 8 hrly. 9α-fluoro-hydrocortisone 0.2 mg. daily.</td>
<td>Very well</td>
<td>-</td>
<td>17</td>
<td>81.4</td>
</tr>
<tr>
<td>Aug. 1959</td>
<td></td>
<td>Well</td>
<td>2.7</td>
<td>11</td>
<td>32.6</td>
</tr>
<tr>
<td>Sept. 1959</td>
<td></td>
<td>Well</td>
<td>2.2</td>
<td>14.4</td>
<td>15.4</td>
</tr>
<tr>
<td>Oct. 1959</td>
<td>Cortisone 7.5 mg. t.d.s. 9α-fluoro-hydrocortisone 0.1 mg. daily.</td>
<td></td>
<td>1.6</td>
<td>23</td>
<td>125.7</td>
</tr>
<tr>
<td>Nov. 1959</td>
<td>Cortisone 5 mg. t.d.s. 9α-fluoro-hydrocortisone 0.1 mg.</td>
<td>Well</td>
<td>4.9</td>
<td>12.3</td>
<td>25</td>
</tr>
</tbody>
</table>
These Tables and the Diagram show that there is a good correlation between the clinical well being of a child and a normal ratio of the Pregnanetriol Fraction II/the 11 oxygenated 17-OH steroids Fraction III. In illness the ratio shows a rise above the normal level. Chromatography of the 17 ketogenic steroids is a reliable guide to the control of treatment. The change in the ratio is also very much more striking than the rise of one or two mg./24 hr. seen in the estimation of the 17 ketosteroids; moreover, for outpatient use single samples may be used.

INVESTIGATION OF SINGLE SAMPLES

Table X shows the ratios obtained from specimens passed at irregular intervals throughout the day by two children - one a normal girl, the other a "salt losing" congenital adrenal hyperplasia on treatment. There is some degree of variance in the ratios throughout the day, but at no time is this great enough to suggest that, in one, the child was not normal, or in the other, that control was not as good as desired. This
### Table X AND DIAGRAM 21

**Use of isolated specimens**

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Fraction No.</th>
<th>ug/10 ml.</th>
<th>Ratio II/III</th>
<th>Clinical details</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II</td>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Four specimens passed in 24 hours and estimated separately.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.8</td>
<td>32.7</td>
<td>0.2</td>
<td>Normal girl.</td>
</tr>
<tr>
<td>2</td>
<td>7.3</td>
<td>20.0</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.7</td>
<td>6.5</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11.0</td>
<td>30.5</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16.5</td>
<td>38.0</td>
<td>0.43</td>
<td>&quot;Salt losing&quot; congenital adrenal hyperplasia on cortisone.</td>
</tr>
<tr>
<td>2</td>
<td>11.3</td>
<td>37.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14.0</td>
<td>30.7</td>
<td>0.46</td>
<td>Male, age 4 years.</td>
</tr>
<tr>
<td>4</td>
<td>10.7</td>
<td>23.3</td>
<td>0.46</td>
<td></td>
</tr>
</tbody>
</table>

Fractions II and III explained in Table I.
O "Salt Looser" Age 4 yrs.
Penile urethra reared as a male. Single Samples

I 2am - 8am
Vol. 165 ml
Ratio II/III 0.43

II 8am - 2pm
Vol. 190 ml
Ratio II/III 0.3

III 2pm - 5pm
Vol. 150 ml
Ratio II/III 0.46

IV 5pm - 1am
Vol. 280 ml
Ratio II/III 0.46

II pregnanetriol
III 11-hydroxylated 170HCS.
Use of isolated specimens.

Results from investigations of patients with congenital adrenal hyperplasia treated with cortisone. One specimen was kept separate from remainder of twenty-four hour collection.

<table>
<thead>
<tr>
<th></th>
<th>Fraction No.</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II ug/10 ml.</td>
<td>III</td>
<td>II mg/24 hr.</td>
<td>III</td>
</tr>
<tr>
<td>A. Total</td>
<td>32</td>
<td>28.6</td>
<td>1.3</td>
<td>1.6</td>
</tr>
<tr>
<td>Single</td>
<td>28</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B. Total</td>
<td>16.2</td>
<td>17.7</td>
<td>0.6</td>
<td>0.65</td>
</tr>
<tr>
<td>Single</td>
<td>11.3</td>
<td>14.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. Total</td>
<td>16</td>
<td>57.5</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td>Single</td>
<td>8</td>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D. Total</td>
<td>13.6</td>
<td>92</td>
<td>0.68</td>
<td>4.6</td>
</tr>
<tr>
<td>Single</td>
<td>13.7</td>
<td>92</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. Total</td>
<td>28</td>
<td>192</td>
<td>5</td>
<td>34.6</td>
</tr>
<tr>
<td>Single</td>
<td>36.5</td>
<td>245</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fractions II and III explained in Table IX.
latter estimation was indeed carried out when the child was recovering from an infection. Other out patients were asked to keep one specimen of urine separate during their routine monthly twenty-four hour collection. 10 ml. of this separate specimen was taken for estimation and the remainder of this separate sample of urine was added to the rest of the twenty-four hour collection. An aliquot of the twenty-four hour sample was then taken for a second estimation. The results of five such coupled estimations are shown in Table XI. Once again the urines demonstrate that a single specimen gives a good indication of the ratio of the whole twenty-four hour collection.

THE DIAGNOSIS OF CONGENITAL ADRENAL HYPERPLASIA ON A SINGLE SPECIMEN OF URINE

Many examples of this have already been given in the diagnosis of the salt losers on a single sample of urine. Table XII and Diagram 22 show how this method was used to screen an adult family, one of whom was under investigation for sterility. Her history, of early rapid growth, of an enlarged
phallus from birth and early and distressing growth of facial hair, was classical of congenital adrenal hyperplasia. Estimation of the steroids confirmed the diagnosis. She showed a raised 17 ketosteroid level, a very high 17 ketogenic level, chromatography of the 17 ketogenic steroids gave a high pregnanetriol excretion with an abnormally high ratio of Fraction II/Fraction III. There are seven siblings. One of these has had miscarriages but has never produced a live child. She too came under investigation for sterility. Another sibling, the youngest member, a boy, was said to have been taller than his elder brother when he was young but was now much shorter. He was also said to have shaved early. From these histories I expected to find three members of this family with an abnormal ratio. The seven siblings sent about twenty ml. of a single specimen of urine in a universal container. Chromatography of the 17 ketogenic steroids was carried out on each of these samples and the results are shown in Diagram 23. Three members are shown to have congenital adrenal hyperplasia by the presence of an abnormally high ratio.
TABLE XII. THE USE OF ISOLATED SPECIMENS OF URINE AS A SCREENING TEST FOR SIBLINGS OF A PATIENT WITH CONGENITAL ADRENAL HYPERPLASIA.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Fraction No. II ug./10ml.</th>
<th>Ratio II/III</th>
<th>Clinical details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>115</td>
<td>3</td>
<td>Congenital adrenal hyperplasia.</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>98</td>
<td>1.3</td>
<td>Clinical findings and history of congenital adrenal hyperplasia.</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>162</td>
<td>2.3</td>
<td>History suggestive of congenital adrenal hyperplasia.</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>5</td>
<td>0.06</td>
<td>Normal.</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>14</td>
<td>0.34</td>
<td>Normal.</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>19</td>
<td>0.47</td>
<td>Normal.</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>25</td>
<td>0.3</td>
<td>Normal.</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>37.5</td>
<td>0.3</td>
<td>Normal.</td>
</tr>
</tbody>
</table>
CHROMATOGRAPHY OF URINARY 17-OH STEROIDS IN SINGLE SAMPLES OF URINE FROM SIBLINGS OF A CONGENITAL ADRENAL HYPERPLASIA.

- PREGNANETRIOL FRACTION II
- 11-OXYGENATED 17-OH STEROIDS

FRACTION III

μg/100ml
INTRODUCTION

The case histories of some of the children are given to demonstrate the use of chromatography of the 17 ketogenic steroids in the diagnosis and control of these children and to illustrate the various difficulties that have arisen in the care of the children.

The weights and heights are compared with those given by Tanner for the United Kingdom Children, 1957.
CASE 19. C.B.

Is the fourth child of healthy unrelated Irish parents. The first child, a girl, was born after a gestation of seven months and died. The genitalia were normal. The other two children are normal. The patient was a full time face delivery. The birth weight was 4.1 kg. (9 lbs.) and the length 53 cm. (21\textquoteleft\textquoteleft). The external genitalia had the appearances of a female pseudohermaphrodite with a large phallus, fused labia and a urogenital sinus at the base of the phallus. No other abnormalities were detected.
A single sample of urine was obtained on the 2nd day for chromatography of the 17 keto­genic steroids. The result was a ratio of the pregnanetriol fraction to the 11 oxygenated fraction of 0.8. This was the lowest ratio found in the cases of congenital adrenal hyperplasia, but was well above that found in normal children and was taken to confirm the diagnosis of congenital adrenal hyperplasia.

The child was transferred from the maternity hospital to the Children's Hospital on the 3rd day of life. Here further confirmation of the diagnosis was obtained by the presence of a chromatin positive buccal smear. Difficulties were encountered in the collection of twenty-four hour urine samples and this was not obtained till the 5th day.

Investigations of this -

Total volume: 180 ml.
17 ketosteroid level: 5.2 mg/24 hr.
17 ketogenic steroids (Norymberski): 7.8 mg/24 hr.
Chromatography of the 17 ketogenic steroids -

Pregnanetriol fraction (II): 0.95 mg/24 hr.

11 oxygenated 17 OH steroids fraction (III): 0.94.

giving a ratio of fraction II/III of 1.0.

Throughout these first six days the infant fed well, was lively, and showed no sign of adrenal insufficiency.

On the 7th day, however, there was a dramatic change. She became lethargic and ceased to suck. Her pulse slowed and she rapidly developed signs of peripheral failure. The systolic blood pressure was 90. A scalp vein infusion was set up and 25 mg. of hydrocortisone was given rapidly, followed by 5 mg. 6 hourly into the infusion. During this twenty-four hour period she passed 645 ml. of urine containing 387 mg.Na. 874 mg.K. 735 mg.Cl. The 17 ketosteroid excretion was 4.7 mg. The pregnanetriol fraction 1.4 mg., and the 11 oxygenated fraction 3.5 mg., the ratio of these two fractions had now fallen to 0.3.

On the 8th day she was much improved but was not yet sucking well enough to take her feeds orally, so intravenous therapy was continued and
hydrocortisone was again added to the drip. In addition 9α-fluorohydrocortisone was given orally because of its salt retaining properties. The serum electrolytes were Na 139 m. Eq/1 K 6.8 m. Eq./1. Urea 51 mg%. A complete specimen of urine was not obtained. The ratio of fraction II/III was 0.28.

The 9th day showed marked improvement. She was lively, with a good colour, the blood pressure was 120/80. The serum electrolytes were Na 141 m. Eq/1. K 6.2 m. eq/1. Urea 30 mg%. A twenty-four hour urine contained 3 mg. of 17 ketosteroids. Chromatography of the 17 ketogenic steroids - Pregnanetriol fraction (II) 0.69 mg/24 hr., 11 oxygenated fraction (III) 2 mg/24 hr., with a ratio II/III of 0.34. The intravenous medication was discontinued and she was given intramuscular cortisone, oral 9α-fluorohydrocortisone and 3 g. of salt was added to her normal intake of milk.

On the 10th day oral cortisone was given but she became so sluggish over her feeds that intramuscular cortisone was again used and for the next two days she had to be fed by tube.
The electrolytes had also shown a deterioration, the Na. had fallen to 137 m. eq/1 and the K risen to 10.2 m. eq/1.

By the 13th day she was taking feeds well but she required 10 mg. of cortisone 6 hourly, 0.2 mg. of 9α-Fluorohydrocortisone and 3 g. salt daily in order to maintain weight gain and normal electrolytes.

At six weeks she weighed 4.15 kg. (9 lbs. 7 ozs.) and was 56½ cm. (22¾") long. Twenty-four hour urines were now estimated at weekly intervals and at this time the Pregnanetriol fraction II had fallen to 0.1 mg/24 hrs., which is within normal limits for this method, the 11 oxygenated 17 ketogenic steroid fraction III was 1 mg. The ratio 0.1. The 17 ketosteroids were 0.2 mg/24 hr.

This baby presented as a problem of sex differentiation. Chromatographic fractionation of the 17 ketogenic steroids of a single sample of urine demonstrated an abnormal ratio of the two fractions confirming the clinical impression that she had congenital adrenal hyperplasia.
The buccal smear was later shown to be chromatin positive. She showed no signs of adrenal insufficiency until the 7th day when this presented quite rapidly as extreme lethargy and failure to suck. There was never any vomiting and no pigmentation was present. It was, however, apparent from the rapid deterioration that had the diagnosis not been known, and this very emergency awaited, she might have died before adequate treatment could have been given.
P.P. BORN 8.7.59.

P.P. is the seventh child of healthy unrelated Irish parents. The mother is blood group Rh. positive and W.R. negative. The fourth child, a boy, died at the age of one week on the day he was admitted to hospital because of vomiting. The sixth child died in the Children's Hospital with a congenital cardiac anomaly. The other four children are well. The patient was a normal delivery, birth weight 3.6 kg. (7 lbs.). He was breast fed. On the 2nd day he was noted to be jaundiced and this increased till the 4th day. By the 14th day he weighed only 3.2 kg. (6⅔ lbs.) and his general condition was unsatisfactory. He was listless and fed poorly. On the 15th day vomiting occurred after every feed and he was admitted to hospital the following day.

Examination showed a moderately jaundiced infant who was marasmic and mildly dehydrated. He weighed 3.15 kg. (6½ lbs.) and was 53.5 cm. (21") long. Beneath the jaundice there appeared to be an excessive pigmentation of the nipples,
scrotum and the perianal area. The penis was rather large and the testis both present, but small and soft. No other abnormalities were found.

A single sample of urine was obtained and showed the presence of chlorides. Chromatography of the 17 ketogenic steroids into Pregnanetriol fraction II and 11 oxygenated fraction III demonstrated the high ratio of Fraction II/III found in patients with congenital adrenal hyperplasia.

Ratio: 1.8

Serum Electrolytes: Na. 128 m.eq/1
                        K11, 3  Cl 99 m.eq/1.

Urea: 42 mg%.

Total bilirubin: 9.9 mg%, direct reacting, 2.4 mg%, indirect 6.7 mg.
The bone age was normal.

A scalp vein infusion was set up; fluids containing 5 g. NaCl were given over the following twenty-four hour period. At the end of this time his condition was much more satisfactory. The serum electrolytes were Na 128 K. 4.6 and Cl 104 m.eq/1. Urea 18 mg%. Estimations on twenty-four hour collection of urine, volume 580 ml., gave Na 696 mg. K. 104 mg. Cl 974 mg. The 17 ketosteroids were 8.5 mg/24 hr. Chromatography of the 17 ketosteroids gave a pregnanetriol fraction II of 5.4 mg/24 hrs. and an 11 oxygenated fraction III of 3.8 mg/24 hr. The ratio II/III was 1.44.

On the second day of treatment, intravenous fluids were continued, but D.O.C.A. 9 mg. was given intramuscularly over the twenty-four hour period. At the end of this period the serum electrolytes were Na. 156 K. 6.9 m.eq/1 and the urea 13 mg%. The urine collection over this period was 590 ml. and contained 596 mg. Na. 47 mg. K. and 975 mg. Cl/24 hr. The 17 keto-steroids were 9 mg/24 hr. Chromatography of the
17 ketogenic steroids, Pregnanetriol fraction II, 4.3 mg/24 hr., the 11 oxygenated fraction III 4.1 mg/24 hr. The ratio fraction II/III, 1.

On the third day cortisone was started, with 50 mg. intramuscularly and D.O.C.A. 4 mg. was also given. Oral feeding was tried. During this period 690 ml. urine was collected with an electrolytic content of Na. 932 mg. K.66 mg. Cl 1467 mg. The 17 ketosteroids were 7.4 mg/24 hr. The pregnanetriol fraction of the 17 ketogenic steroids 2.4 mg. and the 11 oxygenated fraction 2.4 mg. The ratio II/III of 1 being still abnormal.

On the fourth day he was taking his feeds very hungrily and was inclined to ingest large quantities of wind and subsequently vomit. His jaundice was now fading and the serum bilirubin had fallen to 3.5 mg%. The pigmentation was now more definite. The serum electrolytes were satisfactory, Na. 141 m.eq/1 K.5.9 m.eq/1 and the urea 22 mg%. The intramuscular D.O.C.A. was replaced by 9<sup>fluorohydrocortisone</sup>, initially in
a dose of 0.2 mg. twice daily. There was a failure to collect a full twenty-four hour urine, but on an incomplete sample the ratio II/III had now fallen to 0.18.

On the fifth day a complete specimen of 390 ml. was obtained. The 17 ketosteroids were 0.5 mg/24 hr. Chromatography of the 17 ketogenic steroids; Pregnanetriol fraction II 0.38 mg., and the 11 oxygenated fraction III was 1.4 mg. The ratio II/III was 0.34.

Two weeks after admission he was feeding well and weight was 3.2 kg. (7 lbs. 7 ozs.). He was maintained on cortisone, 5 mg. four times daily, 3 g. added salt and 0.5 mg. of 9α-fluorohydrocortisone daily. The latter proved too high a dose for him. He became oedematous and his blood pressure rose, so that during the third week this was reduced to 0.1 mg. daily. This dosage gave fairly satisfactory control, but weight gain was not steady and the serum sodium tended to be low while the potassium tended to be high. He was still a very greedy feeder and took a dislike to his cortisone which made it a little difficult to tell just how much was absorbed. The cortisone
dose was therefore raised to 7.5 mg. four times daily and the mixture made more palatable.

At the age of six weeks, he weighed 3.8 kg. (8lbs.5ozs.) The serum sodium 141 m.eq/1. K. 5.4 m.eq/1 and the urea 47 mg%. Urinary 17 keto-steroids over this period were 0.6 mg/24 hr., and the chromatography of the 17 ketogenic steroids gave a pregnanetriol fraction of 1 mg./ 24 hr., with a ratio of fraction II/III within normal limits. Cortisone was reduced to 5 mg. four times daily and then to three times daily, but once again he failed to gain and then lost weight. There was an apparent increase in pigmentation and in vomiting. Better control was obtained on cortisone 7.5 mg. four times daily, 9α fluorohydrocortisone 0.1 mg. daily, and salt 3 g. in addition to that in his feeds.

**Comment:** This child presented in the commonest way seen in the salt losing type of congenital adrenal hyperplasia. An unusual feature was the presence of jaundice which obscured the pigmentation initially. Later, however, P.P. demonstrated the reappearance of pigmentation whenever his
control was not good. Because of his tendency to vomit, even when well, his control has been particularly difficult, but his progress is now very satisfactory.
CONGENITAL ADRENAL HYPERPERPLASIA

Average Normal

Single Sample

DOCA CORTISONE

mg/100ml

Aug. 2

He is the second child of healthy unrelated Irish parents. The first child is a normal daughter. His birth weight was 3.4 kg. (7 lbs. 10 ozs.). The delivery was normal at term. He fed well on a cow's milk formula and at the age of five weeks weighed 10 lbs.

Between the fifth and eighth week he first ceased to gain and then, with the onset of vomiting, he lost weight. By the eighth week his weight was only 4.15 kg. (9 lbs.). Vomiting continued and at the age of three months he was admitted to hospital with a diagnosis of congenital pyloric stenosis.

On examination his weight was now only 3.4 kg. (7 lbs. 12 ozs.). He was dehydrated with poor circulation shown by cold blue hands and feet and a rather mottled appearance of the skin. There was no suggestion of any abnormal pigmentation and the external genitalia were not remarkable. No pyloric tumour could be felt. On the day following admission, the serum electrolytes were Serum Na. 122 m.eq/1. K. 9 m.eq/1. Cl. 79 m.Eq/1, and the blood urea 98 mg%. A single sample of
urine was obtained and this showed that there was much chloride present by the routine ward test with silver nitrate. Chromatography of the 17 ketogenic steroids was carried out on 5 ml. of urine. The results of this investigation showed 133.5 ug/10 ml. in the pregnanetriol Fraction II and 46 ug/10 ml. in the 11 oxygenated 17-OH steroids. The ratio II/III of 3.4 was diagnostic of congenital adrenal hyperplasia.

While this estimation was being carried out and before treatment was started with corticoids, two complete twenty-four hour collections were obtained. During this time the child was being treated with intravenous fluids. Despite the replacement of the estimated sodium loss, the serum electrolytes remained abnormal. The results are tabulated below.

15-16/12/59: Serum electrolytes: Na.124 m.eq/1. K. 5.9 m.eq/1.

Urine steroids: 17 ketosteroids 5.5 mg/24 hr. 17-OH 40 mg/24 hr.

Chromatography of the 17 ketogenic steroids Fraction II 17.7 mg/24 hr. Fraction III 8.2 mg/24 hr. Ratio II/III 2.1.
16-17/12/59: Serum electrolytes Na. 109 m.eq/1.  
K. 5.1 m.eq/1. Cl. 82 m.eq/1.  
CO₂ 20 vols.%.  
Urine volume 430 ml. steroids  
17 ketosteroids 6.7.  
17-OH steroids 22.4 mg/24 hr.  
Chromatography of the 17 ketogenic steroids Fraction II 11.9 mg/24 hr.  
Fraction III 4.6 mg/24 hr.  
Ratio II/III 2.5

On the 17th, treatment with intravenous hydrocortisone was started in a dose of 5 mg. six hourly following a loading dose of 25 mg. 9α Fluorohydrocortisone 0.1 mg. twice daily was given orally and sodium replacements were given in the infusion. There was a rapid improvement in the child's condition and this was reflected in the improvement in the serum electrolytes and in the reduction in the urinary steroids.

17-18/12/59: Serum electrolytes Na. 122 m.eq/1.  
K. 3.6 m.eq/1. Cl. 90 m.eq/1.  
Blood urea 9 mg%.  
Urine volume 670 ml.  
17 ketosteroids 3 mg/24 hr.

Chromatography of the 17 ketogenic steroids. Fraction II 4.6 mg/24 hr.  
Fraction III 5.3 mg/24 hr.  
Ratio II/III 0.87.

Three days later the child was feeding well and was receiving 10 mg. of cortisone orally at six hourly intervals and 9α Fluorohydrocortisone 0.2 mg. daily, Salt 2 g., was added to his feeds daily.
20-21/12/59:

The urine volume was 335 ml. The 17 ketosteroids 2 mg/24 hr. Chromatography of the 17 ketogenic steroids showed a pregnanetriol Fraction II of 0.8 mg/24 hr., and an 11 oxygenated 17-OH steroid Fraction III of 3.9 mg/24 hr. The ratio was now normal 0.2. His progress continued to be satisfactory with a steady weight gain till the 27th, when he developed pneumonia. By this time his cortisone dose had been reduced to 10 mg. eight hourly, and the 9α-Fluorohydrocortisone 0.1 mg. daily.

28-29/12/59:

A further collection of urine was obtained. The volume was 555 ml. The 17 ketosteroids 0.9 mg/24 hr. and Chromatography of the 17 ketogenic steroids demonstrated 0.6 mg/24 hr. in Fraction II and 2.2 mg/24 hr. in Fraction III. The ratio II/III being 0.27.

He responded rather slowly to antibiotics and there was a temporary failure to gain weight. However there were no signs of adrenal failure and further urine obtained (3-4/1/60) had a volume of 410 ml. with a total 17 ketosteroid of 0.8 mg/24 hr.
Chromatography of the 17 ketogenic steroids
Fraction II 0.4 mg., pregnanetriol and Fraction III 2.6 mg/24 hr. 11 oxygenated 17-OH steroids
the ratio II/III being 0.15. His weight was
now 4.4 kg. (9 lbs. 13 ozs.), and the length
57.7 cm. (22-5/8"). By the following week his
chest infection had resolved. His treatment was
now adjusted to cortisone 7.5 mg. eight hourly,
9α-Fluorohydrocortisone 0.1 mg. daily with three
grams of salt added to his feeds.

Urinary estimation 14-15/1/60. Volume
460 ml. Total 17 ketosteroids 1.1 mg/24 hr.
Chromatography of the ketogenic steroids, Pregnane-
triol fraction 0.7 mg/24 hr. 11 oxygenated 17-OH
steroids 4.2 mg/24 hr., with a ratio of 0.17.
At this time the child weighed 4.9 kg. (10 lbs.
13 ozs.).

Comment: This child presented as a difficult
diagnostic problem. The diagnosis of congenital
adrenal hyperplasia was initially considered un-
likely because of the absence of pigmentation and
the reporting of a normal excretion of sodium and
chloride in the urine by the laboratory. However,
it was quite evident that the child was losing salt. Chromatography of the 17 ketogenic steroids was again diagnostic on a single sample of urine. In this case there was however a delay in receiving the first sample of urine, otherwise treatment could have been started twenty-four hours earlier and much anxiety over the child's condition avoided.
Treatment started

mean normal

16
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

μg/10ml

mg/24hrs.

D.D.

- Pregnanetriol Fraction II
- Oxidated Monosteroids
- Fraction III
- 17 Ketosteroids.
T.Q. BORN 12.10.59.

He is the fourth child of unrelated healthy Irish parents. The eldest girl of seven is well but the other two children died in infancy. The first from a patent ductus arteriosus at four months, the second of transposition of the great vessels at the age of 10 days. This boy was a normal delivery birth; weight 4 kg. (8 lbs.12 ozs.). On discharge he was breast fed but took his feeds slowly and was dyspnoeic. Cyanosis developed and he was admitted to hospital on his 15th day.

He weighed 3.2 kg. (7 lbs. 11 ozs.). He was very dyspnoeic and there was pitting oedema of the legs. Cyanosis was present. There was also a yellowish pigmentation which was considered to be due to jaundice. The hair was black and there was a little pigmentation of the scrotum. The heart was enlarged and there was a Grade Three pan-systolic murmur audible throughout the thorax. The pulmonary second sound was loud and there was a diastolic murmur in this area. The femorals were easily palpable. The liver and spleen were enlarged.

The baby was treated, as a congenital heart
lesion in failure, with digoxin, an oral diuretic, and a salt free feed. An X-ray of the heart showed a very large heart with pulmonary plethora. The electrocardiogram showed extreme right axis deviation and right ventricular hypertrophy.

Two days after admission his weight was 3.6 kg. (8 lbs.). He remained an ill infant and some twitching of the right side was noted. A cineangiocardiogram demonstrated a large right to left shunt probably at ventricular level. Early aortic filling was noted, but probably from the shunt rather than through a patent ductus arteriosus.

On the third day he was feeding better and was now free from oedema, but despite this he had a further convulsion and vomited for the first time.

On the fourth day he was cyanosed and showed signs of peripheral failure. Vomiting had continued. The pigmentation was now much more marked and seemed abnormal even for a dark haired child. A single sample of urine was obtained and this showed an abnormal ratio of 1.5. The electrolytes were Na. 102 meq. K. 6 meq. Blood sugar 90 mg%. 
Treatment was started with oral cortisone 10 mg. six hourly, and his feed was changed to a salt containing one.

On the sixth day a complete twenty-four hour urine estimation was carried out. 17 keto-steroids 3 mg., Pregnanetriol fraction II 3.8 mg., 11 oxygenated 17-OH steroids fraction III 2.8 mg. with a ratio II/III 1.37. Cortisone was continued and in addition 0.1 mg. of 9-alpha fluorohydrocortisone was given once daily, and 2½ g. of salt was added to his feeds.

His condition was much improved by the seventh day. The steroid estimation mirrored this improvement. 17 ketosteroids 0.9 mg., Pregnanetriol fraction II 0.7 mg., 11 oxygenated 17-OH steroids fraction III 2.8 mg. The ratio II/III had fallen to 0.3.

By the end of his first week of treatment the ratio had fallen to 0.13 on a single specimen. He was less dyspnoeic and the pigmentation was not as marked. His weight gain, however, was slow. Weight 3.6 kg. (8 lbs. 2 ozs.). Height 57 cm. (22½"). He was feeding well without vomiting. Two weeks from admission an attempt
was made to decrease the cortisone as his response had been so satisfactory and there was a constant fear that too large a dose of cortisone would cause sodium retention and precipitate heart failure again. He developed a respiratory infection. Vomiting occurred and the cortisone was increased. Antibiotics were given. Serum electrolytes at this period were normal and the steroid pattern was still satisfactory. The deterioration was probably due more to his pulmonary and cardiac condition.

Discussion: This infant is graded as a severe salt loser. His early failure was no doubt precipitated by the salt free milk and the sodium diuretic given for his heart failure. The treatment with cortisone reduced his steroid output rapidly. The control of this child was made difficult by his associated congenital heart lesion. The treatment of the two conditions being exactly opposite to each other. The child showed the tendency of the infants with congenital heart lesions with pulmonary plethora to develop pulmonary infections. During these periods he
required an increase in the corticoid. He finally developed cardiac failure precipitated by a further respiratory infection and died.

Autopsy confirmed the diagnosis of congenital adrenal hyperplasia. The adrenal glands were not very large, this being due to the corticoid therapy. The surface, however, showed the typical convoluted pattern seen in cases of congenital adrenal hyperplasia. The child had a very large heart with a large ventricular septal defect. In addition, there was an absence of the pulmonary valve cusps and pulmonary infection.
A.B.  BORN 16.3.55

This boy was admitted with a history of vomiting and weight loss at the age of three weeks. There was generalised pigmentation which was more marked on the scrotum, the penis and the nipples. He was treated with cortisone, D.O.C.A., and added salt. During his infancy adrenal crises necessitated several hospital admissions.

In October, 1958, at the age of 3¾ years, he weighed 15.9 kg. (35 lbs.) 3rd percentile, and his height was 94 cm. (37") 15th percentile. He was receiving cortisone 10 mg. three times daily, salt 3 g. daily, and a monthly injection of 25 mg. of the long acting trimethly desoxy corticosterone acetate crystules. He was seen monthly and control had been based on electrolytes and the 17 ketosteroid estimation, excretion. Steroid estimation at this time was 17 ketosteroids 1.8 mg/24 hrs.  Pregnanetriol Fraction II 0.8 mg/24 hrs., 11 oxygenated 17-OH steroids Fraction III 5.2 mg.  Ratio II/III 0.1.

At this age it was felt that the monthly injection of long acting D.O.C.A. should be
discontinued. In January, 1959, age 4 years, the child developed a respiratory infection and the parents brought up a twenty-four hour collection of urine, so that this could be estimated at a time when he was unwell. The estimation of this specimen showed a dramatic increase in the 17 ketogenic steroids excretion as estimated by the chromatographic technique, Fraction II Pregnanetriol 17 mg/24 hrs., Fraction III 11 oxygenated 17-OH steroids 22.4, Ratio II/III 0.8. and less by the 17 ketosteroid estimation 18.5. Cortisone was temporarily increased to cover this illness. A week later he weighed 15.9 kg. (35 lbs.). The blood pressure was 110/60. The serum electrolytes were Na. 131 m.eq. Urea 30 K. 5.4 m.eq. He was reported to have been in contact with a case of measles and was given a protective dose of gamma globulin.

It was now obvious that the child had not been so well following the discontinuation of the D.O.C.A. and that his "salt losing" tendency was not sufficiently controlled with cortisone and salt alone. 9α-fluorohydrocortisone 0.2 mg. was started in February and by the end of the month
he was much improved. Weight 17 kg. (38 lbs.) 50th percentile. Height 95 cm. (37½") 10th percentile. Blood pressure 110/60. Steroid excretion was less, 17 ketosteroids 2.2 mg. Pregnanetriol Fraction II 0.6 mg. 11 oxygenated 17-OH steroid Fraction III 10 mg. Ratio II/III 0.06.

In the following month despite a cold he was well and the steroid excretion, 17 ketosteroid 1.7 mg/24 hrs. Chromatography of the 17 ketogenic steroids; Pregnanetriol Fraction II 0.5 mg/24 hr. 11 oxygenated 17-OH steroids Fraction III 6.2 mg/24 hr. Ratio II/III 0.08.

In April he developed a respiratory infection and his parents increased the cortisone. He was admitted to hospital because of continued lethargy and some vomiting. On admission measles was diagnosed. He showed only mild adrenal insufficiency and responded well to an initial intramuscular dose of cortisone and an increase of his oral dose to 10 mg. six hourly. He and his sister, with congenital adrenal hyperplasia, were given protective doses of gamma globulin. During the last two days in hospital the ratio of the 17 ketogenic steroids showed a return to normal.
17 ketosteroids 2.2 mg/24 hrs. Pregnanetriol Fraction II 0.9 mg/24 hrs. 11 oxygenated Fraction III 3.5 mg/24 hrs. Ratio II/III 0.25.

17 ketosteroids 4 mg/24 hrs. Pregnanetriol Fraction II 0.7 mg/24 hrs. 11 oxygenated Fraction III 4.6 mg/24 hrs. Ratio II/III 0.15.

He was discharged weighing 17.6 kg. (38 lbs. 14 ozs.). Height 98 cm. (39"), on a dose of cortisone 7.5 mg. three times daily, salt 3 g. daily, and 9αfluorohydrocortisone 0.2 mg. daily.

In May he was well. 17 Ketosteroids 1 mg/24 hrs. Pregnanetriol Fraction II 1 mg/24 hrs. 11 oxygenated Fraction III 10 mg/24 hrs. Ratio II/III 0.1.

In June, at the age of 4½ years, his height was 98 cm. (38-5/8"), weight 18 kg. (40 lbs.). The penis was 2" and the testis still small and soft. At 4½ years he remains well and is now able to withstand minor infections without vomiting. His height is 101 cm. (39 ¼") 25th percentile, weight 19.5 kg. (43 lbs.) 75th percentile. The bone age is as chronological age and his appearance is not cushingoid. He attends school.
Discussion: This boy, though a severe salt loser, has been relatively easy to control. The stopping of the long acting D.O.C.A. injections, however, soon demonstrated his continued need for a salt retaining hormone. 9α-fluorohydrocortisone has proved an excellent substitute for the injections. The steroid chromatography patterns have correlated well with the clinical evaluation. Serum electrolytes are no longer estimated at out patient visits. This, and the discontinuation of the D.O.C.A. injections, has made it very much easier for this child.
Ratio $\frac{IV}{III}$

- Mean normal

Bronchitis Withdrawal of DOCA.

Recovering from measles 11-Fluorohydrocortisone started

Graph showing changes in pregnanetriol, 11-hydroxylated 17-ketosteroids, and 17 ketosteroids from November 4 to December.
Y.B.  BORN 17.1.58.

The younger sister of A.B.  She was a normal delivery after a normal pregnancy. Abnormal genitalia were noticed at birth, but the child was considered to be a boy, and at the age of five days she was sent to a surgeon for advice on the abnormal genitalia. Arrangements were made for a buccal smear to be carried out, but it was still considered that the child was a boy. However, at the age of ten days, the child started to vomit and took breast feeds poorly. By this time she had lost a considerable amount of weight. It now appeared much more likely that the child suffered from the same syndrome as the sibling and she was sent in to hospital by her own doctor.

On admission she was ten ounces below her birth weight and was mildly dehydrated. There was no excessive pigmentation. The phallus was enlarged and the labia were fused. There was a common urogenital sinus.
Investigations showed the electrolyte changes of adrenal insufficiency with a raised potassium, and urea, and a reduced sodium and carbon dioxide combining power. The 17 keto-steroids were 3 mg. in 24 hrs. The salt content of the urine was high and the total volume large for a dehydrated infant. The buccal smear showed a female chromatin pattern.

Treatment with cortisone, initially intramuscularly, but later orally, was given combined with daily intramuscular D.O.C.A. Three grams of salt were added to her milk feeds. Once she was stabilised, the D.O.C.A. was given as the long acting crystules once monthly, initially in
a dose of 125 mg. monthly. The dose was then gradually reduced so that at nine months she was receiving 75 mg. monthly. During this period only the 17 ketosteroids were estimated and these showed suppression to less than 1 mg/24 hrs. She was admitted to hospital at monthly intervals for these estimations.

At the age of nine months the steroids were estimated by chromatography of the 17 ketogenic steroids. At this time she weighed 8.8 kg. (19 lbs. 6 ozs.) on the 50th percentile, length 66 cm. (26") on the 25th percentile. She was able to sit up despite her rather cushingoid appearance. There was no change in the genitalia. The serum electrolytes were satisfactory, Na. 148 m.eq. K. 5 m.eq./l., urea 28 mg%. The results of the steroid estimations are shown below.

<table>
<thead>
<tr>
<th>Date</th>
<th>17 keto-steroid mg/24 hr.</th>
<th>Chromatography 17-OH steroids mg/24 hr.</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-17/10/58</td>
<td>1.7</td>
<td>0.3</td>
<td>0.17</td>
</tr>
<tr>
<td>17-18/10/58</td>
<td>1.5</td>
<td>0.3</td>
<td>0.18</td>
</tr>
<tr>
<td>19-20/10/58</td>
<td>2.3</td>
<td>0.3</td>
<td>0.16</td>
</tr>
</tbody>
</table>

These results showed very satisfactory control. Her blood pressure was raised. She was discharged on
cortisone 5 mg. eight hourly, salt three grams added to her feeds and the monthly percorten crystules were reduced to 25 mg. during the next month.

In the middle of November she developed a cough and on the 29th of November, aged 10 months, she was readmitted to hospital with a history of lethargy and vomiting for two days. She was moderately dehydrated and very lethargic. The blood pressure was 100/70. The serum electrolytes were surprisingly good with a sodium of 141 m.eq./l., potassium 4.8 m.eq./l., and a blood urea of 45 mg%.

The steroid estimation at this time, however, showed a definite deterioration: 17 ketosteroids 3 mg/24 hrs. Pregnanetriol Fraction II 0.8 mg/24 hrs. amd 11 oxygenated 17-OH steroid Fraction III 2 mg/24 hrs. The ratio II/III 0.4. She was treated with 25 mg. of cortisone intramuscularly and an added 25 mg. of the long acting D.O.C.A. crystules. This mild adrenal crisis appeared to have been precipitated by a respiratory infection and by the reduction of the D.O.C.A. She was discharged on the 3rd December, and when seen again on the 23rd, she had gained two pounds in weight and was very well.
On the 29th January she was given a protective dose of gamma globulin because she had been in contact with a case of measles. She remained well, gaining a satisfactory amount of weight each month on cortisone 5 mg. eight hourly, salt 3 grams daily, and Percorten crystals 50 mg. monthly, during the next three months. In April she was again given a protective dose of gamma globulin because her brother had developed measles. Despite the close contact over the incubation period she avoided the infection.

Progress on this regime was very satisfactory and it was decided to change her to the oral salt retaining hormone, $9\alpha$-Fluorohydrocortisone, already being successful in the treatment of other "salt losers" including her brother. In July no further D.O.C.A. was given and in its place she received $9\alpha$-Fluorohydrocortisone 0.1 mg. twice daily. Daily single samples of urine were collected over this period. Unfortunately, at this time she developed another upper respiratory tract infection and she showed, with one exception, a
high initial ratio. However, at the end of a week the ratio II/III was back to normal and the child was very well. Her height at eighteen months was 77 cm. (29\(\frac{3}{4}\)"\) on the 10th percentile and her weight 11 kg. (24 lbs. 5 ozs.) on the 50th percentile.

In September, when she was twenty months old, she was walking well and had lost the cushingoid appearance. Her bone age was at the lower limit of normal for her age. The blood pressure was 120/80. Though she had another cold she was not showing any signs of adrenal insufficiency. Her change of attitude to hospital visits, which now involved no intramuscular injections, was very striking. Her mother collects single specimens of urine at monthly or two monthly intervals and these have shown normal ratios of the Pregnanetriol Fraction II/III oxygenated 17-OH steroid Fraction III, between 0.32 and 0.08.

This child represents a severe salt loser. She is now satisfactorily controlled on cortisone and 9\(\alpha\)-Fluorohydrocortisone with added salt.
She is able to overcome infections without signs of adrenal insufficiency. Her control has of late been by single samples of urine and she has not had to be admitted to hospital in the past nine months. Her height and weight are normal and her bone age on the lower limits of normal. She will require surgical correction of the genitalia, but this is being postponed until she shows a further diminution of the tendency to adrenal insufficiency. It is planned to have this corrected before the child goes to school. Her parents tell me that her sibling has already mentioned the abnormality of his sister and they are therefore rather anxious that corrective surgery should not be delayed too long.
Y.B.  
Cortisone 5mg. 8hrly.

D.O.C.A.

9a Fluoro hydro cortisone

\[ \text{Ratio} \frac{\text{II}}{\text{III}} \]

2

1

mean normal

mean normal

\[ \text{mg/24hr. October Dec. July Aug. Sept Nov.} \]

\[ \text{Na} \]
The child was admitted to hospital at the age of 6½ months because of failure to thrive. At this time he weighed only 3.6 kg. (8 lbs.) below the 1st percentile. Height 59.5 cm. (23½") on the 1st percentile. The genitalia were abnormal. The "penis" was very small and no testis were palpable.

He was fair haired and no excessive pigmentation was present. The blood pressure was 90/60 and serum electrolytes Na. 123 m.eq. K. 6 m.eq. Urea 16 mg%. The 17 ketosteroids were 2 mg/24 hrs. The bone age was normal. A skin biopsy demonstrated a chromatin positive
pattern. The child was therefore a female pseudohermaphrodite due to the "salt losing" type of congenital adrenal hyperplasia, and treatment with cortisone, D.O.C.A., and salt was started at the age of 7½ months. The difficulties in proving the diagnosis are demonstrated by the findings in this child. A single sample of urine will now give this answer in 24 hours. He was discharged from hospital at the age of 11¼ months. Weight 6.5 kg. (14 lbs.) below the 3rd percentile. Height 64.5 cm. (25½") below the 3rd percentile. His treatment was cortisone 25 mg. in divided doses, salt 3 g. and monthly injections of percorten long acting crystules 125 mg. His serum electrolytes were normal and the twenty-four hour excretion of the 17 ketosteroids 1 mg.

At 15 months - weight 7.4 kg. (16½ lbs.), height 66 cm. (26"). The blood pressure was raised and the serum Na. high. The long acting D.O.C.A. crystules were reduced to 75 mg. monthly.

At 18 months urography was attempted, but this failed to show a vagina. The following
month he was admitted in adrenal failure. He was hypotonic, the skin was mottled and res­pirations rapid and grunting. The serum electrolytes - Na. 135 m.eq., K. 5 m.eq., Urea 116 mg%. He was given extra D.O.C.A. and fluids and made a satisfactory recovery. During the remainder of his second year he remained well on cortisone 5 mg. tds., salt 4 g. daily, and long acting D.O.C.A. crystules 75 mg. at monthly intervals.

At the age of 2½ years serum electrolytes were estimated six weeks after his last dose of D.O.C.A. and as they were normal, no further injections were given. Weight 13 kg. (28½ lbs.) 50th percentile. Bone age normal. 17 keto-steroids less than 1 mg. in 24 hours.

At three years he developed a respiratory infection and again manifested signs of adrenal insufficiency with listlessness, anorexia and vomiting. The cortisone was increased to 10 mg. three times daily for one week and the infection treated with penicillin.

At the age of 3½ years, he was admitted to hospital for assessment. At the time of ad­mission, he had bronchitis and during the first
week he was hypotonic, listless and vomited. He was thirsty and drank large quantities of fluids. The blood pressure was 90/60, lower than his normal reading. Weight 13.6 kg. (30 lbs.) 50th percentile; Height 88 cm. (34\frac{1}{8}"") 3rd percentile. The bone age was on the lower limits of normal. The buccal smear showed a chromatin positive pattern. His steroid excretion during his early illness showed how much more definite are the changes shown by the chromatography of the 17-OH steroids than those shown by the 17 ketosteroids. 17 ketosteroids 4.4 mg/24 hrs., Pregnanetriol fraction II 1.9 mg., 11 oxygenated fraction III 4.5 mg/24 hrs., Ratio II/III 0.4., and the following day, when his condition was worse and his urinary excretion 980 ml., the ketosteroids had only risen to 6.3 mg., but the pregnanetriol was 25 mg. and the 11 oxygenated fraction III 4.6 mg/24 hrs. The ratio of II/III was well in the untreated range for congenital adrenal hyperplasia of 5.7.
When he had recovered from the bronchitis he was subjected to urethroscopy. Again no definite vaginal orifice was seen and no uterus was palpable rectally.

The question of change of sex was discussed with the parents. They were afraid of the social consequences and the father was strongly opposed to a change. For these reasons and also because we had been unable to adequately demonstrate the vaginal orifice, it was decided to continue to rear the child as a boy and later to remove the female organs. The phallus which is not as large as a normal penis will enlarge when cortisone suppression can be reduced.

During the admission the child had shown that despite the slightly retarded bone age, he was unable to develop an infection without developing adrenal insufficiency. The cortisone dose was raised to 7.5 mg. three times daily. A single sample of urine showed improvement of the ratio following this increase. At 3 years 5 months, he developed chickenpox but showed no signs of adrenal insufficiency. Five months
later, however, he had another respiratory
infection accompanied by vomiting. His mother
increased the dose of cortisone to 10 mg. three
times daily and he recovered rapidly.

At four years he was admitted with in-
fluenza. He had been listless and vomiting for
two days. His serum Na. 143 m.eq., Urea 66 mg%.
Blood pressure 120/90. he was given intramus-
cular cortisone and oral fluids containing extra
salt. Steroid patterns were estimated over this
period and again correlated well with his clinical
progress.

It was evident that this child had shown
repeated signs of adrenal insufficiency since
he had stopped having a salt retaining substance.
Cortisone had already retarded growth and bone
age and yet had failed to prevent adrenal in-
sufficiency. He was therefore given 0.2 mg. of
9α-fluorohydrocortisone daily.

He was discharged very well weighing 18.5 kg.
(41 lbs.). The 17 ketosteroids 1.7 mg/24 hrs.
Pregnanetriol fraction II 0.6 and the 11 oxygenated
fraction III 4.1. The ratio II/III 0.14. He
was receiving cortisone 7.5 mg. tds. 9α-fluoro-
hydrocortisone 0.2 mg. daily and salt 3 g.
Two months later he was admitted with measles. He had been vomiting and listless for two days. His colour was poor and he was hypotonic. The blood pressure, however, was 130/90. There were signs of an infection in the lungs. He received 25 mg. of cortisone intramuscularly and the oral dose of cortisone was increased to 10 mg. six hourly. Penicillin was given for the secondary infection. His initial steroid patterns showed an abnormal ratio, the highest being 1.9. With clinical improvement, which was evident by the fifth day, the ratio reverted to normal. He was discharged home on the tenth day on cortisone 10 mg. three times daily, which was decreased to his normal dose, after one week of 7.5 mg., and 0.2 mg. of 9α-fluorohydrocortisone and 3 g. salt.

Since then he has been in excellent health with normal ratios of the two fractions. His 9α-fluorohydrocortisone has been reduced to 0.1 mg. daily because he was gaining weight rapidly and his ratio had fallen to 0.09. The total pregnanetriol fraction to 0.8 mg/24 hrs.
A further decrease in the cortisone recently has, however, shown an increase in the steroid output and the ratio suggesting that we might again run into trouble. Bone age remains rather reduced but his height is normal. Age 4½ years: weight 21 kg. (47 lbs.) 97th percentile; height 105 cm. (39½") 50th percentile.

This child is of particular interest because he is being reared in the wrong sex, and will require careful management from this angle later. At present, he still shows the tendency of the "salt loser" to develop adrenal insufficiency during illness. As long as this tendency persists, he will require sufficiently large doses of cortisone to prevent androgenisation. The aim is to remove the female reproductive organs as soon as his adrenal state permits. He will receive extra cortisone cover over this period. Once this is done the aim of cortisone therapy will be directed at achieving maximum height. Once this is achieved, cortisone will be reduced to allow androgenisation to occur.
P.T. BORN 24.11.56.

This is the ninth child, the other eight children are well. All the children are under the care of the local authorities. He was a normal delivery and his birth weight was 4 kg. (8 lbs. 14 ozs.). Vomiting started at the age of seven days and at the age of fourteen days he was admitted to the Children's Hospital. At this time, though he was not dehydrated, his tone was poor. Over the next ten days, however, it was evident that he was passing large quantities of urine and was losing weight and becoming dehydrated. His serum biochemistry showed a low sodium of 120 m.eq. Potassium was raised 7.8 m.eq. The blood urea was 102 mg%. A twenty-four hour collection of urine gave a slightly raised 17 ketosteroid level of 1 mg. The bone age was normal. The child required intravenous fluids and on 20 Dec. 1956 treatment as a congenital adrenal hyperplasia was started with D.O.C.A. cortisone 5 mg. three times daily. Salt, 3 g., was added to his daily intake. Over the next week this treatment proved inadequate. It was not till he was receiving cortisone
35 mg. orally in divided doses and intramuscular D.O.C.A. 4 mg. daily with the addition of three grams of salt, that his condition improved.

On 9.4.57 the daily D.O.C.A. was discontinued and was given as the long acting trimethyl crystals.

At the age of six months, he was discharged weighing 5.8 kg. (13 lbs.) below the 3rd percentile. He was then receiving 10 mg. of cortisone twice daily, with the 3 g. of salt. Once a month he was given 100 mg. of the long acting D.O.C.A. During the latter part of the first year, this was given at six weekly intervals.

At fourteen months he weighed 9 kg. (20 lbs.) 5th percentile and was 72 cm. (28½") high on the 3rd percentile. Despite a series of respiratory infections he had shown no signs of adrenal insufficiency. At eighteen months his weight was 10 kg. (22 lbs.) 10th percentile; height 72 cm. (28½ ins.) below the 3rd percentile.

The penis was not large. The blood biochemistry was normal but the twenty-four hour 17 ketosteroid was high, 4 mg/24 hrs. Six weeks
following his last injection of the long acting D.O.C.A., the serum sodium and potassium were normal. No further D.O.C.A. was given at this visit, but because the 17 ketosteroids had been high, the dose of cortisone was increased to 7.5 mg. three times daily.

At the age of one year eleven months, he was admitted to hospital for review, and for the first time his condition was investigated by the method of chromatography of the 17 ketogenic steroids. The results of the steroid estimation showed:

<table>
<thead>
<tr>
<th>Day</th>
<th>17 ketosteroids</th>
<th>17 ketone triol II</th>
<th>11 oxygenated 17-OH steroids III</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td>29-30/9/58</td>
<td>2.4</td>
<td>1 mg/24 hr.</td>
<td>2.8 mg/24 hr.</td>
<td>0.36</td>
</tr>
<tr>
<td>30/9</td>
<td>2.7</td>
<td>0.4 mg/24 hr.</td>
<td>3.2 mg/24 hr.</td>
<td>0.11</td>
</tr>
<tr>
<td>1/10/58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2/10/58</td>
<td>6</td>
<td>0.4 mg/24 hr.</td>
<td>3.1 mg/24 hr.</td>
<td>0.14</td>
</tr>
</tbody>
</table>

His weight was 11.8 kg. (26 lbs.) 25th percentile; height 83 cm. (32½") 10th percentile, and the bone age was normal. His general control and his steroid suppression were good, and for a
period an attempt was made to reduce the cortisone to 5 mg. three times daily, but he was said to be drinking more fluids and passing more urine. His weight gain decreased. At the same period he was having repeated colds and otitis media. His cortisone was increased to 7.5 mg. three times daily as this appeared to be the minimum dose necessary for his well being.

At two and a half years he was given a protective dose of gamma globulin intramuscularly because he was in contact with measles in the children's home. He was protected. The steroid excretion was: 17 ketosteroids 3 mg/24 hrs. The chromatography of the 17 ketogenic steroids: Pregnanetriol Fraction II 0.4 mg/24 hrs., 11 oxygenated 17-OH steroids 2.5 mg/24 hrs., the ratio II/III 0.2.

At two years nine months, he was reported to have been in contact with a case of chickenpox but he did not develop it. However, at this time he had bronchitis and became lethargic and pale. He was treated with antibiotics and the cortisone dose was increased temporarily to 10 mg. three times daily.
Now at the age of three years, he weighs 15.5 kg. (34 lbs.) 60th percentile; his height is 90.5 cm. (35½") 15th percentile. The penis is normal in size and he remains very well. The steroid excretion 17 ketosteroids 2.8 mg/24 hrs. Chromatography of the 17 ketogenic steroids: Pregnanetriol II 1.25; 11 oxygenated 17-OH steroids 4 mg/24 hrs., and the Ratio II/III 0.3. His bone age is normal and general control is good.

**Discussion:** This child is a mild salt loser, one of the few in this series who has been able to discontinue a salt retaining hormone completely. The routine examination of the steroid excretion has been hampered by the fact that he was late in being bladder trained. This could in future be overcome by using single specimens of urine. The only difficulty in looking after this child has been the infections with which he has been in contact, because he lives in a home for children. Co-operation between the home and the hospital has been excellent.

This girl is the eldest child of healthy parents. The clitoris was enlarged at birth, but she was considered to be a female. At the age of three years, she was admitted for investigation. Her height was 99 cm. (43") above the 100th percentile, and her weight 18 kg. (40 lbs.), 100th percentile. She had a deep voice and a masculine conformation. The clitoris was enlarged and the labia fused; the common urogenital sinus opened at the base of the phallus.
Pubic hair was present. The bone age was 8-9 years and the 17 ketosteroid excretion 26 mg. in twenty-four hours. Laparotomy was carried out and revealed a normal female reproductive tract. The perineum was split back to expose the vaginal orifice.

At the age of four years, she was started on cortisone. During this year, between operation and treatment, the clitoris had enlarged and the bone age advanced to that of a child of 12-14 years. There was now a heavy growth of pubic hair. She received intramuscular cortisone initially in a dose of 50 mg. daily and this was then changed to oral cortisone in double the dose. The general texture of the skin improved but as she gained weight rapidly and there was very little fall in the ketosteroid output and no decrease in the size of the clitoris, after a trial of four months, cortisone was stopped.

At the age of 6½ years, she was admitted for clitoroidectomy. The bone age had by now advanced to 14-16 years. Cortisone was now freely available and she was given 75 mg. daily and this dose was maintained to the time of
assessing progress in this study. At the age of 10½ years she was 152 cm. (59½") high, 100th percentile and her weight 43 kg. (94 lbs.) 95th percentile. There was a little breast development but menstruation had not yet started. The bone age had advanced to 16-18 years.

Steroid excretion was high:

<table>
<thead>
<tr>
<th>Date</th>
<th>17 keto-steroid</th>
<th>Chromatography of the 17-OH steroid</th>
<th>Ratio 11/11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/24 hr.</td>
<td>mg/24 hr.</td>
<td>11/11</td>
</tr>
<tr>
<td>8-9/10/58</td>
<td>7.3</td>
<td>2.1</td>
<td>0.15</td>
</tr>
<tr>
<td>9-10/10/58</td>
<td>9.2</td>
<td>2.8</td>
<td>0.25</td>
</tr>
</tbody>
</table>

It was apparent that androgen secretion was not being adequately suppressed and if fusion of the epiphyses was not halted, little further height increase could be expected. The dose of cortisone was increased to 100 mg. daily. There was a satisfactory reduction of the steroid excretion but this was unfortunately accompanied by a rapid increase in weight gain. She was given a 1000 calorie diet and prednisolone was substituted for the cortisone in a dose of 5 mg. four times daily. These measures have been only partially successful in reducing the weight gain.
Steroid excretion:

<table>
<thead>
<tr>
<th>Date</th>
<th>17 keto-steroid mg/24 hr.</th>
<th>Chromatography 17 ketogenic steroids mg/24 hrs.</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-12/2/59</td>
<td>6</td>
<td>3.6 8.7</td>
<td>0.4</td>
</tr>
<tr>
<td>2 - 3/6/59</td>
<td>5</td>
<td>1.0 10.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

This girl was given a dose of gamma globulin during the study and was successfully protected from measles.

**Discussion:** She has always been well orientated and has done well at school. Her appearance and her outlook are those of an older child.

Control in her case has been only partially successful because of the late start of treatment. She has only reached the height of 153 cm. (60") with a bone age of 16-18 years and indeed has gained no height in the past year. This may be due to the adequate suppression on the larger dose of cortisone; on the other hand, it is quite possible that she will not grow further. She has not yet started to menstruate and breast development is not yet marked. Though she has shown no definite signs of adrenal insufficiency, her younger sister died of adrenal insufficiency during an acute illness.
S.T. BORN 22.8.42

This girl was first seen at the age of eight because of rapid growth and the development of pubic hair. At this time, 1950, her height was 152 cm. (60") above the 100th percentile; weight 40 kg. (88 lbs.) above the 100th percentile. Her voice was deep and the bodily configuration was male. The skin was coarse and greasy and acne was present on the face. Investigations showed a 17 ketosteroid excretion of 16 mg. in 24 hours and a bone age of 16-18 years.
In 1953, when cortisone became available, she was admitted for a trial of cortisone. The initial dose was 75 mg. daily and this was reduced to 50 mg. daily. There was very little change in the 17 ketosteroid level and therefore cortisone was reduced still further and finally discontinued after a course of three months. Following this, she developed facial hirsutism and cortisone was again given and this time continued in a dose of 75 mg. daily.

At the age of twelve years she was menstruating fairly regularly and had well formed breasts. There had been no increase in height however.

At the age of sixteen she was seen and the steroid excretion studied. She was 155 cm. (61") tall on the 10th percentile; her voice was deep; in other respects she was very feminine. Her only complaint was shortness of stature. The ossification centres had all fused and therefore no further growth was possible.

The steroid excretion showed satisfactory suppression: on one occasion only.
| Date       | 17 ketosteroid mg/24 hr. | Chromatography | Ratio  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17 ketogenic steroids</td>
<td>II/III</td>
</tr>
<tr>
<td>October</td>
<td>19</td>
<td>13.5</td>
<td>25</td>
</tr>
<tr>
<td>September</td>
<td>3.4</td>
<td>22.8</td>
<td>0.14</td>
</tr>
<tr>
<td>December</td>
<td>18.7</td>
<td>4.7</td>
<td>11.5</td>
</tr>
</tbody>
</table>

This girl once again demonstrates the difficulty in preventing early fusion of the ossification centres when treatment with cortisone is started late. The dose of cortisone adequate to suppress the virilising manifestations has been inadequate to prevent continuing advance in bone fusion. She is, however, well orientated and would be accepted as a short, though in other respects normal, female.
D.T.  BORN 12.8.44.

She is the younger sister of S.T. and was first seen at the age of six in 1950. The child was born with an enlarged clitoris with fusion of the labia and a urogenital sinus. Her growth has been abnormally rapid and for the past year facial acne had been present. Her weight at this time was 24.5 kg. (52 lbs.) on the 95th percentile. Her height was 132 cm. (47") greater than the 100th percentile. The 17 ketosteroids were 35 mg/14 hr. and the bone age was 12-14 years.

During this admission, clitoroidectomy and cutting back of the perineum was carried out.

At the age of nine, she was admitted for cortisone therapy. Pubic and axillary hair were now profuse. The height was 139.5 cm. (55") on the 95th percentile. The bone age had advanced to 15 years. A short course of three weeks of cortisone resulted in no change in the excretion of the 17 ketosteroids. Cortisone was in only limited supply at this time and it was discontinued.
However, acne became increasingly troublesome. Cortisone was started again, this time in twice the dose, 100 mg. daily. This dose resulted in such a rapid gain in weight that it was reduced to 50 mg. daily.

At ten years, menstruation started but was scanty and irregular. At the age of thirteen she was entered into this study. Her height was 146 cm. (572\text{\textfrac{1}{2}}") on the 95th percentile and her weight 54 kg. (118 lbs.) above the 100th percentile. She had a deep masculine voice. The skin was coarse and acne was still present on the face. Menstruation was irregular. She was still receiving the same dose of cortisone, 50 mg. daily.

The steroid excretion:

17 ketosteroid: 42 mg/24 hr.

Chromatography of the 17 ketogenic steroids.

Pregnanetriol Fraction II: 50 mg/24 hr.

11 oxygenated 17-OH steroid Fraction III: 14 mg/24 hr.

Ratio II/III: 3.6.

These results suggested completely inadequate control.
She was seen two months later; her height had shown no increase but the weight was 50.5 kg. (122 lbs.). The bone age was 16-18 years. As both clinical and laboratory evidence suggested that she was not receiving an adequate suppressive dose of cortisone, treatment was changed to 25 mg. four times daily. This increase resulted in such a dramatic change in the steroid output that it was wondered how regularly the child had received her cortisone, especially as she lived some distance from the hospital and did not attend regularly.

The results of the steroid estimation:

17 ketosteroid 34 mg/24 hr.

Chromatography of the 17 ketogenic steroids

Pregnanetriol Fraction II 2.2 mg/24 hr.

11 oxygenated 17-OH steroids Fraction III 21.6 mg/24 hr.

Ratio II/III 0.1.

There was, unfortunately, a very rapid gain in weight and the child developed striae. Prednisolone, in a dose of 5 mg. four times daily, was substituted for the cortisone and she was persuaded to have a 1000 calorie diet.

There was a resulting decrease in weight gain and a further fall in the steroid excretion.

17 ketosteroids 5 mg.

Chromatography of the 17 ketogenic steroids

Pregnanetriol Fraction 1.75 mg/24 hr.
11 oxygenated 17-OH steroids 2 mg/24 hr.
At the end of the year on these larger doses of steroids there had been no advance in bone age and no increase in height.

The steroid excretion was very satisfactory, Pregnanetriol fraction 0.5 mg/24 hr., 11 oxygenated 17-OH steroids 2.4 mg/24 hr. and the 17 ketosteroids were 3.8 mg/24 hr. Because of increasing obesity and these very satisfactory results the prednisolone was reduced to 5 mg. three times daily.

**Discussion:** This girl illustrates the unfortunate results of late and inadequate treatment. She was unfortunately too old when cortisone became readily available for treatment. She will always have a deep voice. During the past year she has gained no height. This may be due to the increased dose of corticoids. This would be supported by the failure of further increase of the bone age. However, it is possible that she will grow no further and remain a very short woman.

The steroid estimations in this girl show particularly well the marked change in the pattern of steroids obtained by chromatography of the 17 ketogenic steroids as opposed to the minor difference in the 17 ketosteroid estimation.
Congenital Adrenal Hyperplasia effect of increased cortisone on pattern

II Pregnanetriol
III 11-hydroxylated 170H.S.
IV Oxosteroids
Cortisone Cortisone
30mg 100mg Prednisolone 20mg 15mg 5mg 4ds 5mg 4ds

mg/24hrs

Feb. March May

June Oct Dec
V.A. BORN 23.2.48.

This boy is the only child of healthy parents. He was first admitted to the Children's Hospital at the age of seven weeks because of persistent vomiting and loss of weight. Investigations at this time proved that he was passing large quantities of urine with a high salt content. Desoxy corticosterone acetate and Eucortone did not give such a satisfactory response as the addition of 7-8 g. of salt to his feeds. He was diagnosed as a "salt-losing" nephritis. His progress was satisfactory while he was receiving these large quantities of salt.
In 1952, at the age of four years, he was re-admitted to hospital for further investigations. His height was 116 cm. (46") above the 100th percentile; weight 22 kg. (47 lbs.) 100th percentile. There was scanty pubic hair and the penis was large, measuring 2". The bone age was 12 years. He still manifest a need for salt and would consume 20 g. daily, if allowed. It was very interesting to see him eat, for he would first lick the salt from the side of his plate and then eat his food. He seemed to show an instinctive craving for salt. It was now evident that he was suffering from congenital adrenal hyperplasia of the "salt losing" type and as cortisone was available for treatment, he was started on it. The initial dose was 40 mg., but there was little suppression of the 17 ketosteroids and the dose was raised to 80 mg. a day. During the next five years this dose was reduced to 50 mg. daily.

At nine years, his height was 142 cm. (56") 97th percentile; weight 37 kg. (79 lbs.) 100th percentile. The bone age was 12-16 years and the blood pressure and the serum electrolytes
were normal. The cortisone was reduced to 40 mg. daily. During this period he was thirsty and drank large quantities of water and his salt intake rose. He became lethargic and after fainting at school, it was decided to increase the cortisone to 20 mg. three times daily. On this dose, the 17 ketosteroids were 8-9 mg./24 hrs.

At ten years, his height was 151 cm. (59½") 100th percentile; weight 44.5 kg. (98 lbs.) above the 100th percentile. The bone age was now 16 years, and the 17 ketosteroid output 4-10 mg/24 hr.

At 10½ years his height was 156 cm. (61½"); weight 47 kg. (104 lbs.). The blood pressure was normal and the buccal smear showed a chromatin negative pattern. There was profuse pubic hair and a little facial hair. The penis measured 3". The testis were firm, of normal size. His general health was only fair; he was still taking two teaspoonsful of salt a day; he was lethargic and unable to keep up with his school work. His circulation always seemed poor, suggesting that he was not receiving adequate treatment. He was
given 9α-fluorohydrocortisone initially 0.25 mg. daily without any change in his state. When the dose was increased to 0.5 mg. daily, there was a rise in the serum sodium to 148 m.eq. and a concomitant fall in the potassium to 4 m.eq. The blood pressure, however, did not rise.

Steroid excretion:

<table>
<thead>
<tr>
<th>Date</th>
<th>17 keto-steroid</th>
<th>17 ketogenic steroid</th>
<th>Ratio II/III</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-12/10/58</td>
<td>5.7</td>
<td>2</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>0.5</td>
<td>0.04</td>
</tr>
</tbody>
</table>

His treatment was adjusted to 25 mg. cortisone three times daily, and 0.25 9α-fluorohydrocortisone daily. On this regime he was reported to be better, he was taking a little less salt, and was doing much better at school.

Weight at 10 years 9 months was 50.5 kg. (112 lbs.). The height advanced to 158 cm. (62½") and the bone age had shown no advance in the past six months. Steroid excretion: 17 ketosteroids 18.6 mg/24 hr., 17 ketogenic steroid Fraction II 2.8 mg., Fraction III 15.8 mg/24 hr., Ratio II/III 0.2.
- 187 At 11 years 3 months he was again reported
to he lacking in energy.

The steroid excretion

was highs

Date

10-11/5/59

17 ketosteroid
mg/?4hri
12.6

17 ketogenic
steroid
aw.724 hr.
11
m

10.7

29.7

Ratio
s z m

0.36

The dose of cortisone was increased to 25 mg. four
times daily with the same dose of 9
cortisone.

fluorohydro-

He improved so much that he was able

to win a 100 yards race, and school reports were
good for the first time.
Steroid excretion:

Date

17 ketosteroid
mg/24 hr.

17 ketogenic
steroid
mg-*/<£.•+ Tnr.
fI
II

R atio
J l/ T ll

8 - 9/6/59

21.0

5.0

34.6

0.15

11-12/8/58

18.9

3-5

16.8

0.2

However, increasing obesity and a rise in blood
pressure indicated that this dosage was excessive
At 1 1 years - weight 65*5 kg. (145 lbs.J.;
height 164 cm. (64iM).

The facial hair was more

profuse and though he was not yet shaving, he had
now the appearance of a post pubertal child.


The increase in the steroid excretion was, in part, due to the onset of puberty. The 9α-fluorohydrocortisone has now been discontinued as no dramatic decrease in salt intake resulted from its use. This boy is of great interest because he is one of the few children with the "salt losing" type of congenital adrenal hyperplasia to have lived through infancy without the benefit of cortisone treatment. He has continued to manifest the "salt losing" tendency to the age of 11½ years, which is unusual. During the early part of his treatment, when cortisone was in short supply, he continued to grow at an excessive rate and there was a continued advance in bone age. In the past eighteen months during a period of increase in the cortisone there has been a decrease in height increment and the bone age has not increased. In appearance he is a post pubertal boy of 16 years. However, as time passes, this discrepancy will decrease and he should grow to a satisfactory height. The sudden great increase in the steroid excretion at the time of puberty is well shown in
the chromatography of the 17 ketogenic steroids as is the reduction when the cortisone was increased. The results of the estimation of the 17 ketosteroids show no significant change when the cortisone was increased.
CONCLUSION

Congenital adrenal hyperplasia, an inborn error of metabolism, has been studied from the clinical and the biochemical aspects. A new method for the estimation of pregnanetriol has been expanded and applied to the problem of the diagnosis and the control of treatment of this defect.

The method consists of the chromatography of the 17 ketosteroids formed, following the oxidation of the 17 ketogenic steroids by bis-muthate. Two fractions are estimated as Zimmermann chromogens. One contains the oxidation products of those 17 ketogenic steroids with no oxygen group at the C11 position. In the normal subject little chromogen is found in this fraction. In patients with congenital adrenal hyperplasia, there is a large quantity due to the presence of the oxidation products of 17 hydroxy pregnanolone and pregnanetriol. The second fraction estimated contains the more polar, 11 oxygenated 17 keto-steroids derived from the 11 oxygenated 17 ketogenic steroids. These in the normal subject are
the metabolites of cortisol and cortisone. It is suggested that in patients with congenital adrenal hyperplasia another steroid, 11 keto-pregnanetriol, accounts for the high steroid content of this fraction.

The ratio of these two fractions in the urine of normal people is shown to differ from that in the urine of patients with congenital adrenal hyperplasia. The mean ratio in the former is 0.24; in the latter, 2.0. The lowest abnormal ratio was 0.8, and this was found in an infant. The difference in the ratio in infancy and in the older child, is considered to be due to the relatively higher excretion of 11 keto-pregnanetriol to pregnanetriol in the infant.

The importance of estimating 17α-hydroxy-pregnanolone in the pregnanetriol fraction is discussed.

The change in the ratio found in the patients with congenital adrenal hyperplasia, when the abnormal adrenals are stimulated with exogenous or endogenous corticotrophin, is considered to be indicative of their inability to synthesise cortisol.
When these patients are treated with corticoids, the abnormal ratio is reversed. The method is shown to give results that correlate well, with the adequacy of the control of treatment, as judged clinically.

The major advantage of this method, over all others at present available, is that the diagnosis of congenital adrenal hyperplasia can be made on a single sample of urine. Useful control of treatment is also obtained from a single sample when a complete twenty-four hour collection is not available.

It is suggested that this metabolic defect is more common than indicated by hospital records. That many cases are missed is supported by the finding of six children ascribed to the wrong sex in this series.

Chromatography of the 17 ketogenic steroids is shown to be a reliable method suitable for the routine laboratory. It should provide the clinician, faced with the problem of the differential diagnosis of congenital adrenal hyperplasia, with the answer.
Non adrenal pseudohermaphrodites


Chromosomal sexing


Genetic aspect


The clinical picture


The metabolic Defect


The Enzyme systems of the adrenal cortex


The treatment of congenital adrenal hyperplasia


The surgical correction of the masculinized genitalia

Congenital adrenal hyperplasia and measles

Chickenpox and Cortisone

Change of sex


The Biochemical Methods


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