CONGENITAL ADRENAL HYPERPLASIA

The clinical problem, and the application of a
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I N D E X

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 cortico-trophin 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 speciali-
sing 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, gastro enteritis, 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 treat-
ment 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, $\triangle$ 5 pregnenolone or a

C19 nucleus with no side chain, dehydroepiand-

rosterone.   The former is then converted into an

active steroid progesterone($\triangle_4$-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.

CHOLESTEROL

$\triangle$ 5 PREGNENOLONE
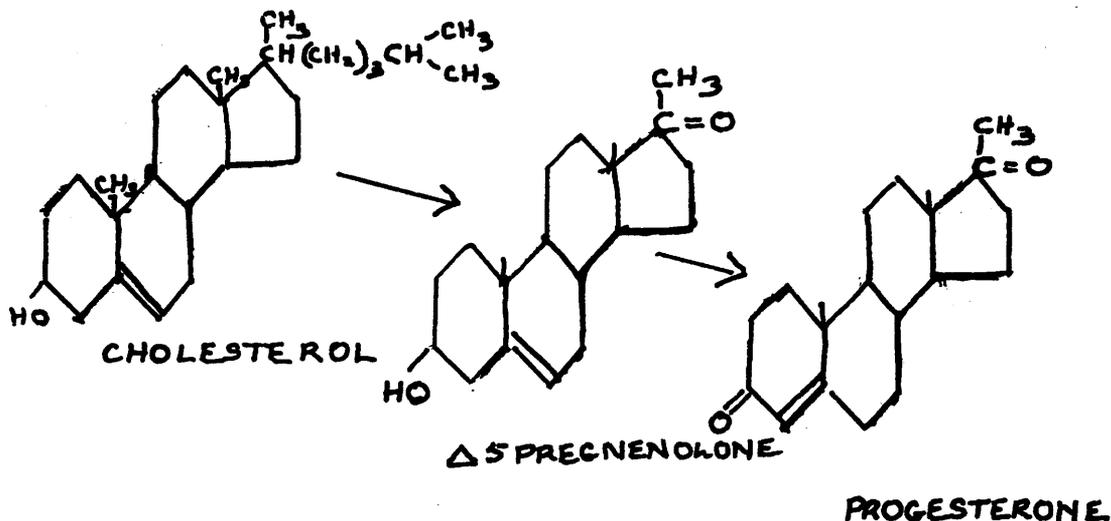
PROGESTERONE

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 α hydroxylase, 11β hydroxy-
lase and 21α hydroxylase.

17 Hydroxylase

CH₃
C=O

Progesterone

CH₃
C=O
-OH

17 hydroxy progesterone

11β Hydroxylase

CH₂
C=O
-OH
HO

21 desoxy 17 hydroxy corticosterone

21 Hydroxylase

CH₂OH
C=O
-OH

17 hydroxycorticosterone
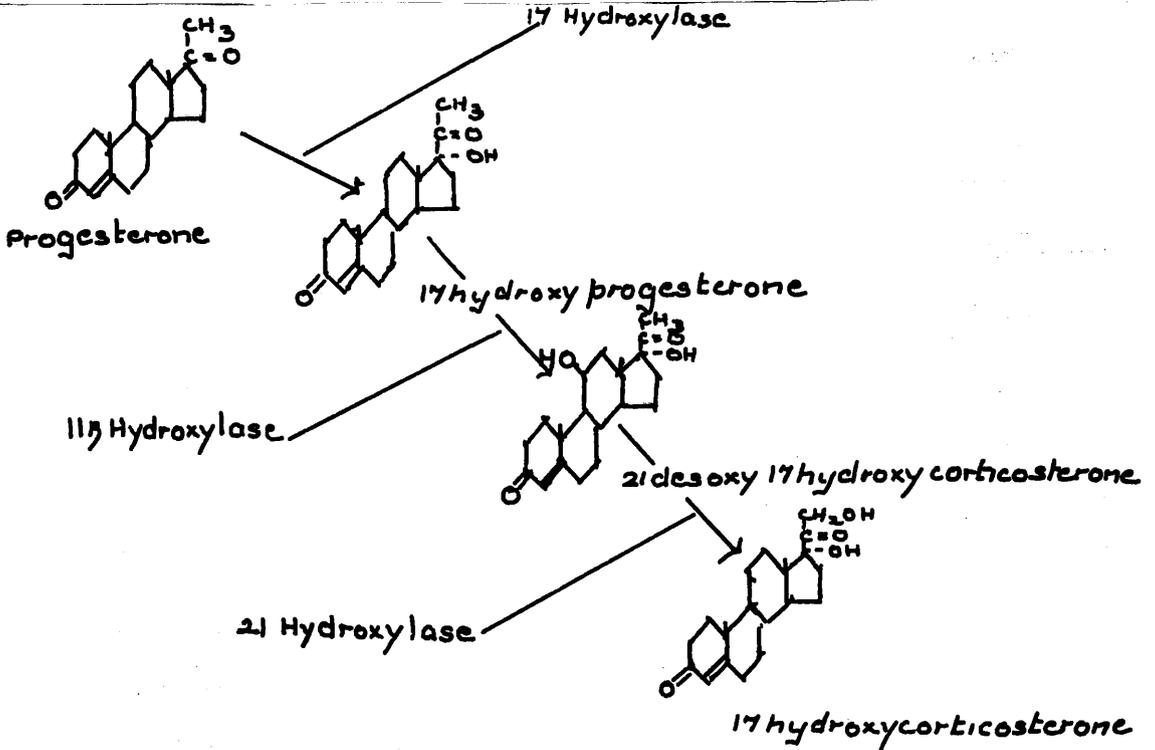
Jailer, in 1953, suggested that the inability to synthesise cortisol was due to an enzymatic failure and in 1954, Dorfman, postulated that congenital adrenal hyperplasia was due to a failure of the enzyme 21 hydroxylase. Evidence has accumulated to prove this aetiology in the "virilising" and the "salt losing" types.

Evidence to show that the rarer "hypertensive" type is due to a lack of 11 β 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 5B pregnane-3∝ 17∝20∝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 con-
trast, 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 17αhydroxy 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 17αhydroxy
progesterone to patients with congenital adrenal
hyperplasia, to those with a low excretion of
steroids, and to normal subjects.

The intermediary metabolite between
17αhydroxy progesterone and pregnanetriol,
(Δ4 pregnene-17α-ol-3,20-dione) 17 hydroxypreg-
nanolone, 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 17αhydroxypreg-
nanolone 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, where as 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-3α17α 20α-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 pregnane-triol.   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 hydroxy-lation 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.

PROGESTERONE

CH_2OH
$C = O$

Desoxycorticosterone

Hypertension

17 Hydroxy progesterone

11β Hydroxylase

CH_2OH
$C = O$
$C - OH$

HO

11 desoxy 17hydroxycorticosterone
Compound S.

Block at

5β Pregnane 3α 17α 20α triol
OH

17 hydroxy corticosterone
Cortisol
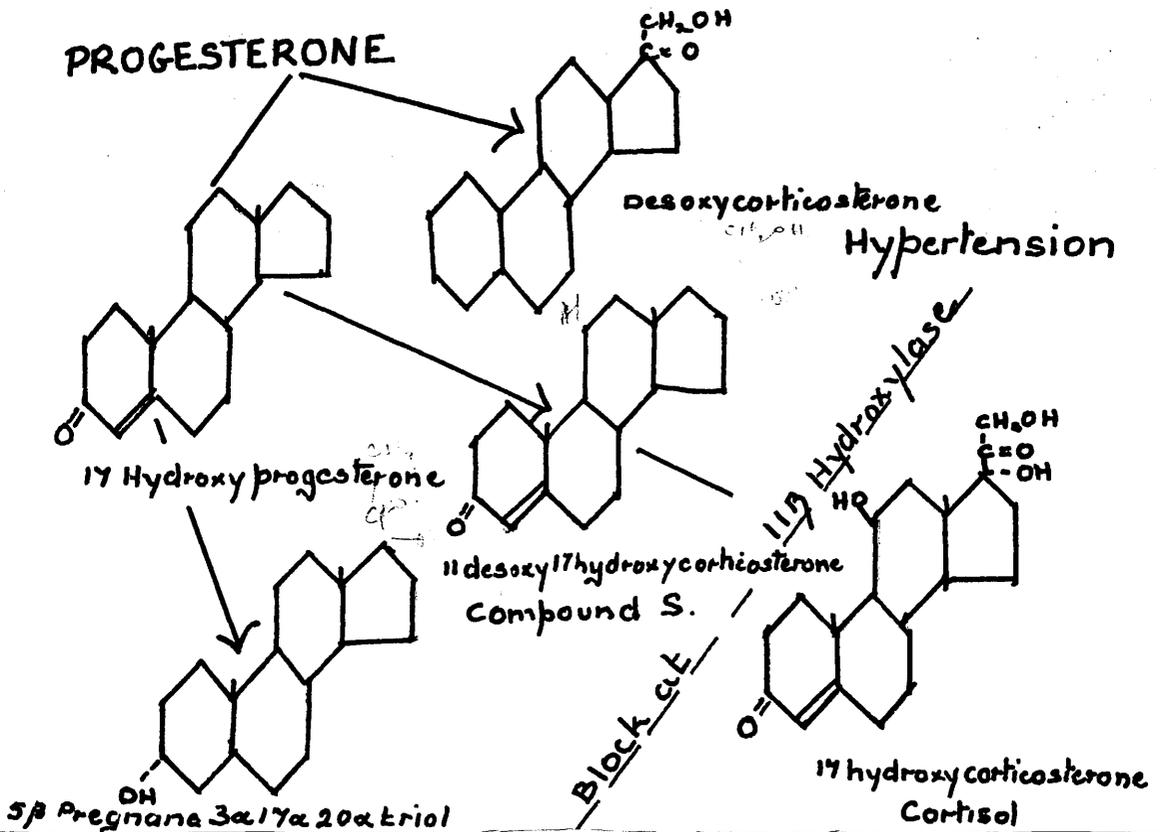
Evidence that hydroxylation at the C21
position did not occur was not substantiated.
Steroids with hydroxylation at the C21 position
were found in normal or only slightly reduced
amounts in the patients with the virilising type
of congenital adrenal hyperplasia. However, in
those showing signs of electrolyte 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 pre-
cursors 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 un-
treated 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 pregnane-
triol 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 adrenacortical
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 cortico-
trophin, 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 ham-
pered 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.

(i) 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 stilbeostrol, 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 dehydra-
tion.   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 in-
sufficiency and not to the hypoglycaemia it may
cause, Butler, Ross and Talbot [1938].   Pigmen-
tation, 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 in-
sufficiency.   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 exami-
nation 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 abnor-
mality 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 elec-
trolyte 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.   Treat-
ment can then be given without any risk of com-
plicating 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 excre-
tion should always be estimated.   The 17 keto-
steroids 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 17OH 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 confir-
mation 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 hyper-
plasia.

AIMS OF TREATMENT

(i) 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 pro-
cedures and all illnesses.

(ii) 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 cushing-oid 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. Post natally 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 inter-
current 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 cortico-
trophin by the pituitary, the adrenal overactivity
diminishes and the hypertrophied adrenal cortex
is reduced in size. Life depends chiefly on the
adminstered cortisone and during periods of
stress, illness or operations, larger doses of
cortisone must be administered. For the con-
venience 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 child-
ren 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.   Desoxycorti-
costerone acetate was the drug of choice and can
be administered as a daily intramuscular injec-
tion, as a monthly injection of the trimethyl
acetate crystules, or as a subcutaneous implant.

The daily injection in doses 2-5 mg., and the
monthly injection of the trimethyl crystules in
doses of 25-250 mg., have been used in children
during this study. Latterly 9 Fluorohydro-
cortisone with a salt retaining property some
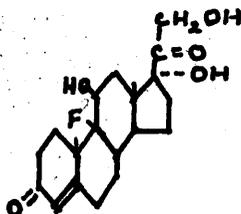
50 times as great as cortisone has been found to
be very successful in doses of 0.1-0.2 mg. daily
in combination with cortisone. When given in
larger doses it produced hypertension. In one
infant oedema occurred with a dose of 0.5 mg.
daily. The action of this corticoid is sustained
for twenty-four hours 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 hyper-
plasia 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 intra-
venous 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 electro-
lyte 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 discon-
tinued.   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 demon-
strated 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 diag-
nosis 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 proce-
dure 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.

INFECTIOUS DISEASES

All the children in this study have been
advised to have the routine immunological inocula-
tions.   Two specific exanthemata, chickenpox and
measles, are considered to be of particular impor-
tance.

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 globu-
lin, 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 treat-
ment 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 manage-
ment 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 un-
surmountable.   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 per-
suaded 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 im-
balance, 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 diffi-
culty 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 investi-
gations. 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. Corti-
sone 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. Con-
sultation 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, suggest-
ing 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

STUDY OF THE URINARY STEROIDS

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 hydroxypregnanolone. The
metabolite of 11 desoxycorticosterone is also
measured in this fraction, and this is the
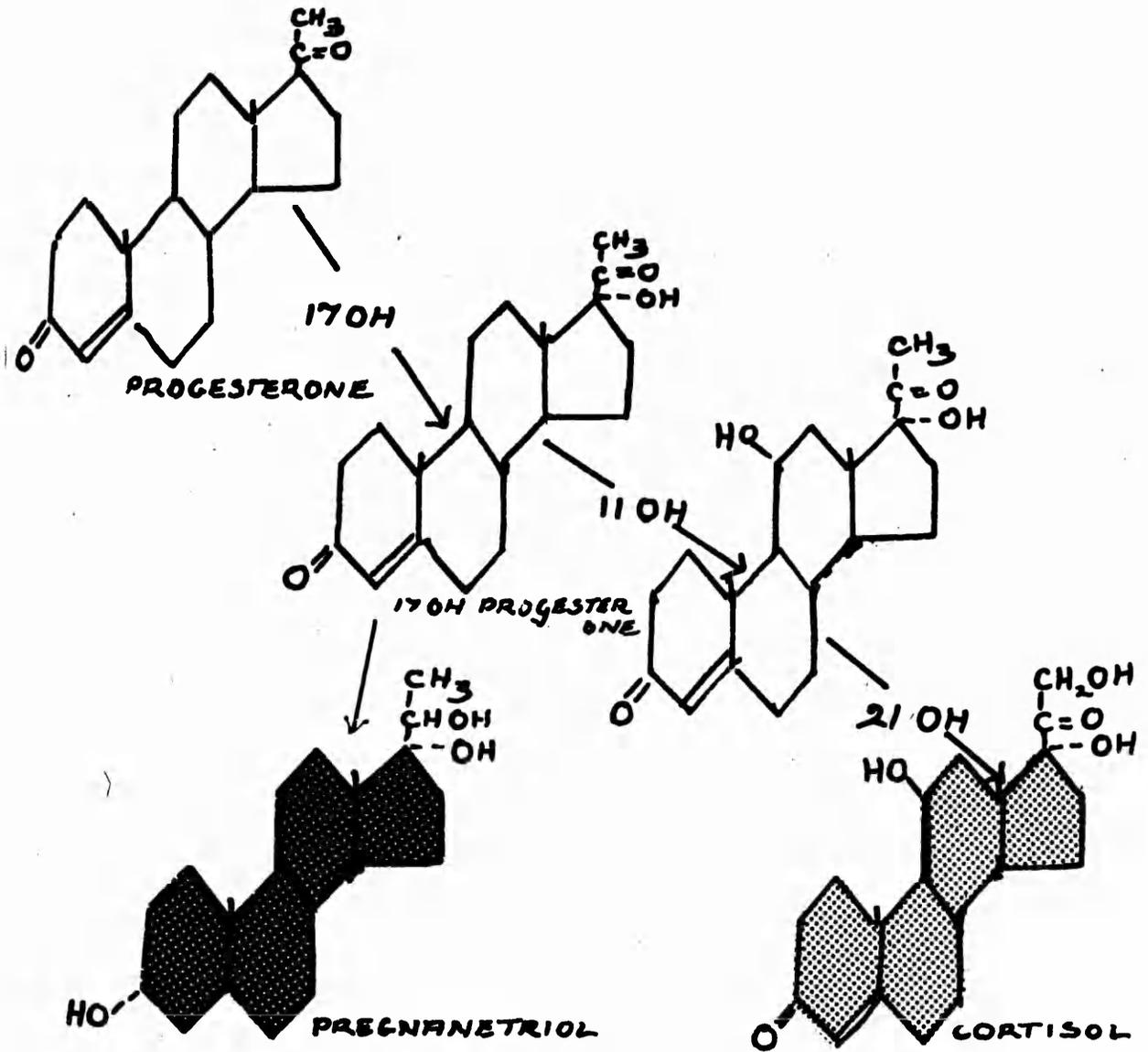important metabolite of the hypertensive type.

In the third fraction, the 11 oxygenated
17OH 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 quanti-
tative 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 chroma-
tography of the 17 ketogenic steroids is illus-
trated 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 hydroxypreganolone
were estimated.

DIAGRAM I

<u>THE NORMAL SYNTHESIS OF CORTISOL AND</u>

<u>THE RESULT OF CHROMATOGRAPHY OF THE</u>

<u>17 KETOGENIC STEROID IN NORMAL CHILDREN</u>

NORMAL SYNTHESIS OF CORTISOL

PROGESTERONE

17 OH

17 OH PROGESTERONE

11 OH

21 OH

PREGNANETRIOL

CORTISOL

CHROMATOGRAPHY OF THE KETOGENIC STEROIDS.

FRACTION II
FRACTION III

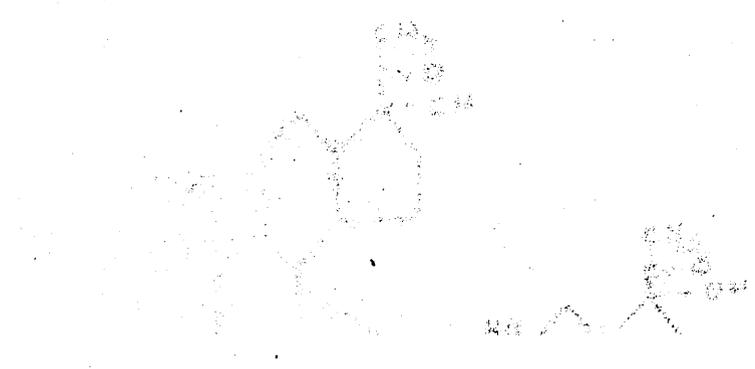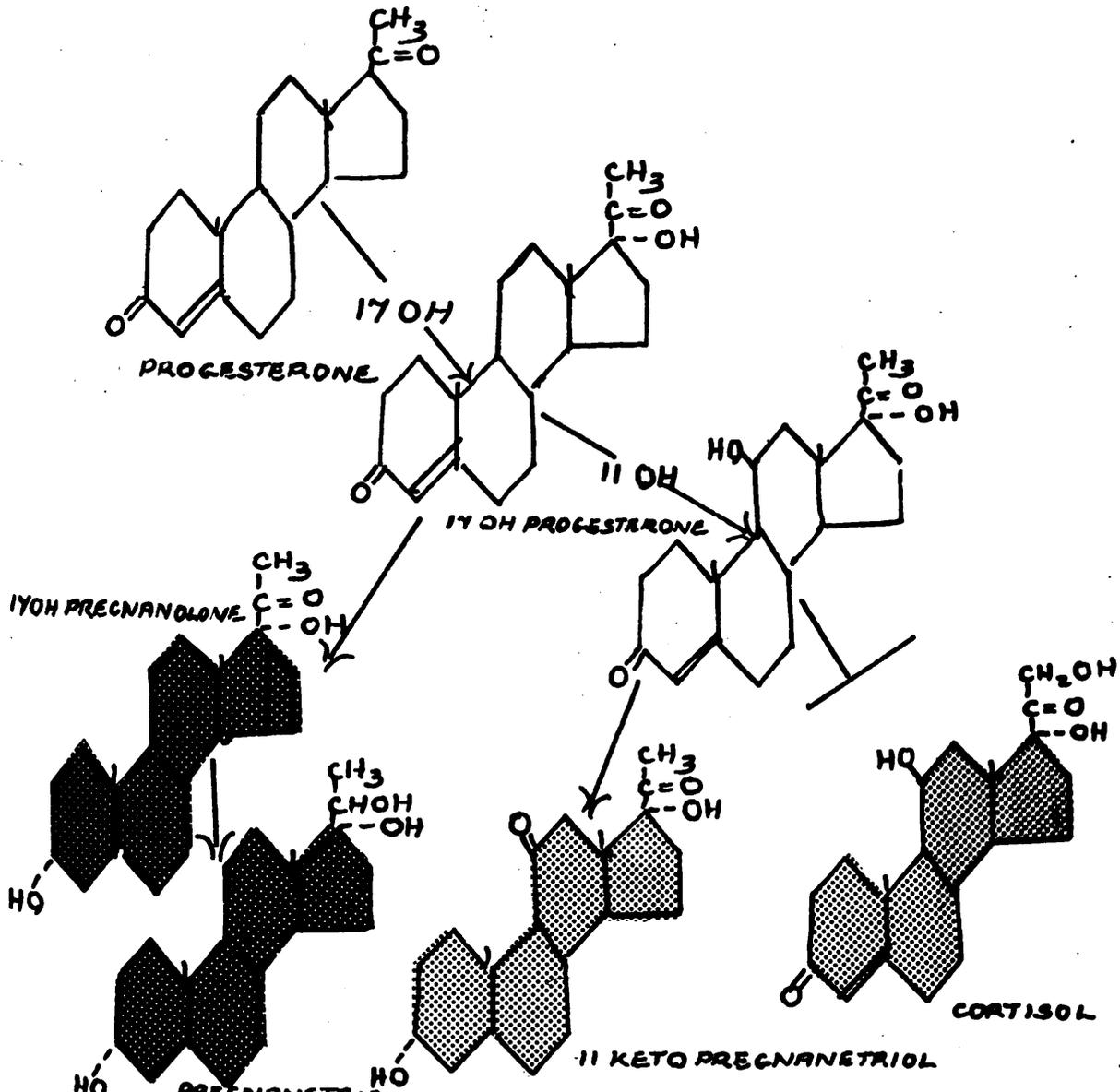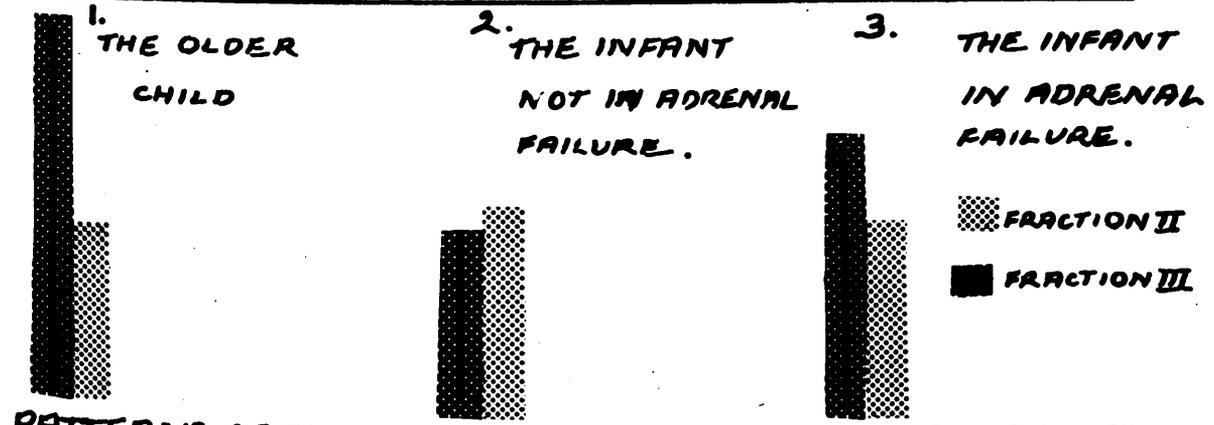PATTERN OBTAINED IN NORMAL CHILDREN

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.

PROGESTERONE

17 OH

14 OH PROGESTERONE

11 OH

14OH PREGNANOLONE

PREGNANETRIOL

11 KETO PREGNANETRIOL

CORTISOL

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.

THE ESTIMATION OF THE URINARY EXCRETION
OF STEROIDS IN THE DIAGNOSIS AND CONTROL
OF 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 in-
creases 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.
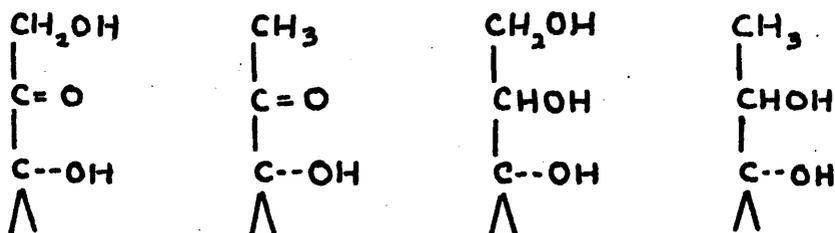
The results are read on the Unicam S P 600 spectrophotometer. Dehydro isoandrosterone 100 µg. is used as a standard and a blank of reagents alone is set up. The reagents are those

of Zimmermann, 2% alcoholic solution of meta

dinitro benzene and an 8N KOH in alcohol.

The correction factor $\dfrac{E520 - (E430 \times 0.6)}{0.73}$ 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:-

$$
\begin{array}{cccc}
CH_2OH & CH_3 & CH_2OH & CH_3 \\
| & | & | & | \\
C=O & C=O & CHOH & CHOH \\
| & | & | & | \\
C\text{-}\text{-}OH & C\text{-}\text{-}OH & C\text{-}\text{-}OH & C\text{-}\text{-}OH \\
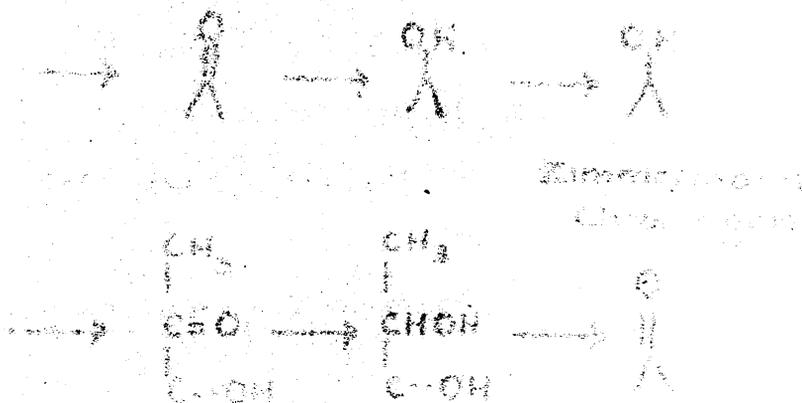\wedge & \wedge & \wedge & \wedge
\end{array}
$$

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 oxydised 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 keto-
steroids 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

$NaBiO_3$ $NaBH_4$ $NaBiO_3$

CH₃
CHOH
C--OH
→   ⟶   ⟶   ⟶

CH₂OH
CHOH
C--OH
→   ⟶   ⟶   ⟶

CH₂OH
C=O
C--OH
→   ⟶   ⟶   ⟶

Zimmermann Chromogen

CH₃
C=O
C--OH
→

CH₃
C=O
C--OH
→

CH₃
CHOH
C--OH
→
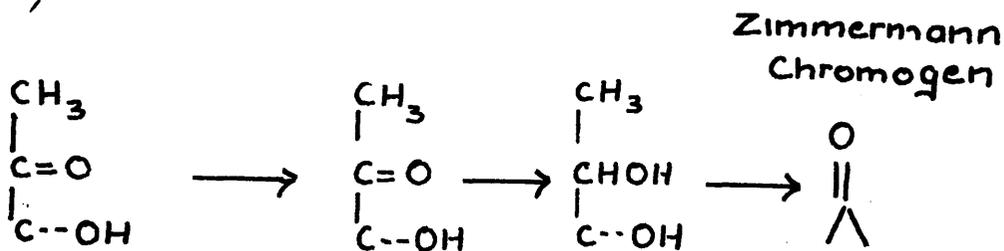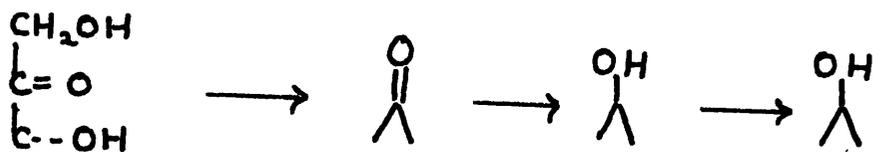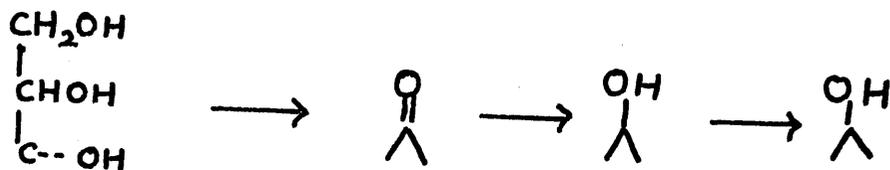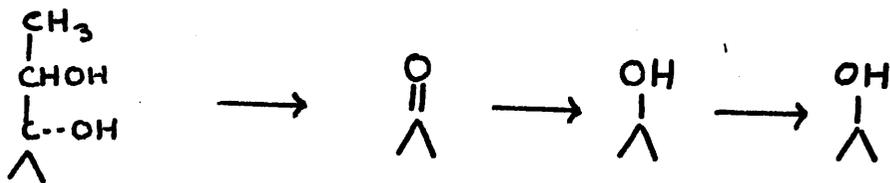
Blank estimation as control

$NaBH_4$ $NaBiO_3$ $NaBH_4$ $NaBiO_3$

CH₃
C=O
C--OH
→

CH₃
CHOH
C--OH
→   ⟶
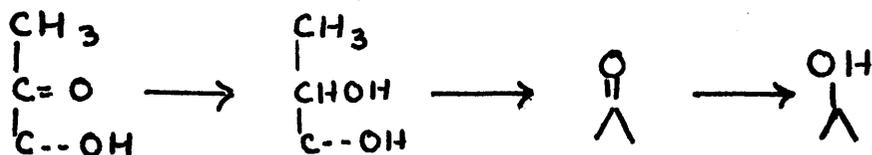
<u>Details of the method used for the estimation</u>

<u>of 17-hydroxypregnanolone</u>

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 $\frac{1}{2}$ 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.

17OH PREGNANOLONE IN FRACTION II

Legend:
- Fraction II Pregnanetriol + 17OH Pregnanolone
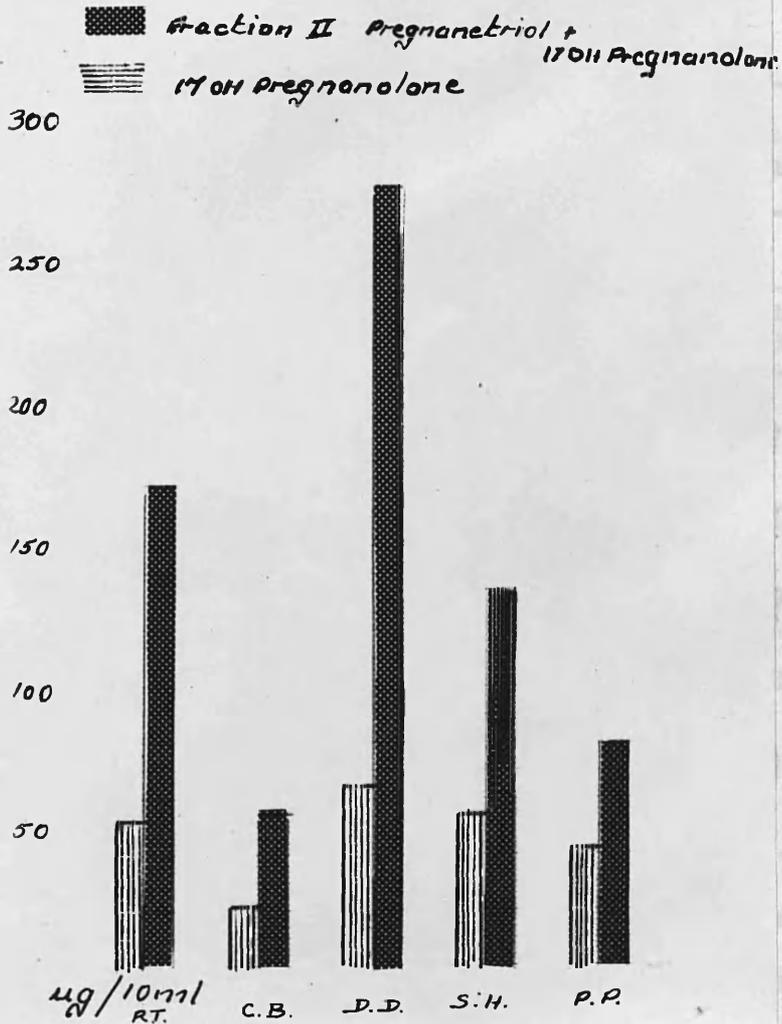- 17OH Pregnanolone

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

	17 ketogenic steroids				21-deoxyketols (17 OH Pregnanolone)			
	II		III		II		III	
	ug/ 10ml.	Mg./ 24hr.	ug/ 10ml.	Mg./ 24hr.	ug/ 10ml.	Mg./ 24hr.	ug/ 10mL.	Mg./ 24hr.
P_P.	79.5	4.7	58.5	3.5	42	2.5	7	0.4
R_T.	171.5	23.8	64.5	9	55.2	8.3	7.7	1.2
C_B.	54.8	0.95	52.2	0.94	21.2	7.6	0.38	0.14
D_D.	277.2	108.6	11.9	4.6	64	16.5	2.75	0.7
S_H.	135	91	4.2	2.8	55.4	3.3	1.7	0.1

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 pregnane-
triol 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.
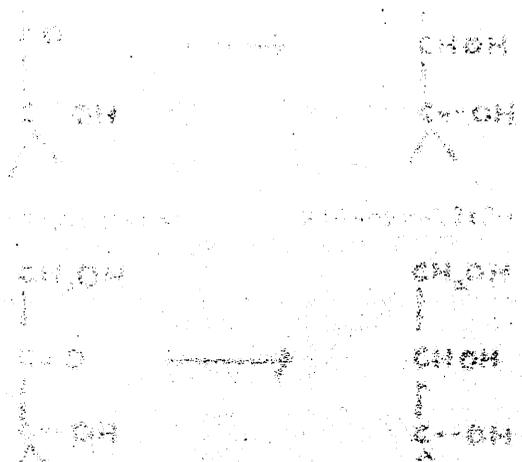
THE CHEMISTRY OF THE METHOD

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.

$$\text{O=C} \longrightarrow \text{OH–CH}$$
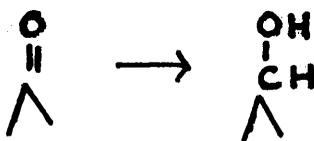
At the same time the C21:17:20 ketols are reduced to glycols, as described by Appleby Gibson, Norymberski and Stubbs [1955].

$$\begin{array}{c} CH_3 \\ | \\ CO \\ | \\ C\text{-}\text{-}OH \end{array} \longrightarrow \begin{array}{c} CH_2OH \quad ?CH_3 \\ | \\ CHOH \\ | \\ C\text{-}\text{-}OH \end{array}$$

2Ideoxy-I7:20 Ketol   2Ideoxy-I7:20 glycol.

$$\begin{array}{c} CH_2OH \\ | \\ C=O \\ | \\ C\text{-}\text{-}OH \end{array} \longrightarrow \begin{array}{c} CH_2OH \\ | \\ CHOH \\ | \\ C\text{-}\text{-}OH \end{array}$$

2Ihydroxy-I7:20 Ketol   2Ihydroxy-I7:20 glycol.
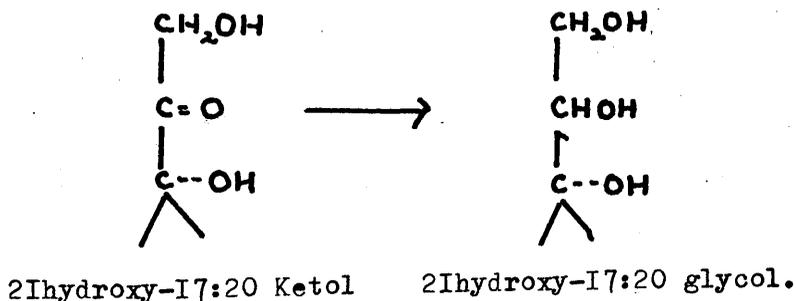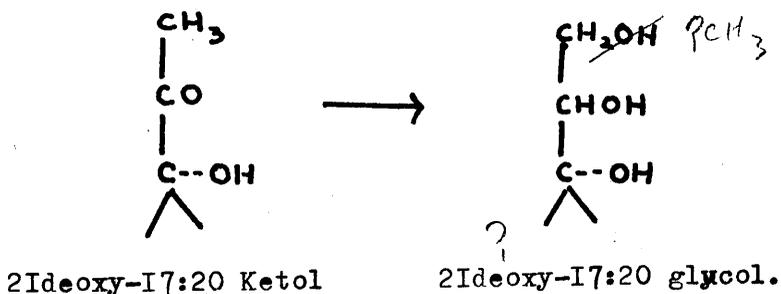
The second reaction, oxidation with sodium bismuthate in the presence of acetic acid, results in the oxidation of the C21 glycols thus formed, and those already present in the urine, to 17 ketosteroids.

$$
\begin{array}{c}
\overset{|}{C}H_2OH \\
\overset{|}{C}HOH \\
\overset{|}{C}\text{--}OH \\
\wedge
\end{array}
\quad + \quad
\begin{array}{c}
\overset{|}{C}H_3 \\
\overset{|}{C}HOH \\
\overset{|}{C}\text{--}OH \\
\wedge
\end{array}
\quad \longrightarrow \quad
\begin{array}{c}
O \\
\| \\
\wedge
\end{array}
$$
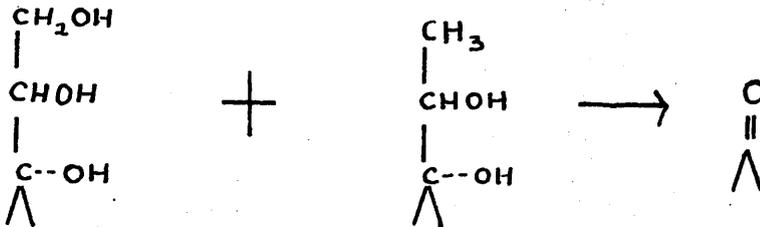
The 17 ketosteroids are then extracted with ethylene dichloride, washed and evaporated to dryness. The extract contains the 17 keto-steroid, Aetiocholanolone (30∝ hydroxy-5β - androstan-17-one) which is the oxidation product of pregnanetriol, and 17 ketosteroids with an oxygen grouping at the C11 position, (3∝ 11β di-hydroxy-5β -androstane-17-one and 3∝ -hydroxy-5β - andostrane 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
ehanol 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

DIAGRAM 3

One month old ♂ congenital adrenal hyperplasia c.J.
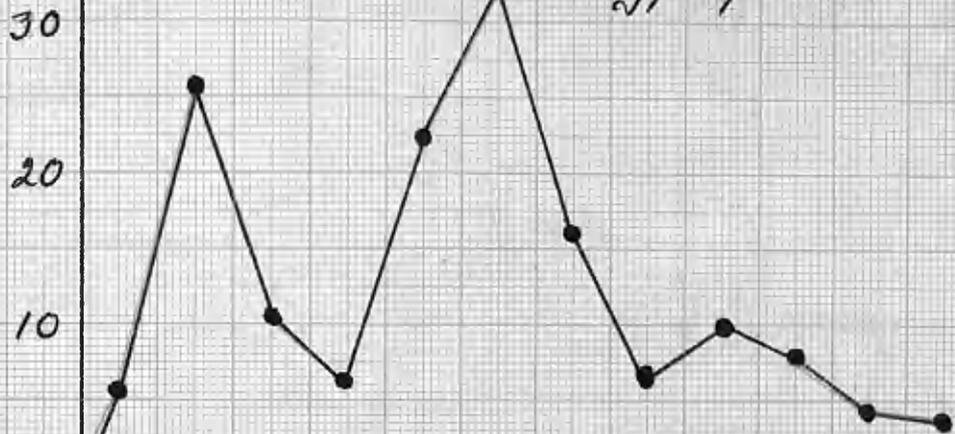
Five day old ♀ congenital adrenal hyperplasia c. B.
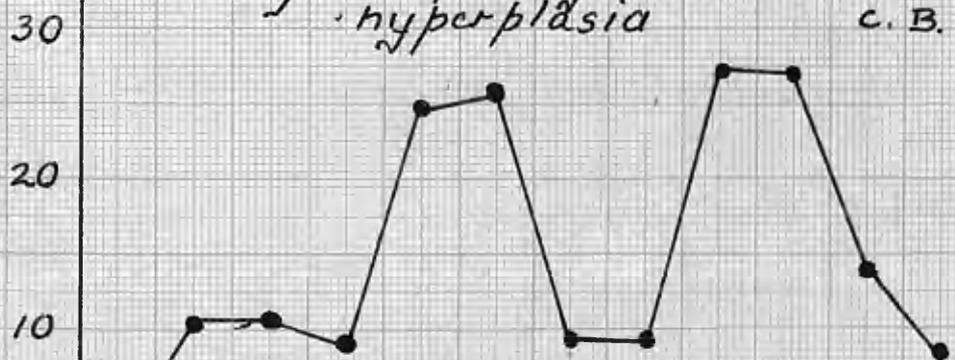
25% ethyl acetate   35% ethyl acetate   65% ethyl acetate

20μg aetiocholanolone   10μg 11OHandrosterone

μg/5ml
Fraction.   I   II   III

DIAGRAM 4

S. H.

♀ infant congenital adrenal hyperplasia

25% ethyl acetate : 35% ethyl acetate : 65% ethyl acetate

μg/5 ml Fraction
I   II   III

D. D.

♂³/₁₂ in adrenal failure

μg/5 ml. Fraction
I   II   III

DIAGRAM 5

♂ Congenital adrenal hyperplasia
on 75mg cortisone daily   D.

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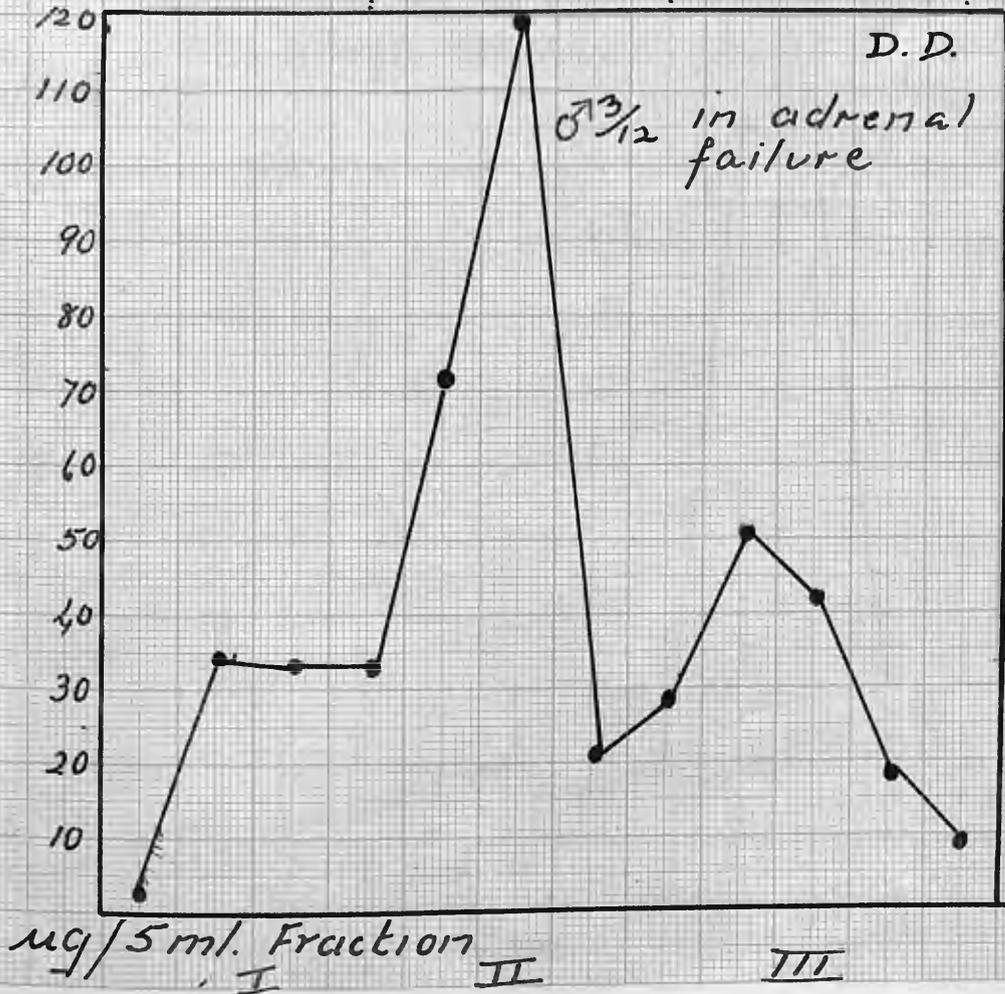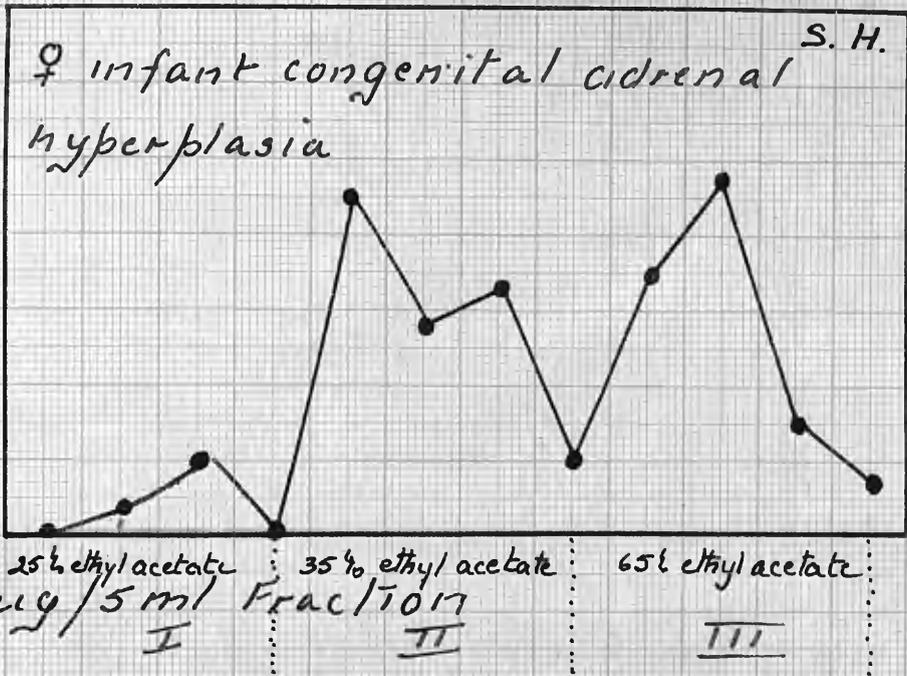25% ethyl acetate 35% ethyl acetate 65% ethyl acetate
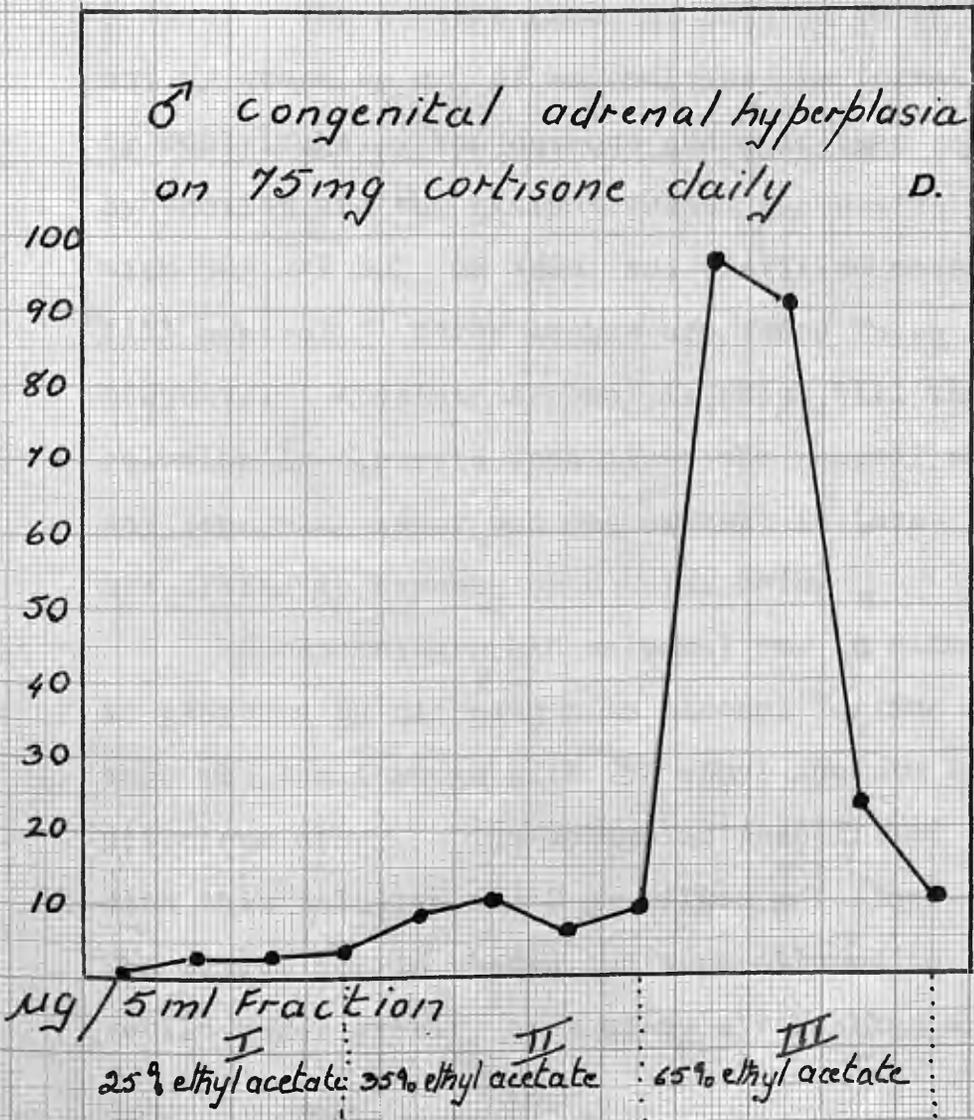
is shaken till all the bismuthate is reduced,
5-30 mins. is the length of time necessary
depending on the quality of the bismuthate used.
To the resulting solution, 4 ml. of concentrated
hydrochloric acid is added.

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 20μ g. 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 NKOH 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 inter-
feres 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 mu.
on a Unicam S.P.600 spectrophotometer.   A
correction factor $E520 - \frac{1}{2}$ ($E440 - E600$) 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 aetio-
cholanolone 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 un-
treated patients with congenital adrenal hyper-
plasia 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 child-
ren, the ratio of the pregnanetriol fraction II
to the 11 oxygenated 17OH 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 17OH 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.

| Sex | Age | Fraction No. | | | | Ratio II/III |
| | | II | III | II | III | |
		ug/10 ml.		mg/24 hr.		
Female	8 days	3.4	6	0.06	0.1	0.57
Female	12 days	2.6	8	0.07	0.2	0.32
Male	4 weeks	4.5	14	0.1	0.4	0.32
Male	6 weeks	7.3	44.1	0.18	1.06	0.17
Male	6 weeks	0.45	8	0.14	0.24	0.06
Male	3 months	0.6	8	0.025	0.33	0.07
Female	4 months	4.3	12	0.2	1	0.35
Male	5 months	2.0	15.2	0.05	0.4	0.12
Female	8 months	8.6	32.6	0.28	1	0.27
Male	8 months					
Male	10 months	4.3	20.6	0.18	0.86	0.20
Female	2½ years	8.1	45	0.18	1	0.18
Male	2½ years	1.6	19.9	0.08	0.9	0.10
Male	2½ years	7.2	53.3	0.2	1.4	0.14
Male	3 years	8.2	47.3	0.41	24	0.18
Male	4 years	6.2	30	0.25	1.26	0.20
Male	5½ years	7	31	0.4	1.5	0.27
Male	6½ years	6	34	0.6	3.4	0.18
Male	6.11/12 yrs.	5.6	25	0.34	1.5	0.23
Female	7 years	14.3	87	0.4	2.6	0.16
Male	7 years	7	28.5	0.35	1.25	0.28
Female	8 years	9.3	45.7	0.36	1.9	0.2
Female	8½ years	5	21	0.5	2	0.25
Female	10 years	2.4	12	0.3	1.65	0.18
Male	11 years	4.5	15	0.25	0.8	0.37
Male	11 years	4.8	13	0.4	1.2	0.33
Male	11 years	6.7	21.4	0.9	2.9	0.30
Male	12 years	7.5	23.8	0.46	2.0	0.31
Male	12 years	8.0	21	1.28	3.4	0.38
Male	12 years	7.0	41.8	0.9	5.4	0.17
Female	12 years	20	56.6	1.6	4.6	0.35

DIAGRAM 7

THE RESULTS OF CHROMATOGRAPHY OF THE

17 KETOGENIC STEROIDS IN NORMAL CHILDREN

Normal Children

II   Pregnanetriol
IV. 11oxygenated 17OH.Steroids
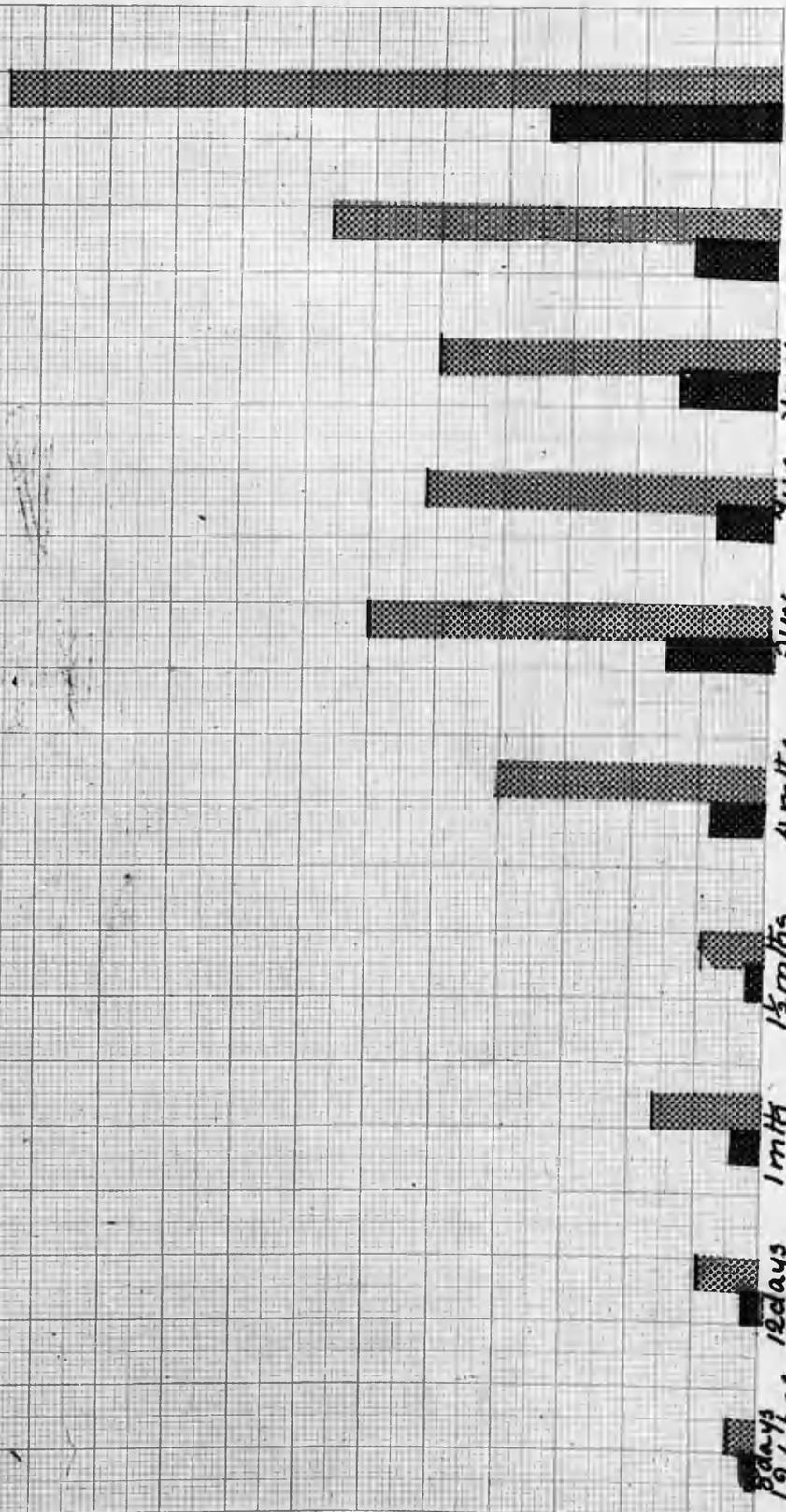
II. CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA

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 dispro-
portionate 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 III

CHROMATOGRAPHY OF THE 17 KETOGENIC STEROIDS

RESULTS ON PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

Sex		Age	24 hr. vol.ml.	Fraction No.				Ratio II/III
				II ug/10 mns.	III	II mg/24 hrs.	III	
	C.B.	2 days	single	58	75.4			0.8
		5 days	180	54.8	52.2	0.95	0.94	1
	B.C. 8 days started on I.V. drip with hydrocortisone during collection of urine.		290	51	21	1.5	0.6	2.4
	S.H.	12 days	single	50	56.5	-	-	0.9
	P.P.	15 days	single	317	105	-	-	1.8
	S.B.	15 days	200	174	136.4	5	4	1.25
	T.Q.	19 days	single	90.6	58.6	-	-	1.5
	C.J.	1 mth.	single	180	80	-	-	2.2
	P.C. 5 wks. On I.V. drip and cortisone		-	146.7	100.8	3.4	2.3	1.5
	H.B.	2½ mths.		16.2	17.7	0.6	0.65	1
	D.D.	3 mths.	single	133.5	46.0			3.4
	C.M.	15 mths.		261.6	68.6	11.4	2.9	4
	L.M.	6 yrs.	430	200	51.5	8.6	2.2	3.2
	C.T.	9 yrs.	1000	111	56	25.4	12.8	2
	K.P.	10 yrs.	720	1190	362	85.7	25	3.4
	R.T.	15 yrs.	1020	411	116	42	11.8	3

Adults	1.	single	115	36				3
	2.	"	98	75				1.3
	3.	"	162	69.5				2.3

DIAGRAM 8

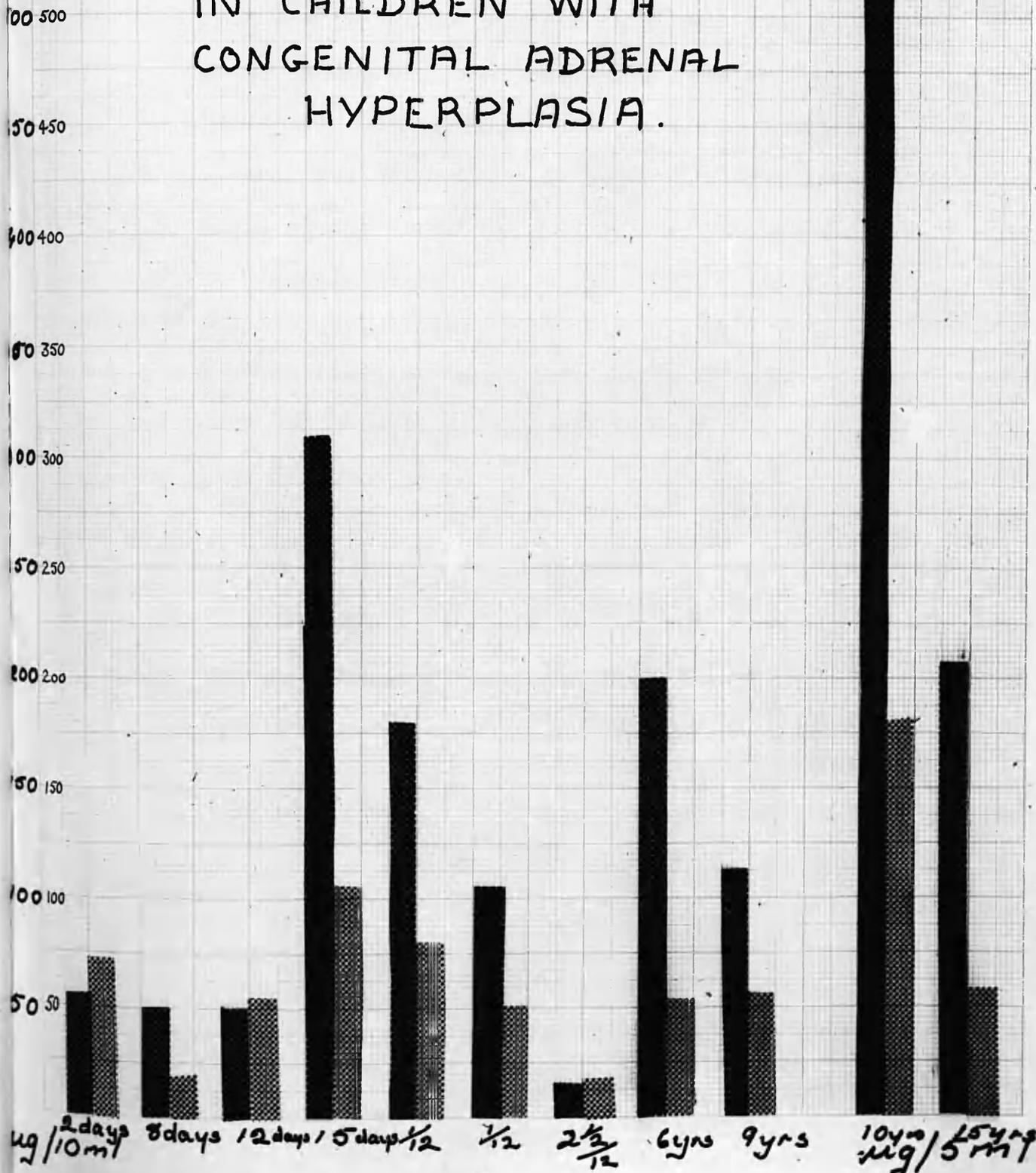THE RESULTS OF CHROMATOGRAPHY OF THE

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 IV

A. Infants not in adrenal failure	B. Infants in adrenal failure	C. Older children
0.8	1.8	4.0
0.9	1.2	3.2
1.0	1.5	2.0
	2.2	3.4
	1.5	3.0
	3.4	
0.9	1.9	3.3

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.
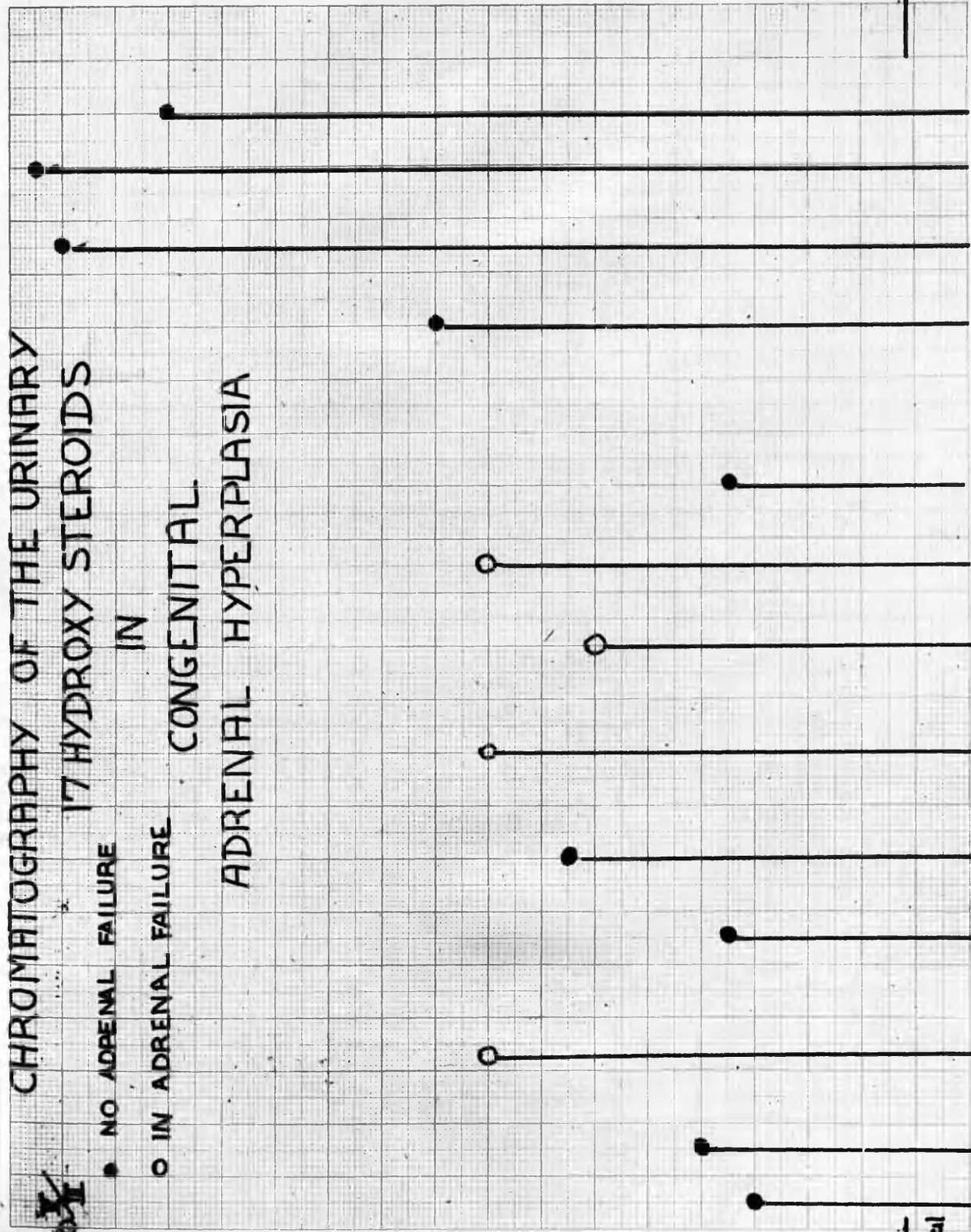
DIAGRAM   9

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 suf-
ficient 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 ketopregnane-
triol 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 ketopregnane-
triol 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 17αhydroxy 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 17αhydroxypregnanolone 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 17αhydroxy-
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 preg-
nanetriol 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 chroma-
tography 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 con-
genital 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 hypo-
spadias.   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

age.   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. stimu-
lation 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 $11\frac{1}{2}$ 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 andro-
genization 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 dehydra-
tion 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 sup-
port 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 corti-
coids 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 hyper-
plasia was confirmed.

TABLE V Diagnostic problems

	AGE	PROVISIONAL DIAGNOSIS	FRACTION NO. II. ug/10 ml.	III.	II. mg/24.hr.	III.	RATIO II/III	DIAGNOSIS
1	2 days	?female pseudo hermaphrodite	58	75.4	single sample		0.8	Female Congenital adrenal hyperplasia
	3 days	?female pseudo hermaphrodite	11	23.4	0.03	0.065	0.46	
2	4 days	ditto	1.7	10	0.025	0.15	0.17	Male Hypospadias
	5 days	ditto	2.4	8.8	0.02	0.07	0.3	
3	4 days	Breech delivery oedema of genit- alia ?enlarged phallus	10	32	single sample	0.3		Normal child
4	12 days	Hypospadias ?con- genital adrenal hyperplasia	50	56	single sample		0.9	Female congenital adrenal hyperplasia
5	6 days	?hypospadias or	3.8	11.2	0.038	0.112	0.34	17KS
	7 days	?congenital adrenal	0.86	2.1	0.03	0.87	0.4	0.2 Male 0.4
	8 days	hyperplasia	3	16.3	0.02	0.13	0.4	0.22 Hypospadias
6	2 wks.	congenital heart V.S.D. pigmented ?cong. adrenal hyperplasia	90.6	58.6	single		1.5	Male congenital adrenal hyperplasia
7	15 days	Male ?pyloric stenosis. High serum potassium low sodium. Jaundiced.	317	166.2	single sample		1.8	Male congenital adrenal hyperplasia. Jaundiced
8	3 wks.	?congenital adrenal hyper- plasia. Failure to thrive.	12	57.2	single sample		0.22	Feeding problem
9	3 wks.	Failure to thrive ?congenital	0.28	2.1	0.074	0.5	0.14	Gastro- enteritis
	6 wks.	adrenal hyperplasia serum potassium high, sodium low 128 meq./l.	0.45	8.1	0.014	2.24	0.05	
10	3 wks.	Vomiting	6.9	21	single		0.3	Feeding problem

TABLE V Continued

	AGE	PROVISIONAL DIAGNOSIS	II.	III.	II.	III.	RATIO II/III.	DIAGNOSIS
11	3 wks.	Vomiting Na. 135. *m.eq./l* K. 5.9. *m.eq./l.* Cl 108 *m eq./l.*	5.5	23.3	0.09	0.4	0.22	Milk allergy
12	1 mnth.	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.	180 105	80 52.5	single sample 9.9	 4.8	2.25 2	Male congenital adrenal hyperplasia
13	5 wks.	?Male pyloric stenosis.	146.7	100.8	3.4	2.3	1.5	Female congenital adrenal hyperplasia
14	2½ mnths.	Hypospadias ?congenital adrenal hyperplasia.	16.2	17.7	0.6	0.65	1	Female congenital adrenal hyperplasia
15	3 mnths.	Vomiting, weight loss. No pigmentation Electrolyte changes.	133.5	46	single sample		3.4	Congenital adrenal hyperplasia
16	4 mnths.	Vomiting, failure to thrive. Congenital adrenal hyperplasia.	2.4	10	0.1	0.5	0.2	Gastro-intestinal upset
17	7 mnths.	Enlarged phallus fused labia. Mother given progesterone during pregnancy.	0.03	0.091	single sample		0.3	Non adrenal female pseudo hermaphrodite
18	15 mnths.	Precocious development ?Cong. adrenal hyperplasia.	264.6	68.6	11.4	2.9	4	Male congenital adrenal hyperplasia
19	2 yrs.	Enlarged phallus ?congenital adrenal hyperplasia.	1.6 5.4	57.5 32	0.5 6.3	1.7 1.9	0.3 0.16	Female masculinised external genitalia

TABLE V Continued

	AGE	PROVISIONAL DIAGNOSIS	FRACTION NO. II.	III.	II.	III.	RATIO II/III.	DIAGNOSIS
20	3 yrs.	Hypospadias ?congenital adrenal hyperplasia	7 4	15.5 15	0.3 0.25	0.7 0.95	0.45 0.3	Male hypospadias
21	5 yrs.	Hypospadias? female pseudo-hermaphrodite.	2.3	20	0.1	0.9	0.1	Male hypospadias
22	6 yrs.	Slightly enlarged phallus.	1.74	26.9	.1	2	.05	Within normal limits
			A.C.T.H. showed no increase in ratio					
23	7 yrs.	Precocious development ?cong. adrenal hyperplasia.	4.6	56	0.2	2.6	0.08	Precocious development. Normal bone age
24	9 yrs.	Male precocious puberty.	111	56	25.4	12.8	2	Macrogenitosoma praecox. advanced bone age.
25	10 yrs.	Repaired hypospadias. Precocious puberty. ?female pseudo-hermaphrodite.	190	362	85.7	25	3.4	Female pseudo hermaphrodite
26	12 yrs.	Enlarged clitoris. Masculinisation.	12	56.6	1.5	7	2	Male pseudo-hermaphrodite or male gonadal dysgenesis with androgenisation

CHROMATOGRAPHY OF THE URINARY 17 KETOGENIG STEROIDS IN CHILDREN WITH ABNORMAL GENITALIA

CHROMATOGRAPHY OF THE 17-KETOGENIC STEROIDS IN CHILDREN WITH VOMITING
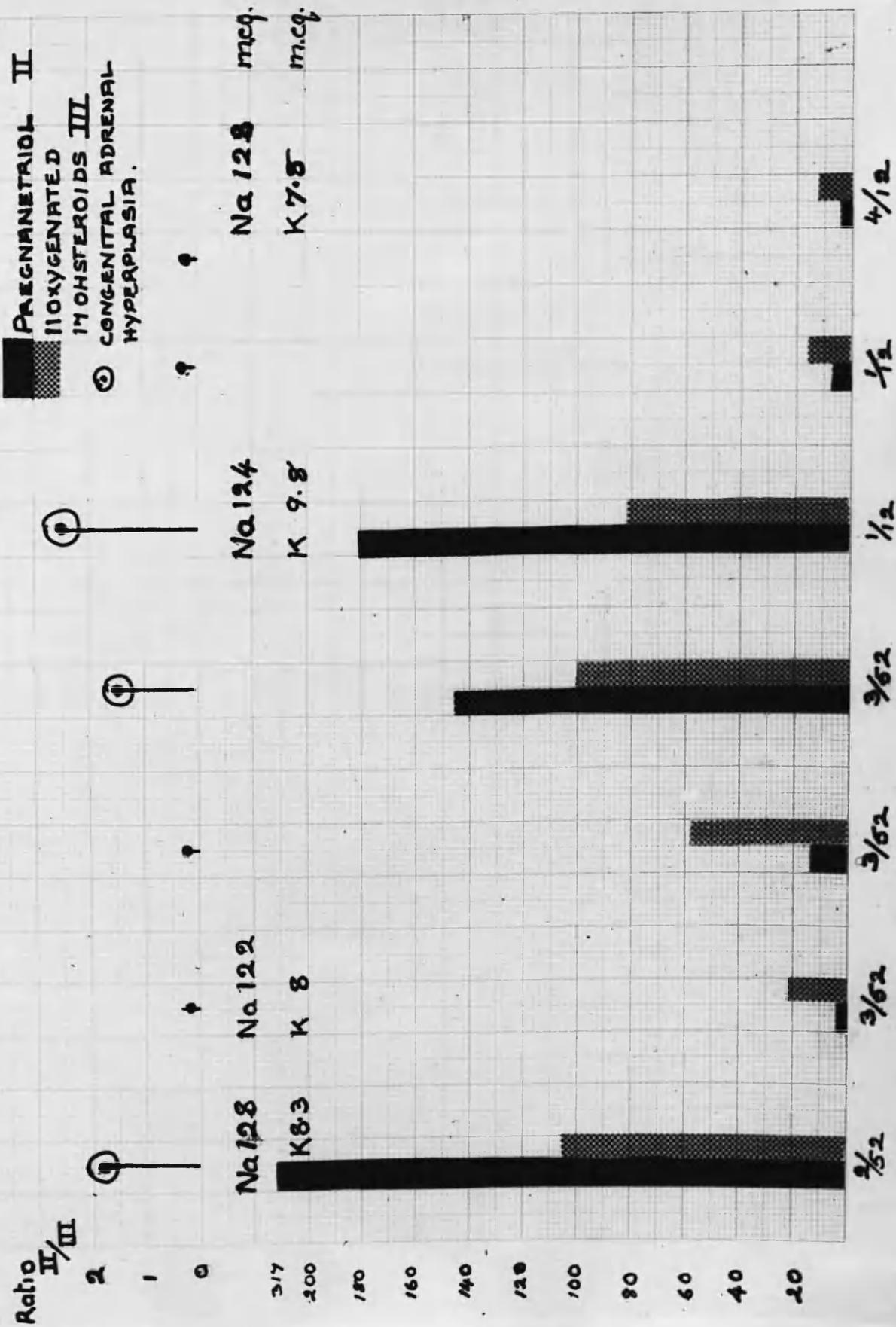
Diagnostic Problems.

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

♀ 10yrs
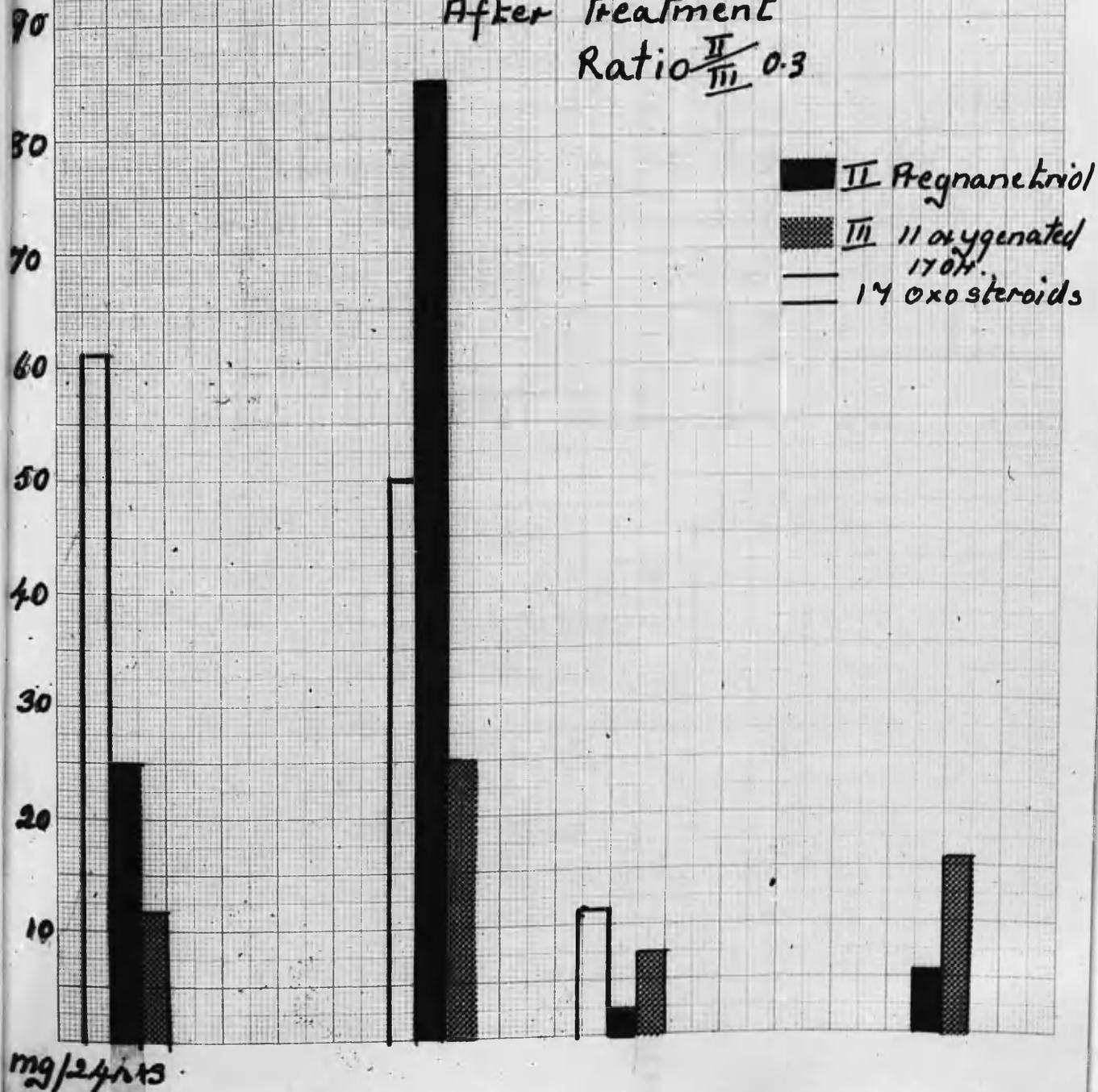Repaired
Hypospadius
Bone age 18yrs
Ratio $\frac{II}{III}$ 3.4

After Treatment
Ratio $\frac{II}{III}$ 0.3

♂ 11yrs
? Cushing
Ratio $\frac{II}{III}$ 0.4

■ II Pregnanetriol

▨ III 11 oxygenated
17OH.

▭ 17 oxosteroids

mg/24hrs

EFFECT OF STIMULATION WITH ACTH. ON
THE STEROID PATTERN

In five children the effect of stimulation
of the adrenal glands with A.C.T.H. was inves-
tigated (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 hypo-
spadias 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 dispro-
portionate 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 hy-
droxylase 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

Day	24 hr. vol. ml.	Fraction No.				Ratio II/III
		II ug/10 ml.	III	II mg/24 hrs.	III	
I. 4 yr. old female with congenital hyperplasia receiving 25 mg. Cortisone daily.						
Control 1	400	700	250	28	10	2.8
2	630	612.5	312.5	38.5	19.7	2
4	500	384	274	19.2	13.7	1.4
ACTH gel 10 units b.d.						
1	1200	600	232	72	27.8	2.6
2	500	744	232	37.2	11.6	3.2
3	1400	398	147	55.7	20.6	2.7
4	1750	349	184	61.1	31.2	2.0
II. 3 yr. old chromatin negative child with scrotal hypospadias (Diagram 13).						
Control 1	460	7	15.5	0.3	0.7	0.43
ACTH gel 10 units b.d.						
1	300	10	24.8	0.3	0.7	0.43
2	230	41.3	140	0.9	3.2	0.3
3	360	35	73.6	1.26	2.6	0.48
4	620	37.8	68	2.3	4.2	0.55
ACTH discontinued						
1	440	17.7	47.3	0.78	2	0.39
4	630	4	15	0.25	0.95	0.26
III. 2½ months old female with congenital adrenal hyperplasia (Diagram 14).						
Control 1	360	14	24.5	0.5	0.9	0.55
2	420	32	38.6	1.3	1.6	0.8
3	370	16.2	17.7	0.6	0.65	1
4	450	22	24.4	1	1	1

TABLE VI (Continued)

Day	24 hr. vol. ml.	Fraction No. II ug/10	III ml.	II mg/24	III hrs.	Ratio II/III
ACTH gel 5 units b.d.						
1	490	23.9	19	1.2	0.9	1.2
2	470	55.5	27.7	2.6	1.3	2
3	575	27	29.5	1.7	1.7	1
4	625	37	19.4	2.3	1.2	1.9
IV. 4 yr. old chromatin positive child with masculinisation of the external genitalia. (Diagram 15).						
Control 1	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.						
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						
1	288	7.4	40.5	0.2	1.2	0.17
V. 3 weeks old child with congenital adrenal hyperplasia (Diagram 16).						
Control 1	Single speci- men	50	56			0.9
ACTH gel 5 units b.d.						
1	310	135	91	4.2	2.8	1.5
2	✓ 94	185	86			2.1
3	✓ 73	186	84			2.2

✓ = Not complete 24 hr. specimens.

♀ ? Congenital Adrenal Hyperplasia 4 yrs
Masculization of external genitalia No I
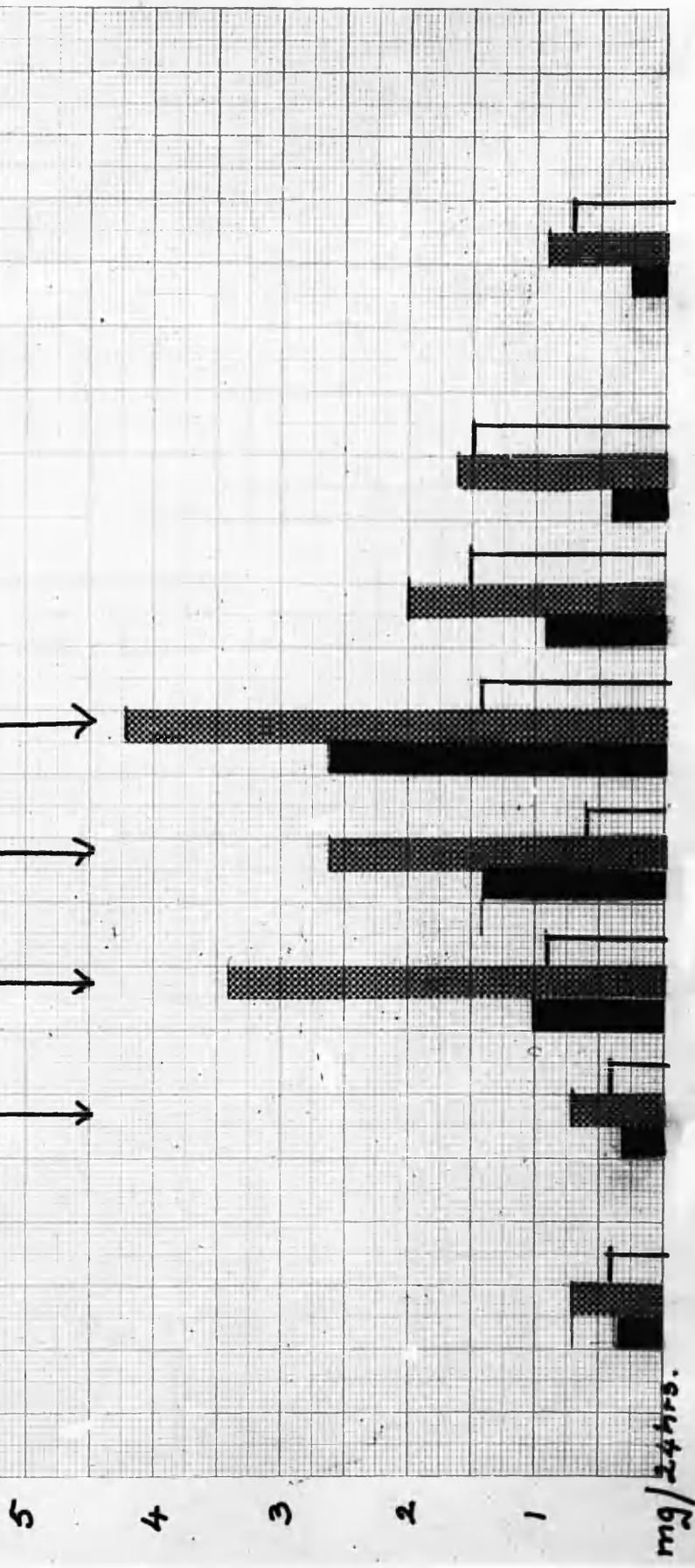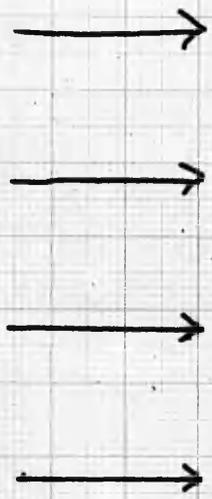Ratio normal.
Normal response to

ACTH ———
10 units
B.D.

PREGNANETRIOL FRACTION II
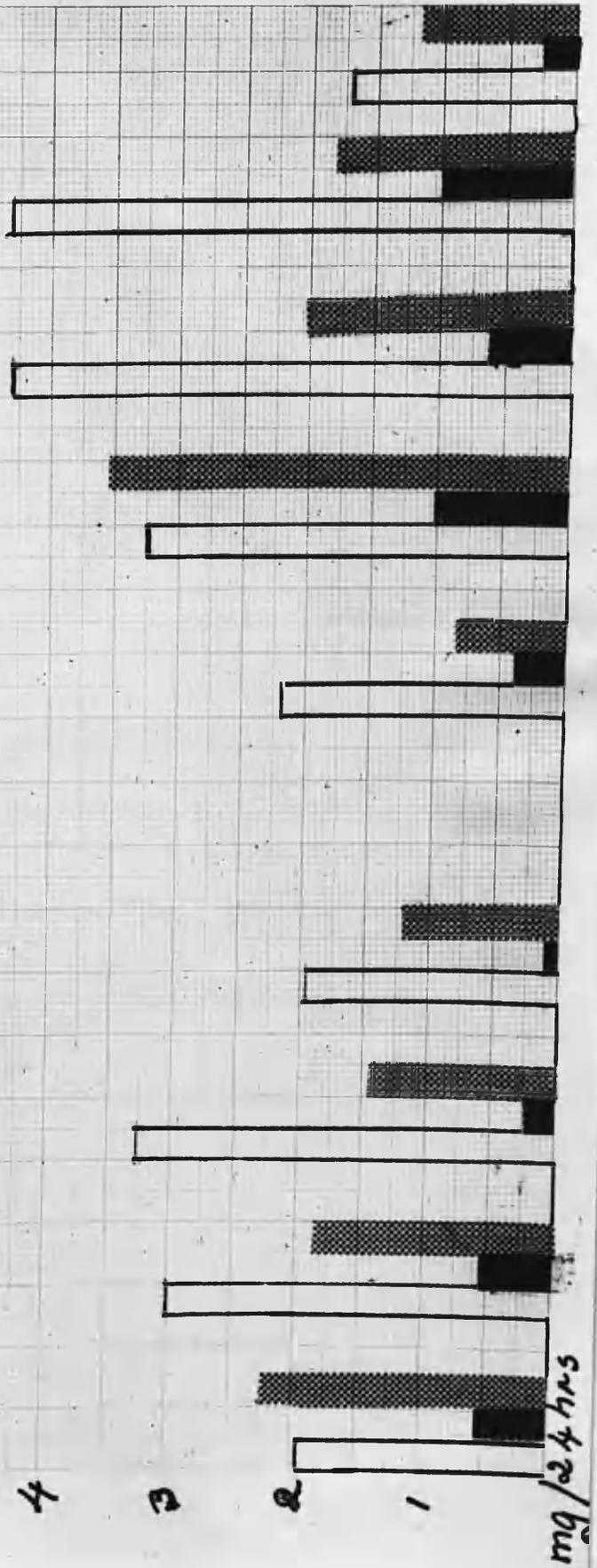
11 OXYGENATED 17 OH STEROIDS
FRACTION III

17 KETO STEROIDS

mg/24 hrs

3yr Male with Hypospadias. No II

PRAEGNANETRIOL FRACTION I
11 OXYGENATED 17OH STEROID FRACTION III
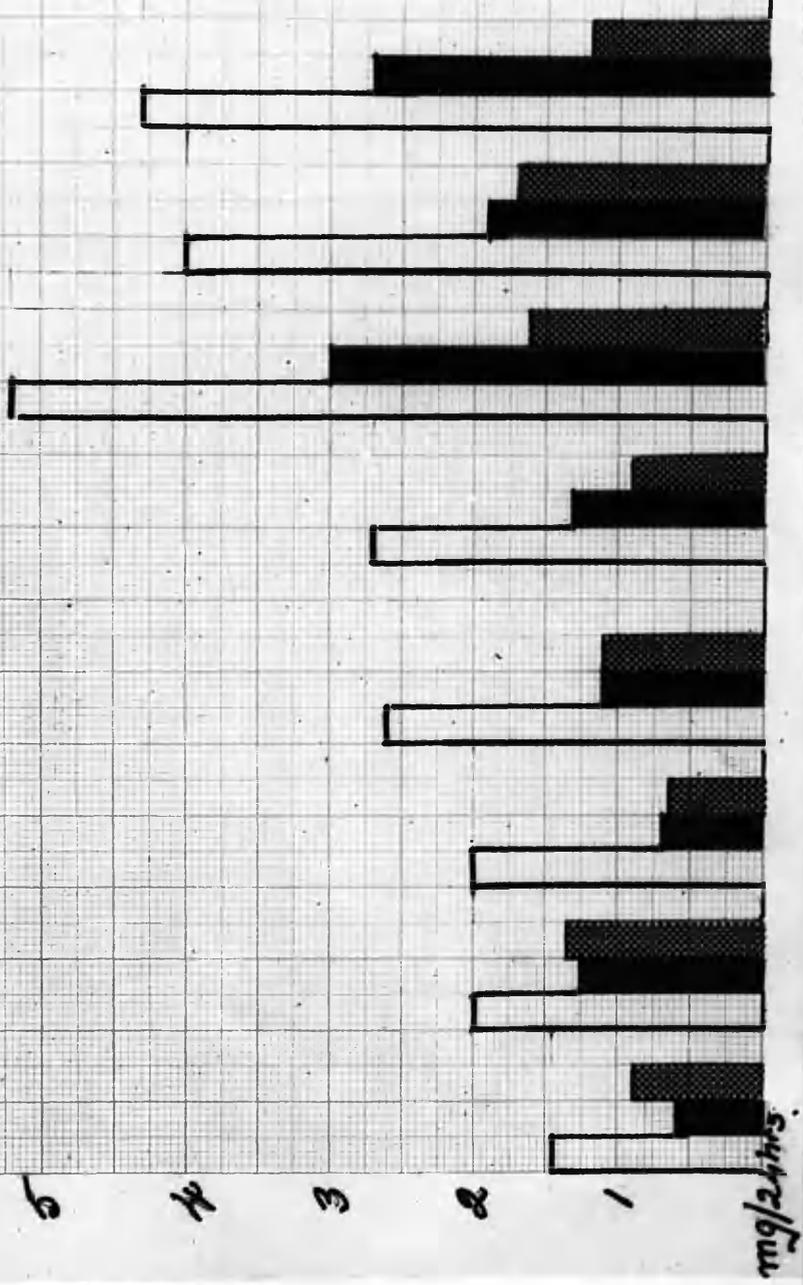17 KETOSTEROIDS

control period.

ACTH 10 UNITS B.D.

mg/24hrs.

♀ Congenital Adrenal Hyperplasia age 2½ mths

Ratio abnormal with abnormal response to ACTH

No III

Pregnanetriol Fraction II
11 oxygenated 17 OH steroid
Fraction III

17 Ketosteroids.

ACTH
5 units
B.D.

mg/24 hrs.

Effect of corticotrophin on a 4 yr old girl with congenital adrenal hyperplasia.

No IV

Fraction II
Fraction III

Ratio II/III

Mean Normal

Mean Normal

mg/24h

Control Period
Day 1 2 3

ACTH 20 units gel daily
1 2 3 4
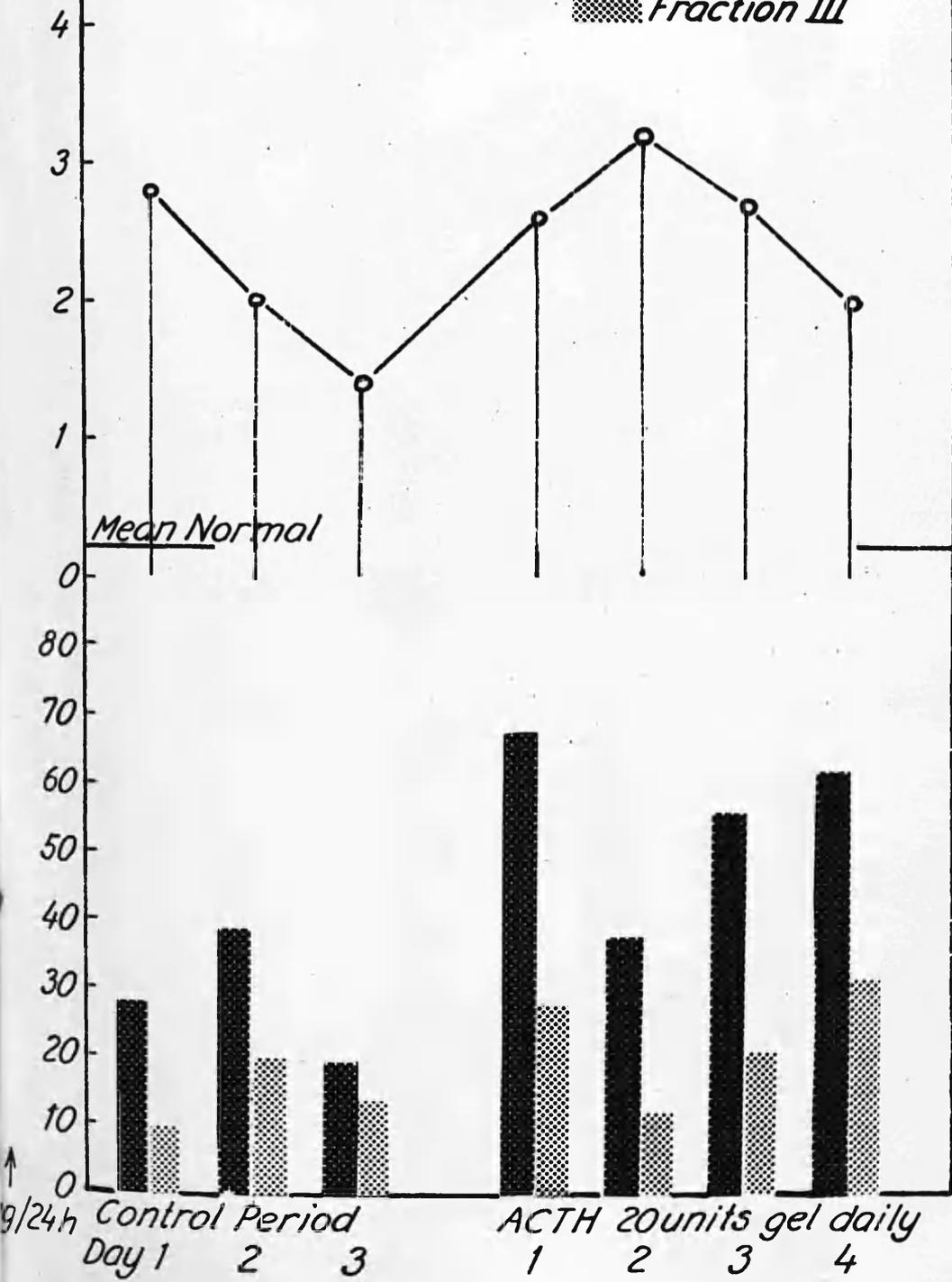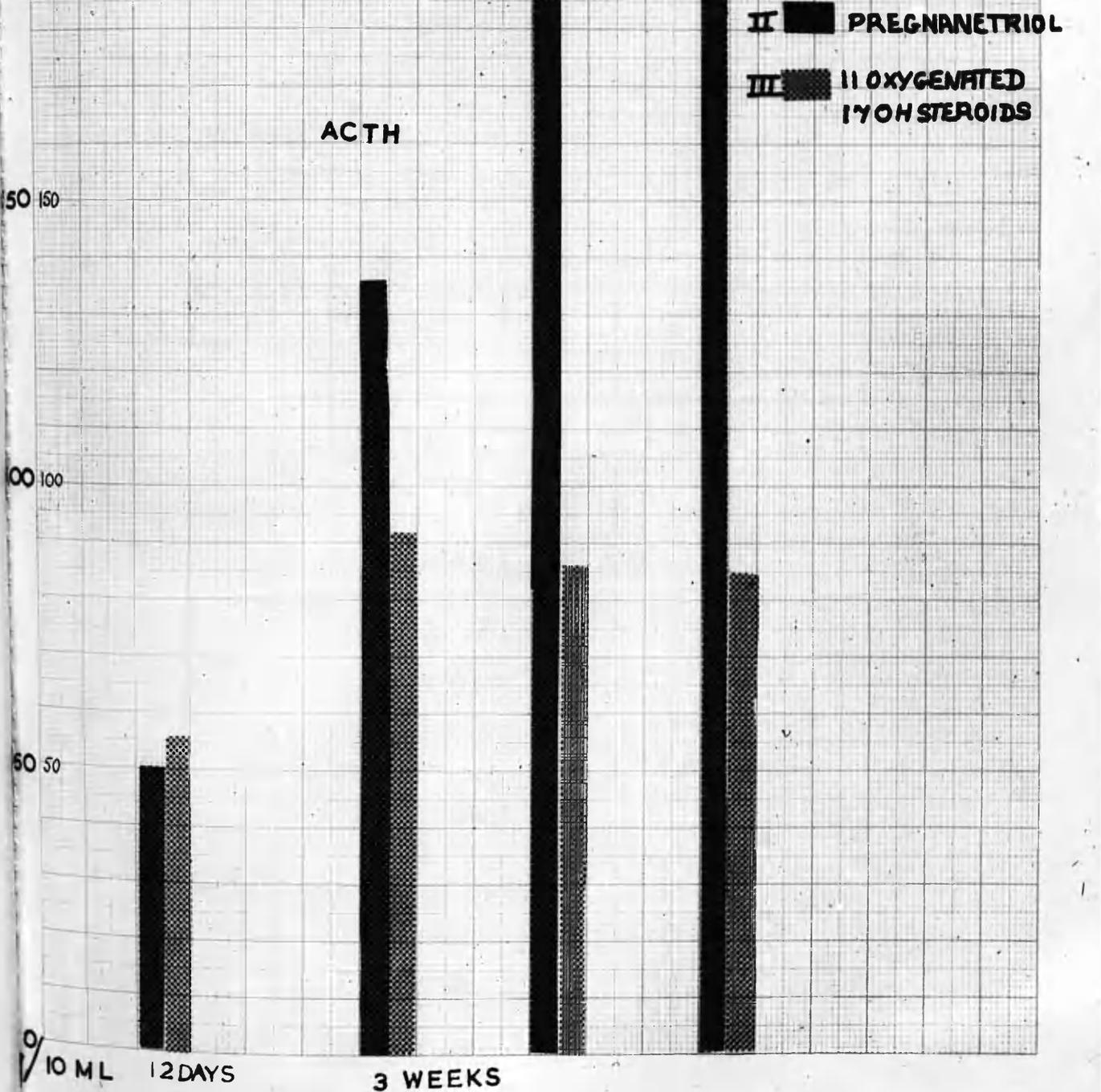
CONGENITAL ADRENAL HYPERPLASIA 3 WEEKS
RESPONSE TO A.C.T.H.

No V

RATIO II/III

II ■ PREGNANETRIOL

III ▓ 11 OXYGENATED
17OH STEROIDS

A.C.T.H.   A.C.T.H.

ACTH

10 ML   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 adreno-cortical 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 hyper-
plasia 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 cortico-
trophin suppressive power is greater. Treatment
with Prednisone or Prednisolone given in one-fifth
of the dose of cortisone or hydrocortisone in-
creases 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 suppres-
sion 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 9αFluorohydrocortisone alone.
In this child, as in those of the former group,
there was a high ratio despite adequate pregnane-
triol 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.

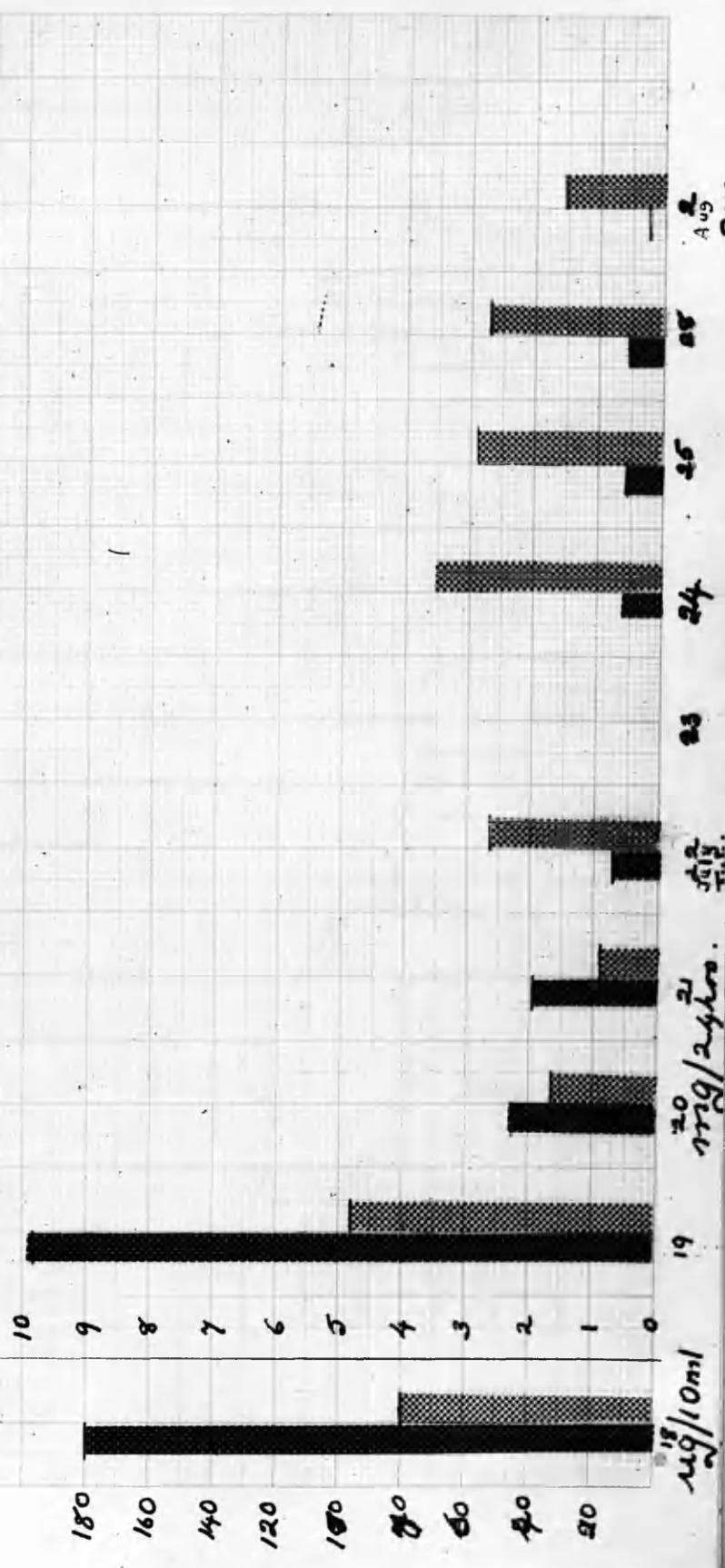C. J. ½

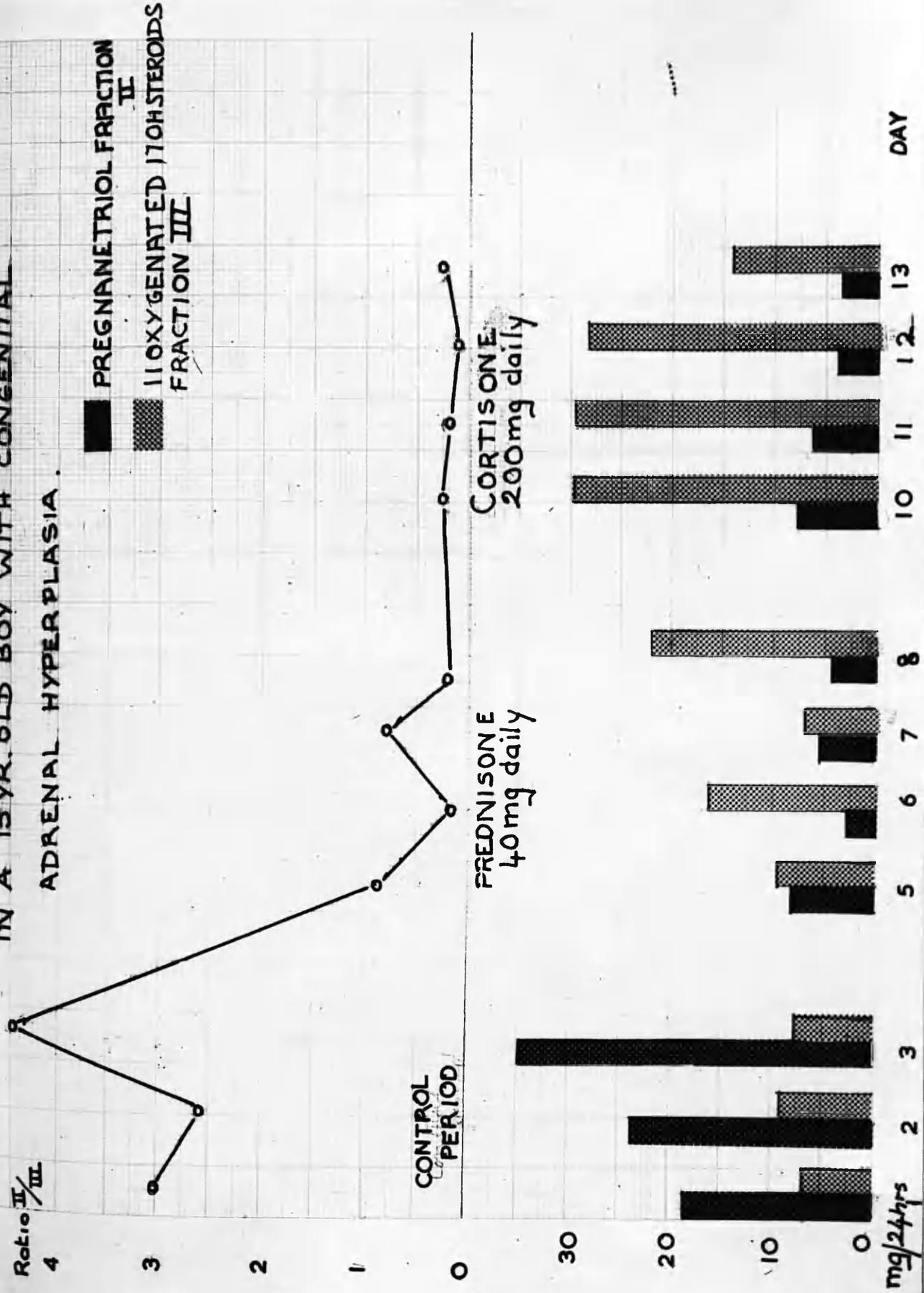CONGENITAL ADRENAL HYPERPLASIA ♂

Ratio II/III

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

Day	24 hr. vol.	17-oxo-steroids mg/24 hr.	Fraction No. II ug/10 ml.	III	II mg/24 hrs.	III	Ratio II/III
	Control period						
1	750	33	246	90	18.5	6.7	3
2	1390	61.9	171.5	64.5	23.8	9	2.6
3	1070	41.5	325	71.5	34.8	7.6	4.5
4	Prednisolone 10 mg. 6 hourly.						
5	1350	37.5	61	70	8.2	9.5	0.9
6	1280	20.7	22	128	2.8	16.4	0.17
7	1620	23.8	34	44.5	5.5	7	0.8
8	1441	15	31	155	4.5	22	0.2
9	Cortisone 50 mg. 6 hourly.						
10	1700	40	45	175	7.7	29.7	0.26
11	1280	56.8	48.5	232	6.2	29.7	0.2
12	1400	54.6	26.6	202	3.7	28.3	0.13
13	1280	23.6	27.5	110	3.5	14.1	0.25

THE EFFECT OF CORTICOIDS ON THE HYDROXYSTEROID PATTERN IN A 15 YR. OLD BOY WITH CONGENITAL ADRENAL HYPERPLASIA.

Adult Congenital Adrenal Hyperplasia

♀ 39 yrs
Attended Sterility
clinic

Short stature
Male build
Hirsute
Ratio abnormal
enhanced by ACTH
returned to normal
on Prednisolone

ACTH   ACTH

Prednisolone
20mg 8hrly

Prednisolone
10mg 8hrly

Prednisolone
5mg 8hrly

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. crystules 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. 9αfluorohydrocorti- sone 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.

DATE	TREATMENT	CLINICAL ASSESSMENT	17-oxo steroids mg/24 hr.	FRACTION NO.				RATIO II/III
				II	III	II	III	
Oct. 1958	Cortisone 10 mg. 8 hrly. Percorten crystules 50 mg. 4 wkly. Salt 3 G.	Well	1.8	11.5	81	0.8	5.2	0.14
Nov. 1958	Cortisone 30 mg. Percorten 25 mg. 4 wkly. Salt 3 G.	Well	3	20	80	1.6	7.5	0.25
Dec. 1958	Cortisone 30 mg. Salt 3 G.	Cough & cold Occasional vomiting	6.7	54.6	90.5	3.5	5.9	1.6
Jan. 1959	do. do.	Cough, cold, listless, vomiting.	8.5	178.7	230	17	22.4	0.8
Feb. 1959	do. do. 9αfluorohydrocortisone 0.25 mg.	Much better, gained 3 lbs.	2.2	14	218	0.64	10	0.06
March 1959	Cortisone 22.5 mg. Salt 3 G. 9αfluorohydrocortisone 0.25 mg.	Well	1.7	6.4	80	0.5	6.2	0.04
April 11th 1959	Cortisone 30 mg.	Measles, recovering	2.2	17	67.5	0.88	3.5	0.25
12th	9αfluorohydrocortisone	Discharged	4	13.6	92	0.7	4.6	0.15

♂ "Salt Looser"
5 yrs
Demonstrating
change of pattern
on discontinuing
D.O.C.A. and minor
illness

Improvement on
adding
9α Fluorohydrocortisone

← Cortisone 30mg.
Urine brought up because
child unwell. Bronchitis
Vomiting.

Cortisone 30mg
9α Fluoro hydro C.
·25mg.

Cortisone 30mg   Cortisone
                   30mg.

DOCA.

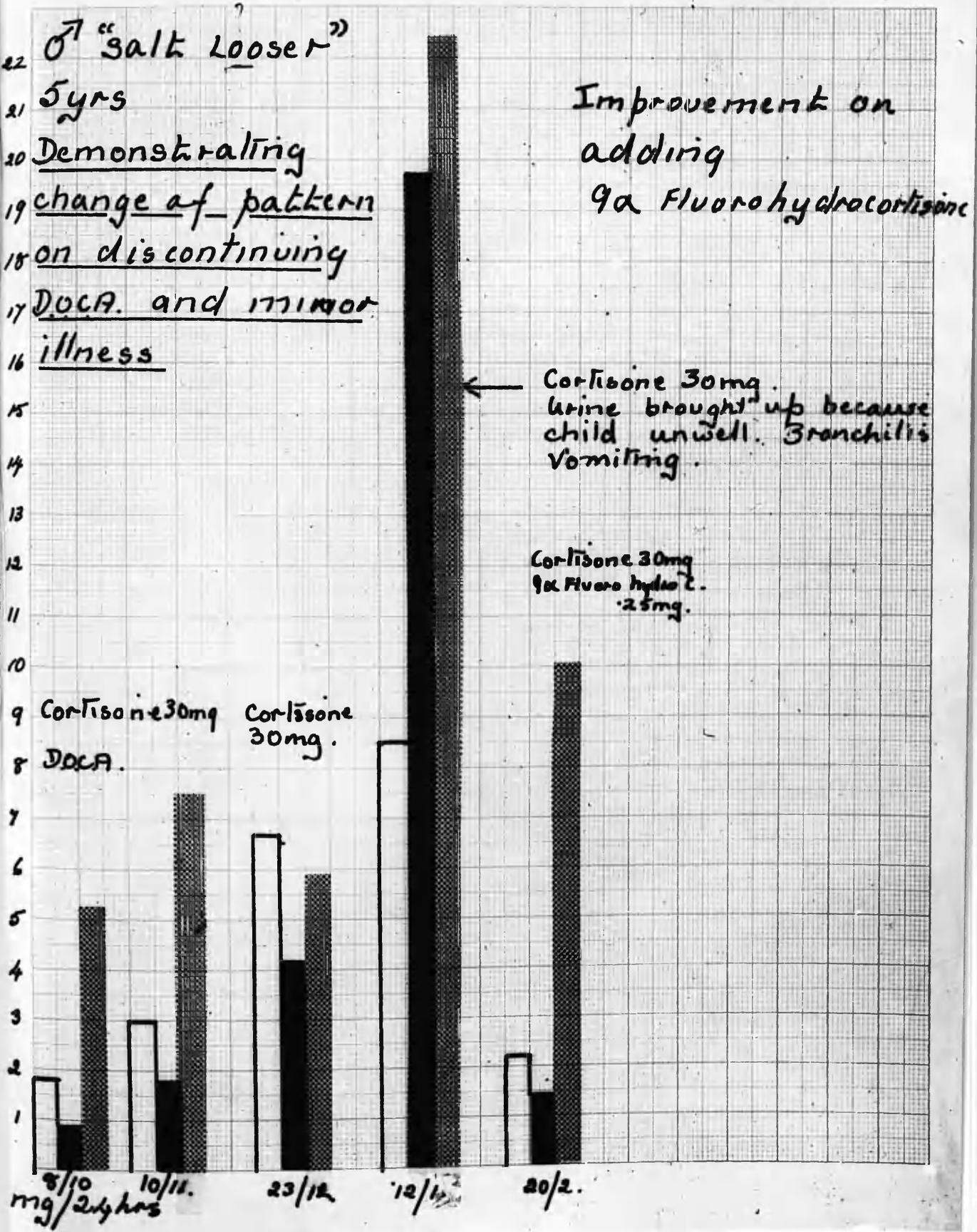8/10   10/11.   23/12   12/1   20/2.
mg/24hrs

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 9αfluorohydrocortisone was
added. Well until he developed measles.
Patterns throughout correlate with clinical
assessment of the child.

DATE	TREATMENT	CLINICAL ASSESSMENT	17-oxo steroids mg/24 hr.	FRACTION NO.		II	III	RATIO II/III
				II	III			
Oct. 1958 Age - 3 yrs. 7 mnths.	Cortisone 5 mg. 8 hrly. Salt 3 G.	Admitted because of recurrent evidence of adrenal insufficiency with infection.	4.4	22	51	1.9	4.5	0.4
	Cortisone 7.5 mg. 8hrly. Salt 3 G.	Developed respiratory infection & vomiting in hospital.	6.3	267	47	25	4.6	5.7
Feb. 1959	do.	Well single sample		18.7	55.7			0.34
March 1959	Cortisone 10 mg. 8hrly. 9α fluorohydrocortisone. 0.2 mg. daily	Admitted influenza 3rd day		76.25	67	6.25	5.5	1.15
		7th day		16.3	43.3	1	2.7	0.4
		8th day		6.9	46.6	0.6	4	0.15
	Cortisone 7.5 mg. 8hrly. 9α fluorohydrocortisone 0.2 mg. daily	Well						
May 1959	do.	Very well	1.7	8	57.3	0.58	4.1	0.14
	Cortisone 25 mg. i.m. Cortisone 10 mg. 6 hrly. 9α fluorohydrocortisone 0.2 mg. daily	Admitted with measles with clinical evidence of adrenal failure 3rd day		41	31	1.4	1	1.4
		4th day		52	29	1.3	0.7	1.9
		5th day		55	196	1.4	4.9	0.3
		Well 6th day		9	170	0.15	0.42	0.35
	Cortisone 10 mg. 8 hrly. 9α fluorohydrocortisone 0.2 mg. daily.	7th day		11.5	50	0.8	3.65	0.2
		8th day		5.5	37.2	0.8	5	0.15
		9th day Discharged		8.4	45.5	0.4	3.8	0.1

DATE	TREATMENT	CLINICAL ASSESSMENT	17-oxo steroids mg/24.hr.	FRACTION NO.				RATIO II/III
				II	III	II	III	
June 1959	Cortisone 10 mg. 8 hrly. 9αfluoro-hydrocortisone 0.2 mg. daily.	Very well	2	7	62.6	0.5	4.3	0.1
July 1959	Cortisone 7.5 mg. 8 hrly. 9αfluorohydro-cortisone 0.2 mg. daily.	Very well	-	17	81.4	0.8	3.4	0.2
Aug. 1959	" "	Well	2.7	11	32.6	0.74	2.2	0.34
Sept. 1959	"	Well	2.2	14.4	15.4	0.8	9.4	0.09
Oct. 1959	Cortisone 7.5 mg. t.d.s. 9αfluoro-hydrocortisone 0.1 mg. daily.		1.6	23	125.7	1.6	9.6	0.17
Nov. 1959	Cortisone 5 mg. t.d.s. 9αfluoro-hydrocortisone 0.1 mg.	Well	4.9	12.3	25	0.98	2	0.5

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 out patient 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

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

Fractions II and III explained in Table I.

♂ "Salt looser"   Age 4 yrs.
Penile urethra reared as a male. Single Samples

II Pregnanetriol
III 11 oxygenated 17OH cs.

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

II 8am – 2pm
Vol. 140 ml
Ratio IV/III 0.3

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

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

µg/10ml   I   II   III   IV

40
30
20
10
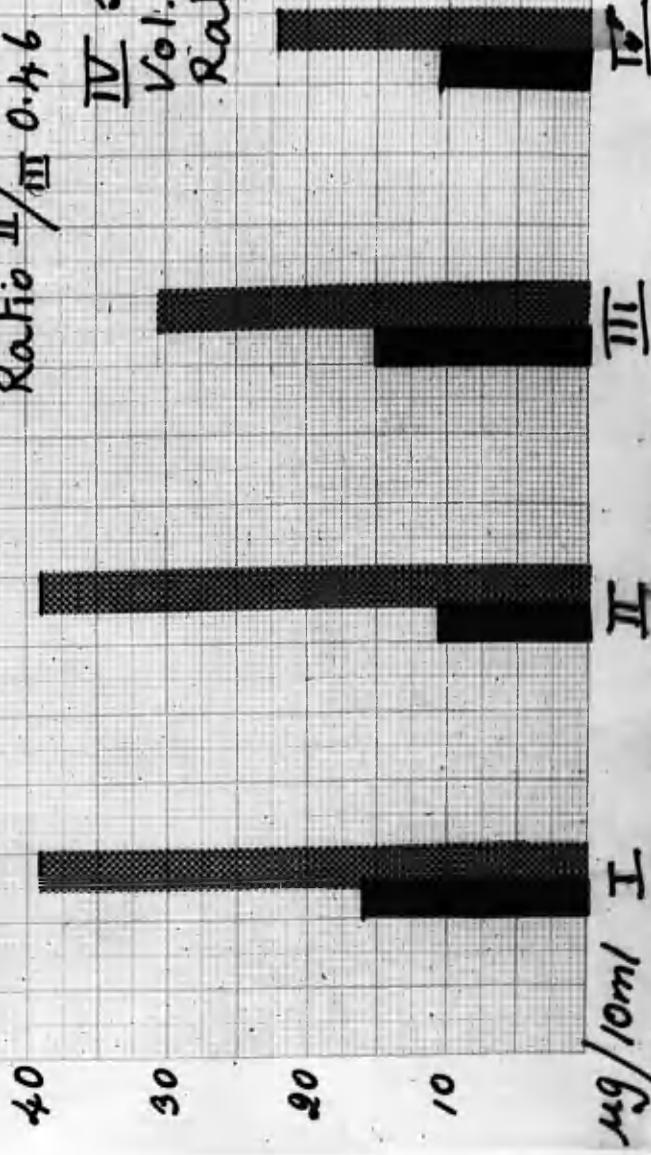
TABLE XI   ' Use of isolated specimens.

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

| | | Fraction No. | | | RATIO |
| | II | III | II | III | II/III |
	ug/10 ml.		mg/24 hr.		
A. Total	32	28.6	1.3	1.6	0.8
Single	28	20	-	-	1.4
B. Total	16.2	17.7	0.6	0.65	1
Single	11.3	14.4	-	-	0.8
C. Total	16	57.5	0.5	1.7	0.3
Single	8	20	-	-	0.4
D. Total	13.6	92	0.68	4.6	0.15
Single	13.7	92	-	-	0.15
E. Total	28	192	5	34.6	0.15
Single	36.5	245	-	-	0.1

Fractions II and III explained in Table II.

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, chromato-
graphy of the 17 ketogenic steroids gave a high
pregnanetriol excretion with an abnormally high ratio
of Fraction II/Fraction III.   There are seven sib-
lings.   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.

Patient	Sex	Fraction No. II ug./ 10 ml.	III	Ratio II/III	Clinical details
1	Female	115	36	3	Congenital adrenal hyperplasia.
2	Female	98	75	1.3	Clinical findings and history of congenital adrenal hyperplasia.
3	Male	162	69.3	2.3	History suggestive of congenital adrenal hyperplasia.
4	Male	5	80	0.06	Normal.
5	Male	14	41	0.34	Normal.
6	Male	19	40	0.47	Normal.
7	Female	25	80	0.3	Normal.
8	Female	37.5	117.5	0.3	Normal.

CHROMATOGRAPHY OF URINARY 17OH STEROIDS IN SINGLE SAMPLES OF URINE FROM SIBLINGS OF A CONGENITAL ADRENAL HYPERPLASIA.

PREGNANETRIOL FRACTION II

11 OXYGENATED 17OH STEROIDS FRACTION III
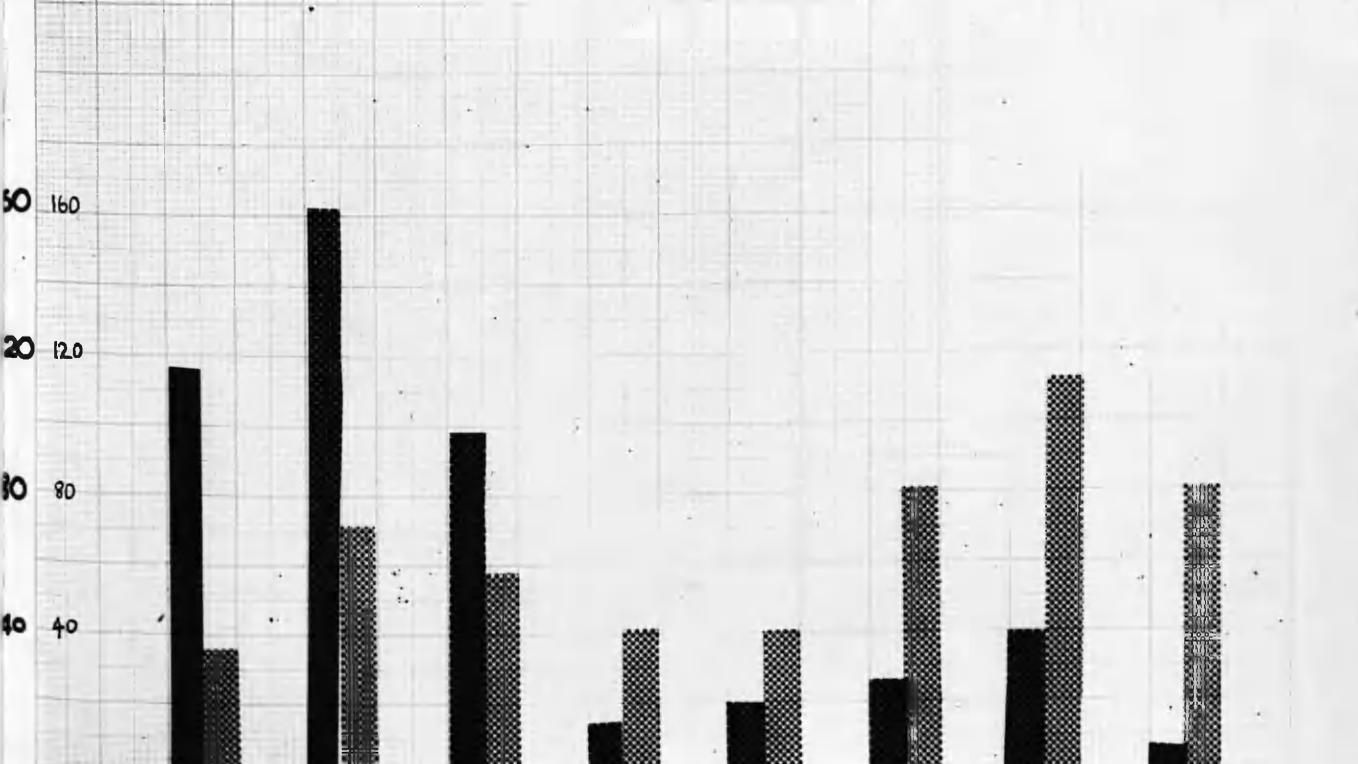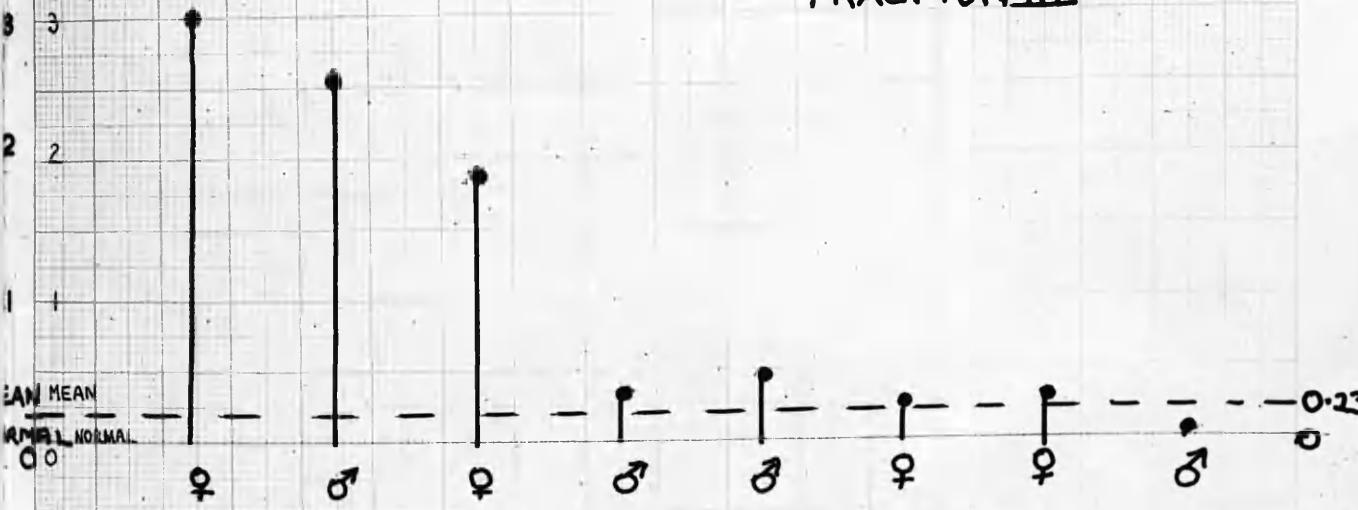
µg/10ml

C A S E   H I S T O R I E S

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").   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 keto-genic 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.C1. 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 9∝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 9∝ fluoro-
hydrocortisone 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 9αFluorohydrocortisone 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 56$\frac{1}{2}$ cm. (22$\frac{1}{4}$") 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 keto- genic 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.
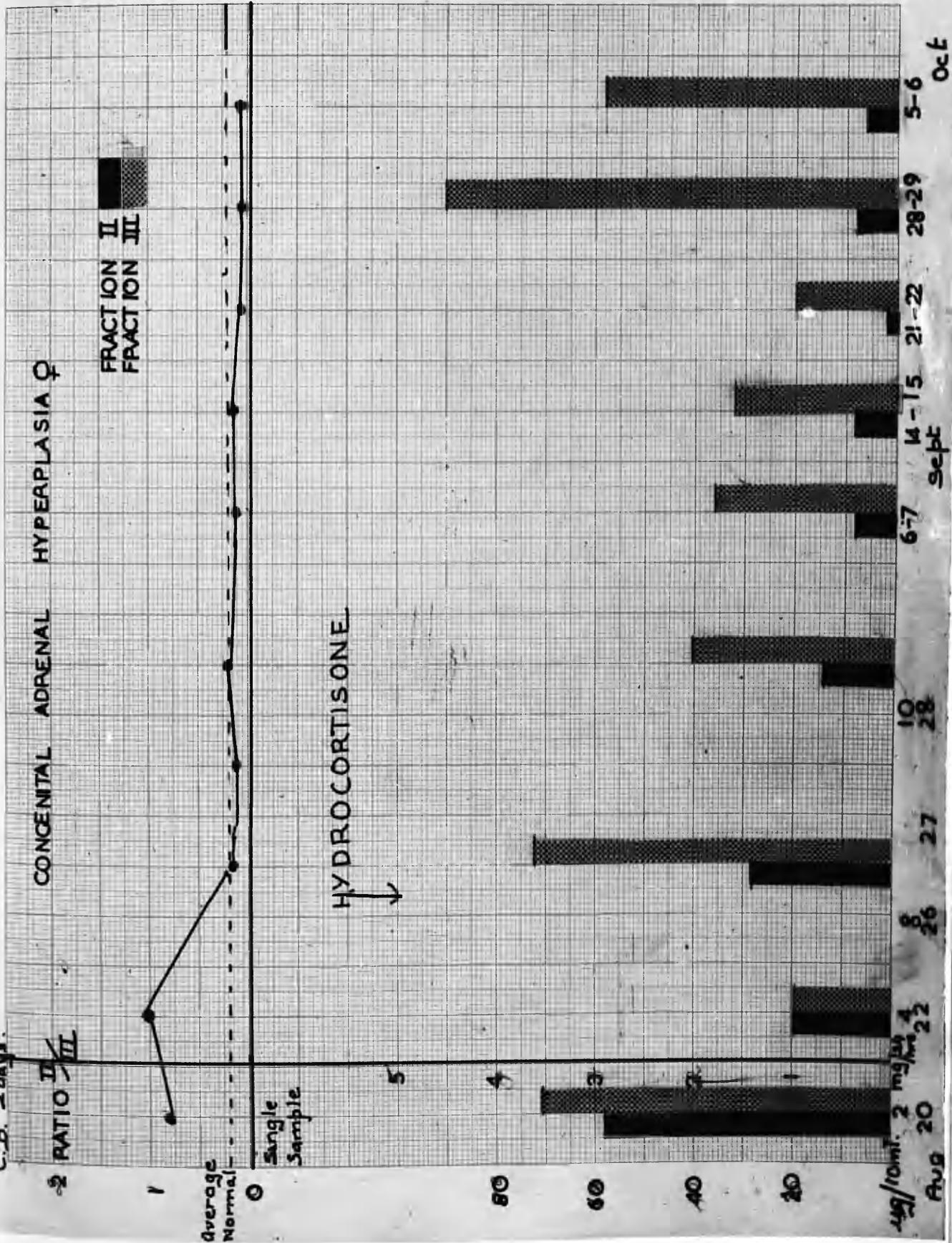
P.P. BORN 8.7.59.

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. (6¾ 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.
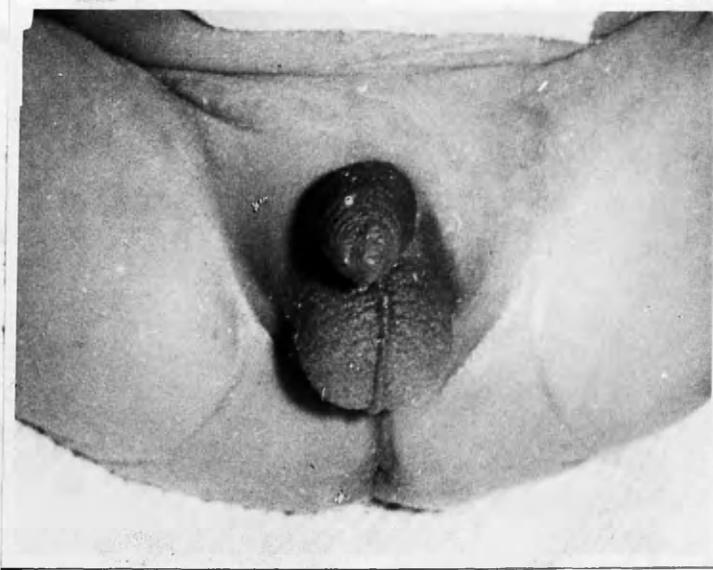
A single sample of urine was obtained and
showed the presence of chlorides.   Chromatography
of the 17 ketogenic steroids into Pregnanetriol
fraction II and 11 oxygenated fraction III demon-
strated the high ratio of Fraction II/III found
in patients with congenital adrenal hyperplasia.

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. NaC1 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 ketosteroids 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.
C1 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 ab-
normal.

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∝fluorohydrocortisone, 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 main-
tained on cortisone, 5 mg. four times daily, 3 g.
added salt and 0.5 mg. of 9$\propto$ 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 pigmenta-tion 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
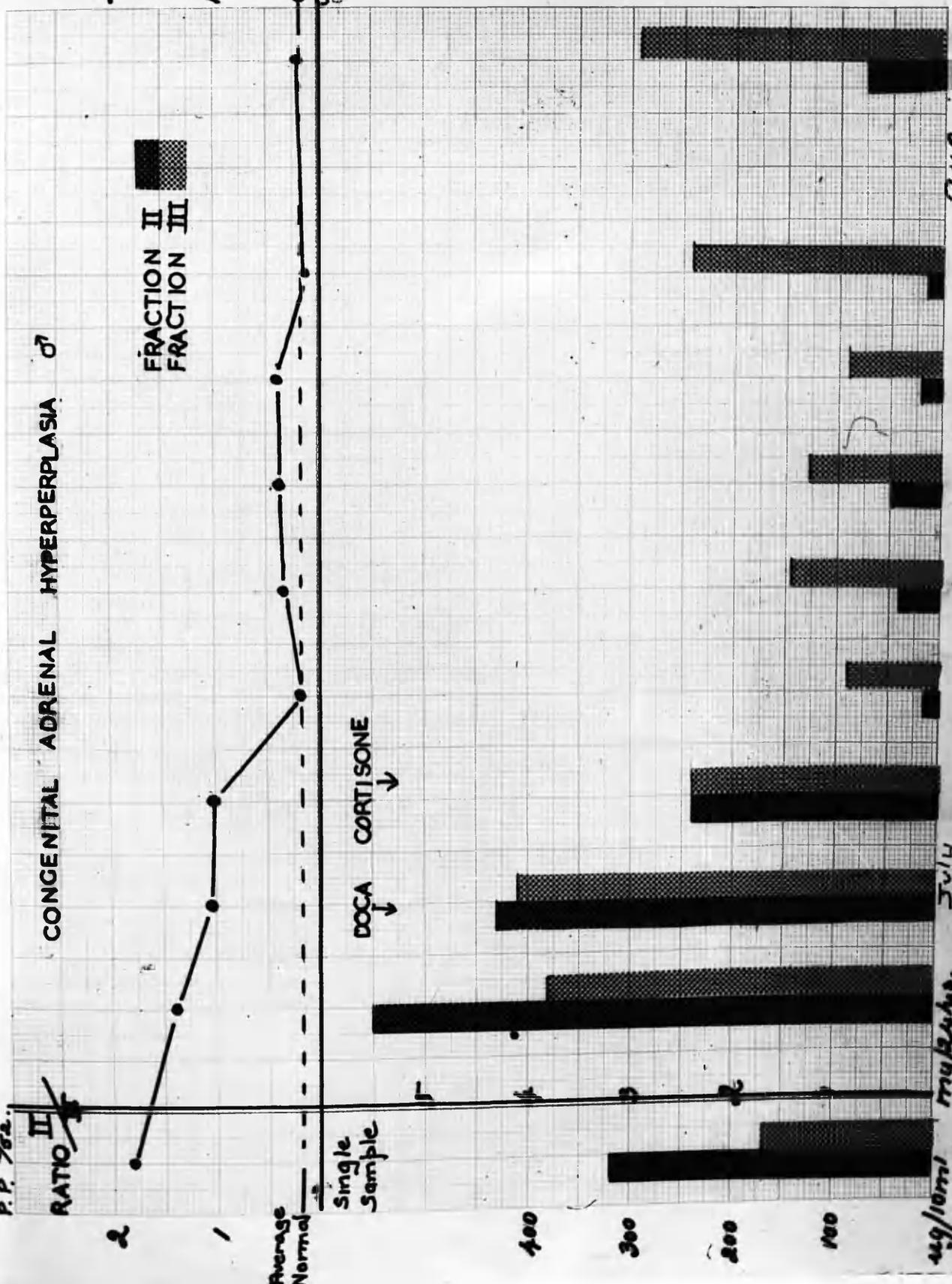very satisfactory.

CONGENITAL ADRENAL HYPERPLASIA ♂

D.D. BORN SEPTEMBER, 1959.

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 vomit-
ing, 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 pigmenta-
tion 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.   C1. 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. C1. 82 m.eq/1.
CO_2 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. C1. 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 9∝ 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 keto-
genic 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
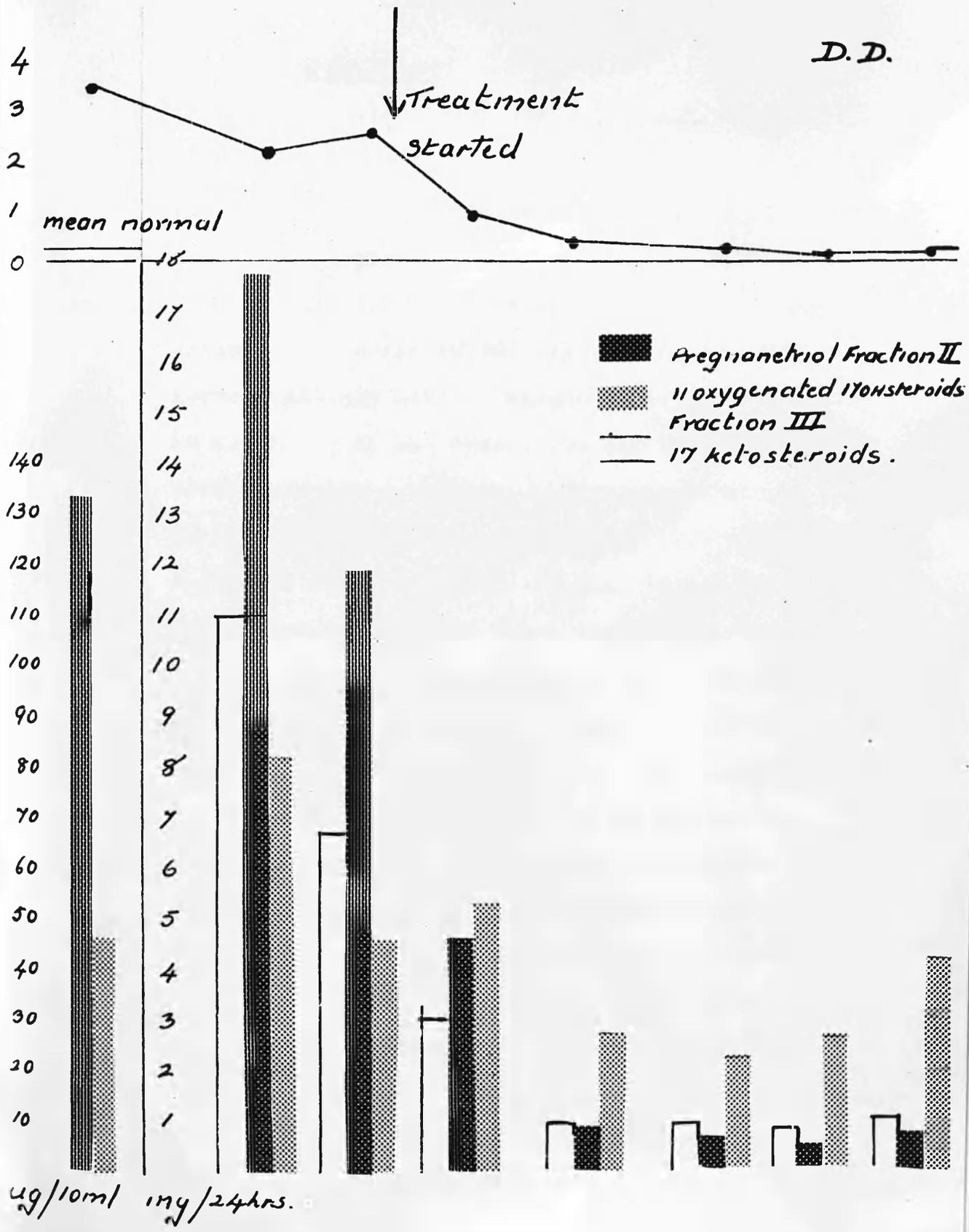of 410 ml. with a total 17 ketosteroid of 0.8 mg/24 hr.

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,
9∝ 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 ketonic 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.

D. D.

4
3
2
1
mean normal
0

Treatment started

Pregnanetriol Fraction II
11 oxygenated 17oxsteroids Fraction III
17 ketosteroids.

18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1

140
130
120
110
100
90
80
70
60
50
40
30
20
10

ug/10ml   mg/24hrs.

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
arteroisus.

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 con-
tinued.   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 ketosteroids 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∝ fluorohydrocortisone was given once daily, and $2\frac{1}{2}$ 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. ($22\frac{1}{2}$").   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 con-
genital 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 in-
fection.
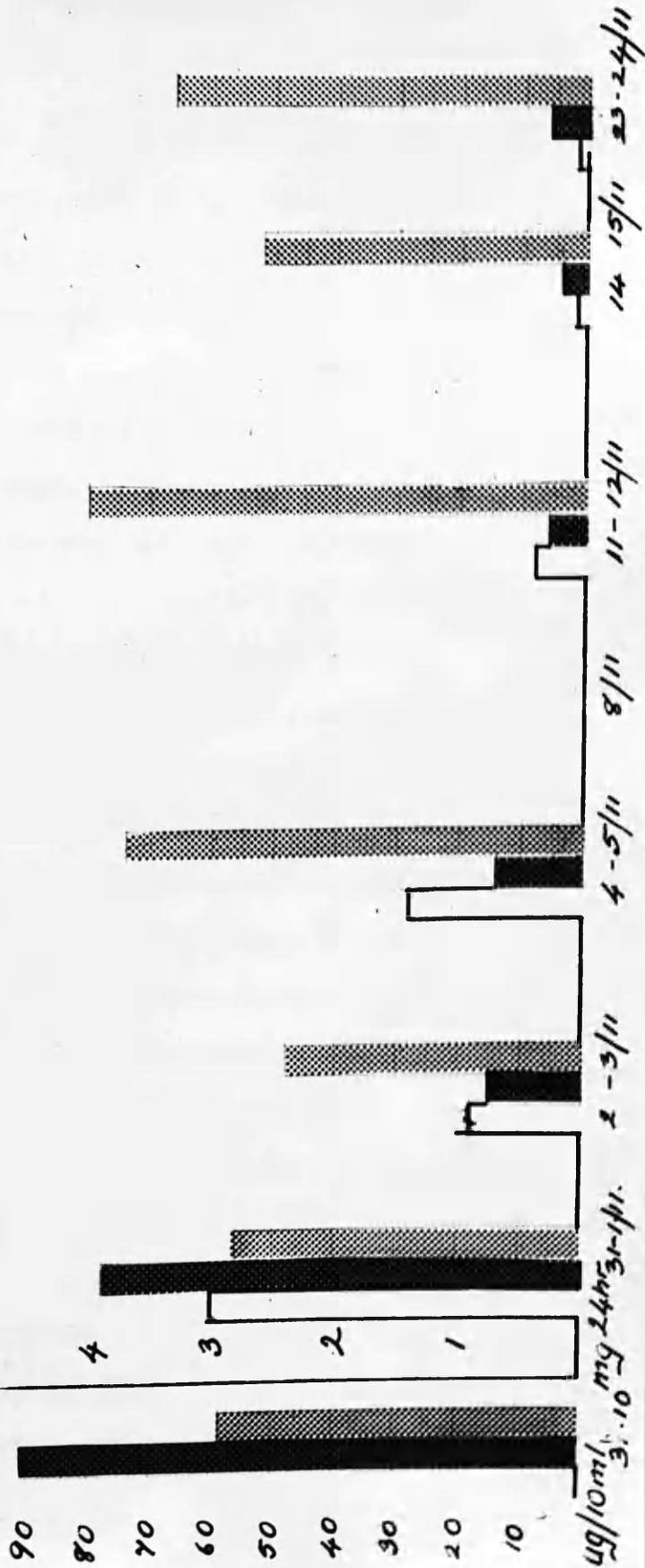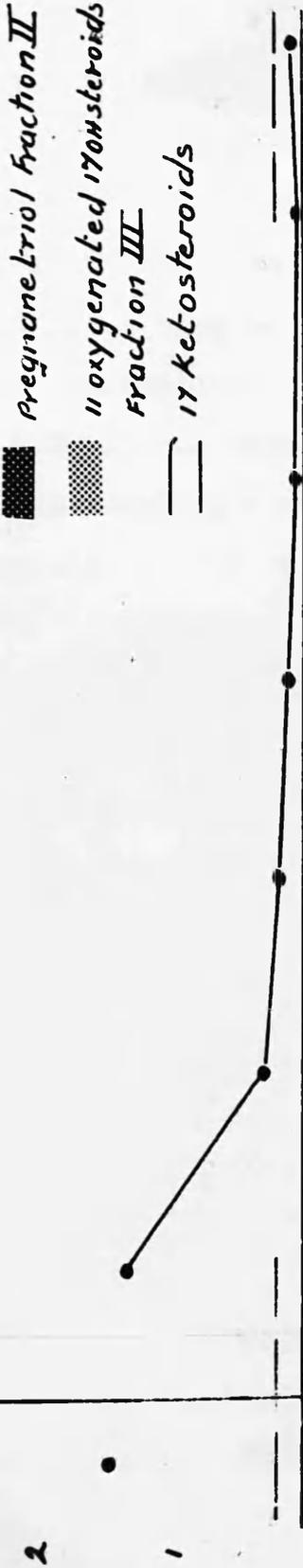
A.B. BORN 16.3.55

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 $3\frac{1}{2}$ 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 estima-
tion 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.* Preg-
nanetriol 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 infec-
tion 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 9 αfluorohydrocortisone 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 4¼ 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. (39¼") 25th per-
centile, 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. in-
jections, however, soon demonstrated his
continued need for a salt retaining hormone.
9∝ fluorohydrocortisone has proved an excellent
substitute for the injections.   The steroid
chromatography patterns have correlated well
with the clinical evaluation.   Serum electro-
lytes 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.
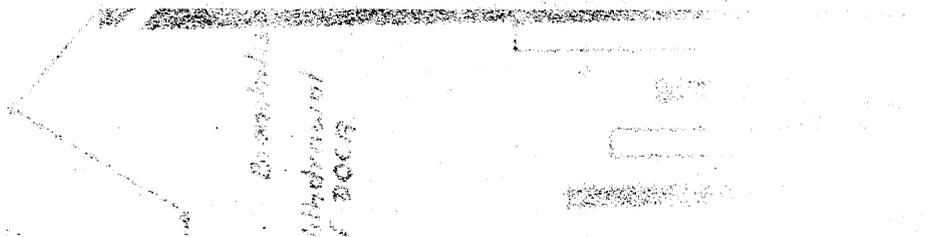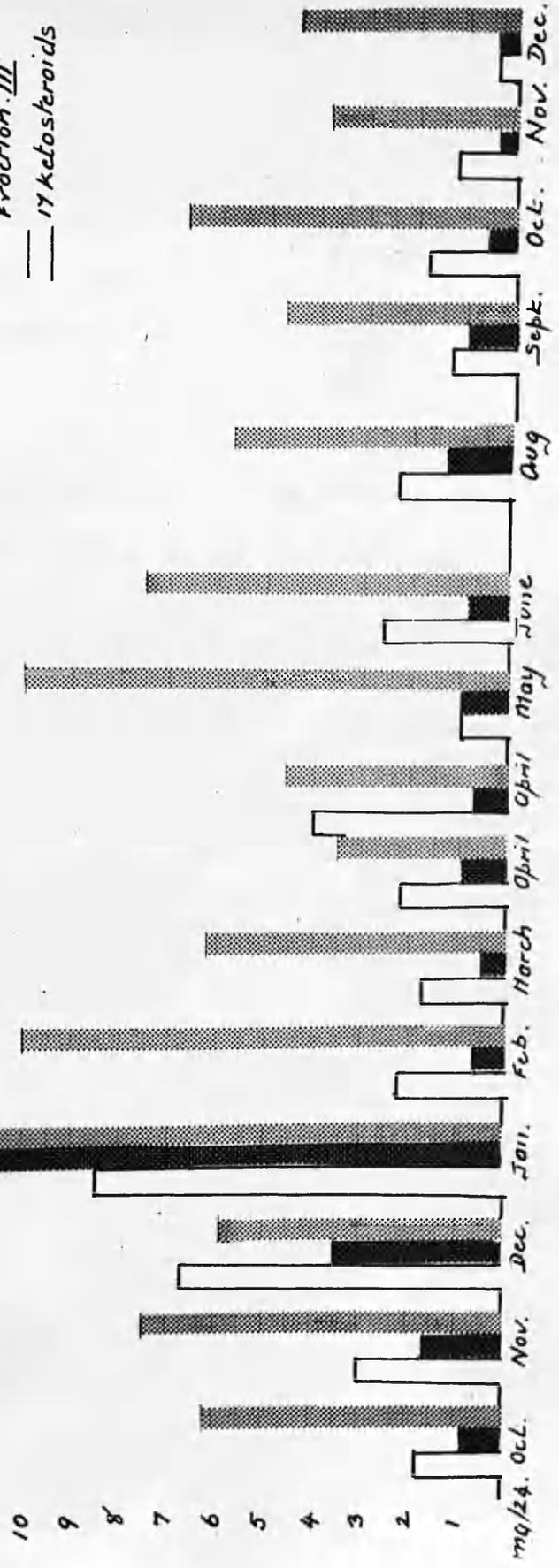
Y.B. BORN 17.1.58.

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.
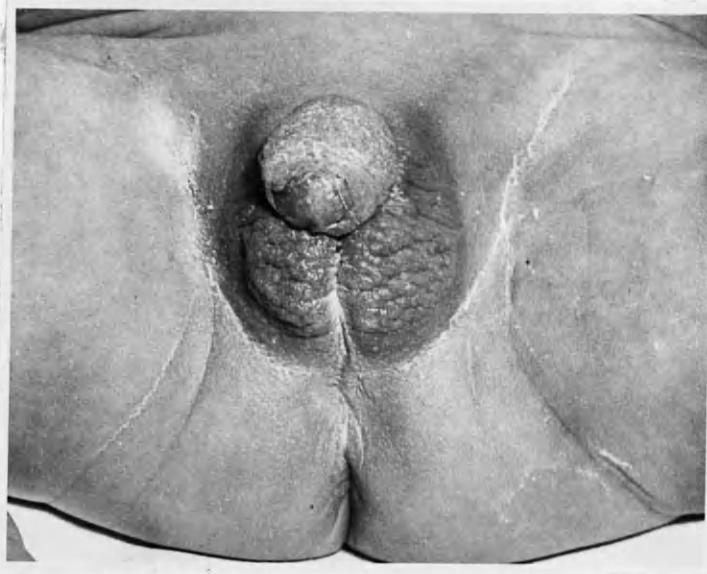
Investigations showed the electrolyte changes of adrenal insufficiency with a raised potassium, and urea, and a reduced sodium and carbon dioxide combining power. The 17 keto-steroids were 3 mg. in 24 hrs. The salt content of the urine was high and the total volume large for a dehydrated infant. The buccal smear showed a female chromatin pattern.

Treatment with cortisone, initially intra-muscularly, 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 keto-
genic 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./1., urea 28 mg%.   The results
of the steroid estimations are shown below.

17 keto-steroid mg/24 hr.	Chromato-graphy II mg/24 hr.	17-OH steroids III mg/24 hr.	Ratio II/III
16-17/10/58	1.7	0.3	1.8	0.17
17-18/10/58	1.5	0.3	1.6	0.18
19-20/10/58	2.3	0.3	1.9	0.16

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./1., potassium 4.8 m.eq./1., 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 crys-
tules 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 in-
fection.

Progress on this regime was very satis-
factory and it was decided to change her to the
oral salt retaining hormone, 9∝ Fluorohydrocorti-
sone, 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∝ 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 insuf-
ficiency.   Her change of attitude to hospital
visits, which now involved no intramuscular in-
jections, 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/11 oxygenated 17-OH
steriod Fraction III, between 0.32 and 0.08.

This child represents a severe salt loser.
She is now satisfactorily controlled on cortisone
and 9$\propto$ 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
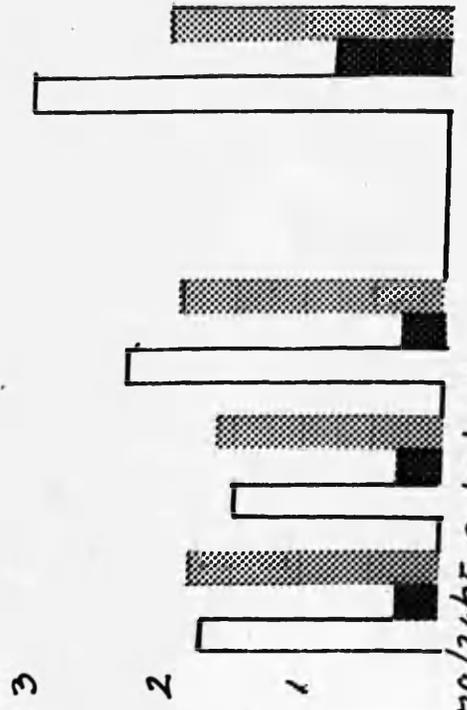surgery should not be delayed too long.

Y.B. Cortisone 5mg. 8hrly.
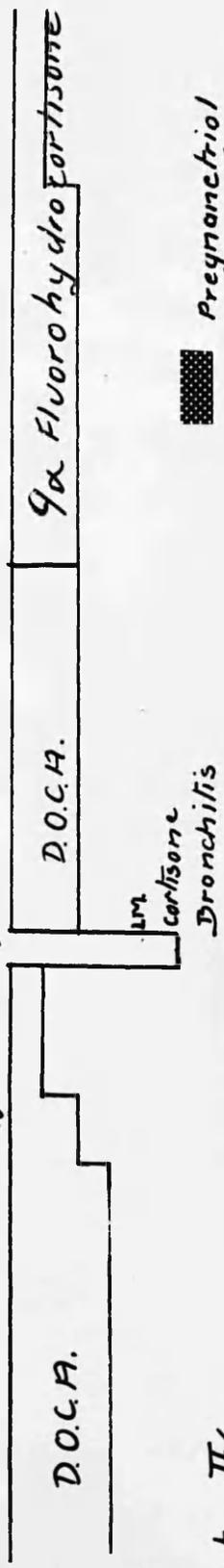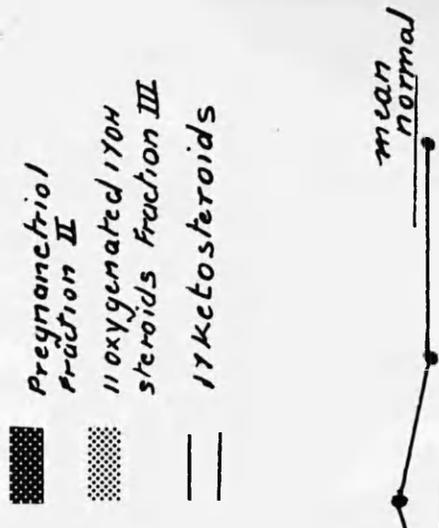
J.H. BORN 13.3.55.

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 demon-
strated 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 11½ 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. (16¼ 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 res-
pirations 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. (28¾ 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 ad-
mission, 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. (34½")

3rd percentile.   The bone age was on the lower

limits of normal.   The buccal smear showed a

chromatin positive pattern.   His steroid ex-

cretion 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 oxy-

genated 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 9∝ fluorohydrocortisone and 3 g. salt.

Since then he has been in excellent health with normal ratios of the two fractions. His 9∝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
$4\frac{1}{2}$ years: weight 21 kg. (47 lbs.) 97th percen-
tile; height 105 cm. ($39\frac{1}{4}$") 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 insuf-
ficiency during illness. As long as this
tendency persists, he will require sufficiently
large doses of cortisone to prevent androgeni-
sation. The aim is to remove the female repro-
ductive 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
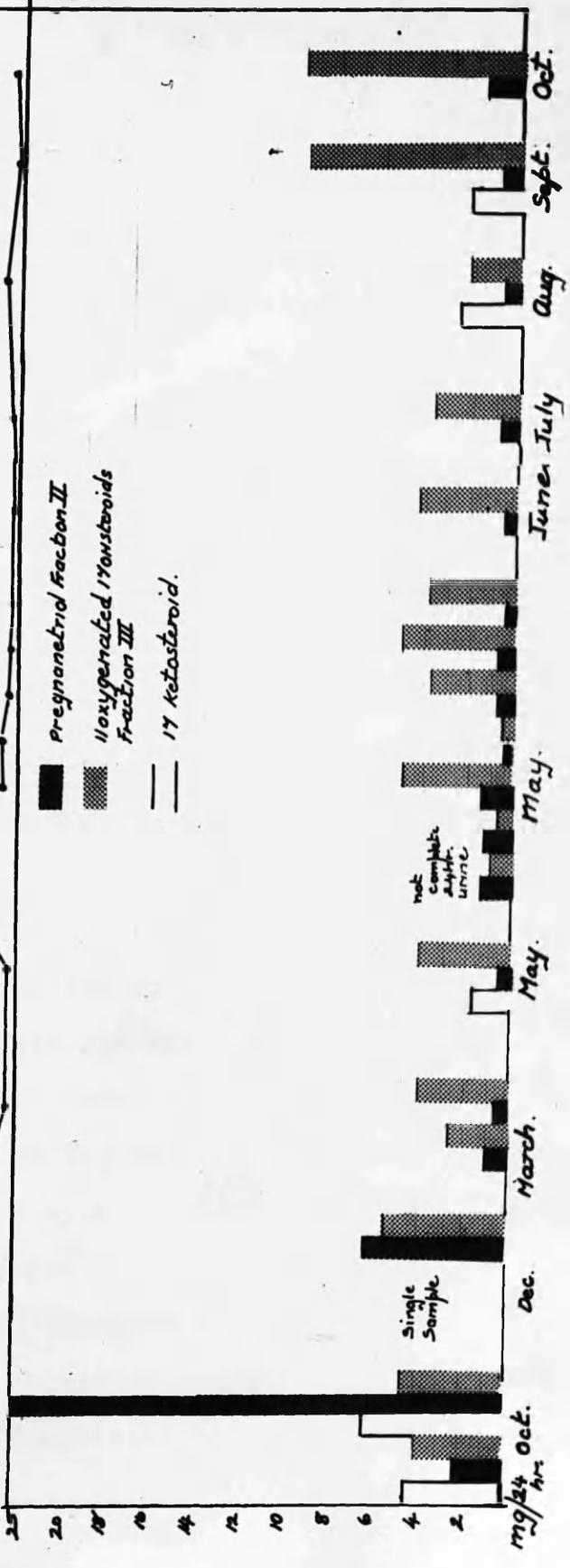will be reduced to allow androgenisation to occur.

Infabant Measles   J.H.

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 de-
hydrated.   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 con-
genital 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 inade-
quate.   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 discon-
tinued and was given as the long acting trimethyl
crystules.

At the age of six months, he was discharged
weighing 5.8 kg. (13 lbs.) below the 3rd percen-
tile. 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 in-
sufficiency. 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 bio-
chemistry 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 nor-
mal.   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:-

Day	17 keto-steroids	Pregnane-triol II	11 oxygen-ated 17-OH steroids III	Ratio II/III
29-30/9/58	2.4	1 mg/24 hr.	2.8mg/24hr.	0.36
30/9 - 1/10/58 }	2.7	0.4 mg/24hr.	3.2 mg/24hr.	0.11
1-2/10/58	6	0.4 mg/24hr.	3.1 mg/24hr.	0.14

His weight was 11.8 kg. (26 lbs.) 25th per-
centile; 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.

M.W. BORN 23.12.48.
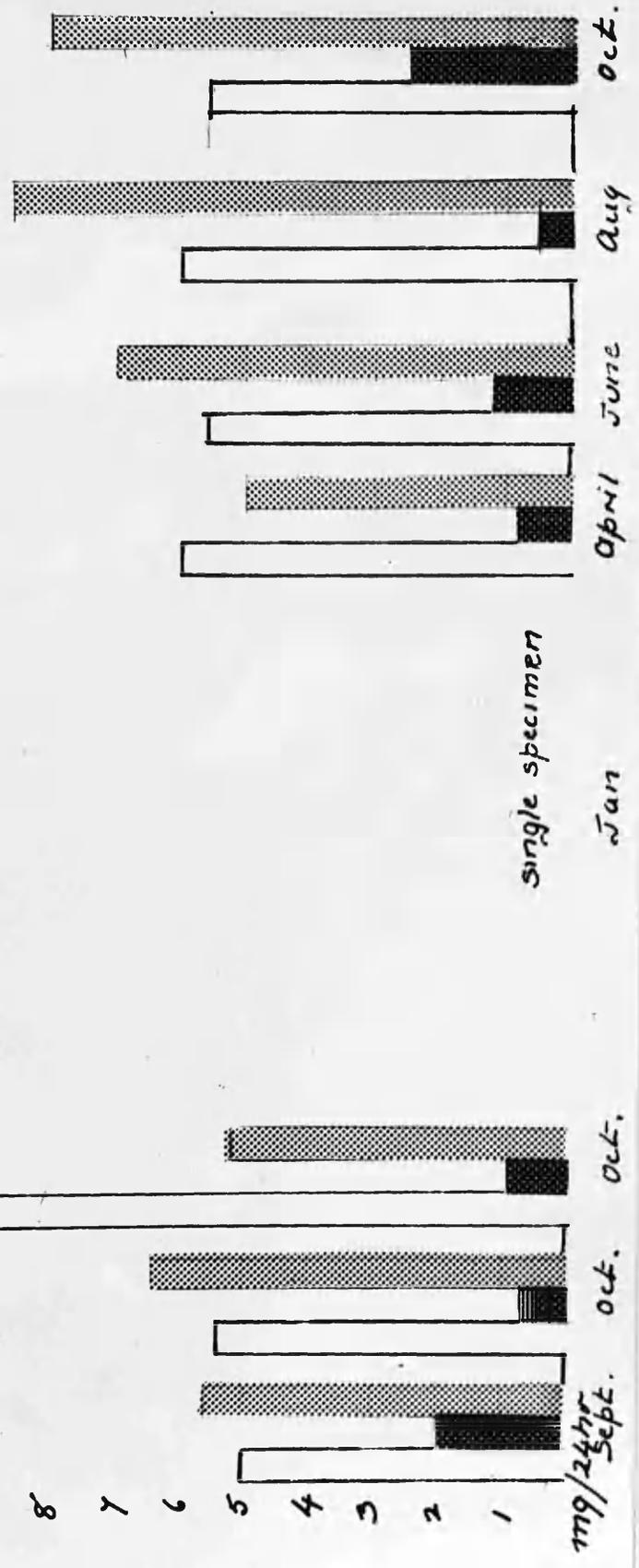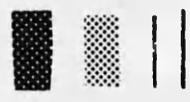
This girl is the eldest child of healthy
parents.   The clitoris was enlarged at birth,
but she was considered to be a female.   At the
age of three years, she was admitted for inves-
tigation.   Her height was 99 cm. (43") above
the 100th percentile, and her weight 18 kg.
(40 lbs.), 100th percentile.   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.
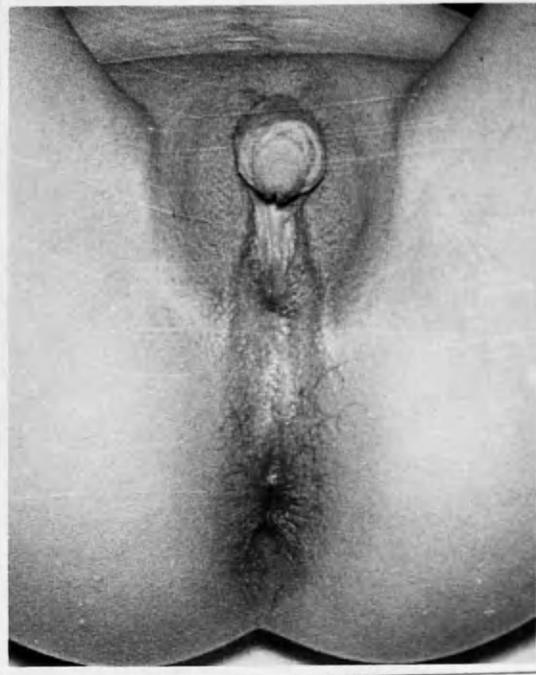
Pubic hair was present.   The bone age was 8-9
years and the 17 ketosteroid excretion 26 mg.
in twenty-four hours.   Laparotomy was carried
out and revealed a normal female reproductive
tract.   The perineum was split back to expose
the vaginal orifice.

At the age of four years, she was started
on cortisone.   During this year, between opera-
tion 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 corti-
sone 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 im-
proved 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 develop-
ment but menstruation had not yet started. The
bone age had advanced to 16-18 years.

Steroid excretion was high:

Date	17 keto-steroid mg/24 hr.	Chromatography of the 17-OH steroid mg/24 hr.		Ratio II/III
		II	III	
8 - 9/10/58	7.3	2.1	14.4	0.15
9-10/10/58	9.2	2.8	11	0.25

It was apparent that androgen secretion was
not being adequately suppressed and if fusion of
the epiphises 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 ex-
cretion but this was unfortunately accompanied by
a rapid increase in weight gain. She was given
a 1000 calorie diet and prednisolone was substi-
tuted 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:-

Date	17 keto-steroid mg/24 hr.	Chromatography 17 ketogenic steroids mg/24 hrs.		Ratio II/III
		II	III	
11-12/2/59	6	3.6	8.7	0.4
2 - 3/6/59	5	1.0	10.5	0.1

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 keto-steroid mg/24 hr. | Chromatography 17 ketogenic steroids | | Ratio II/III |
		Fraction II	Fraction III	
October	19	13.5	25	0.5
September		3.4	22.8	0.14
December	18.7	4.7	11.5	0.4

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 keto-steroids 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. (57½") 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/24hr.

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 predniso-lone was reduced to 5 mg. three times daily.

<u>Discussion</u>: 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   D.T.
Hyperplasia effect of increased
                    corisone on pattern
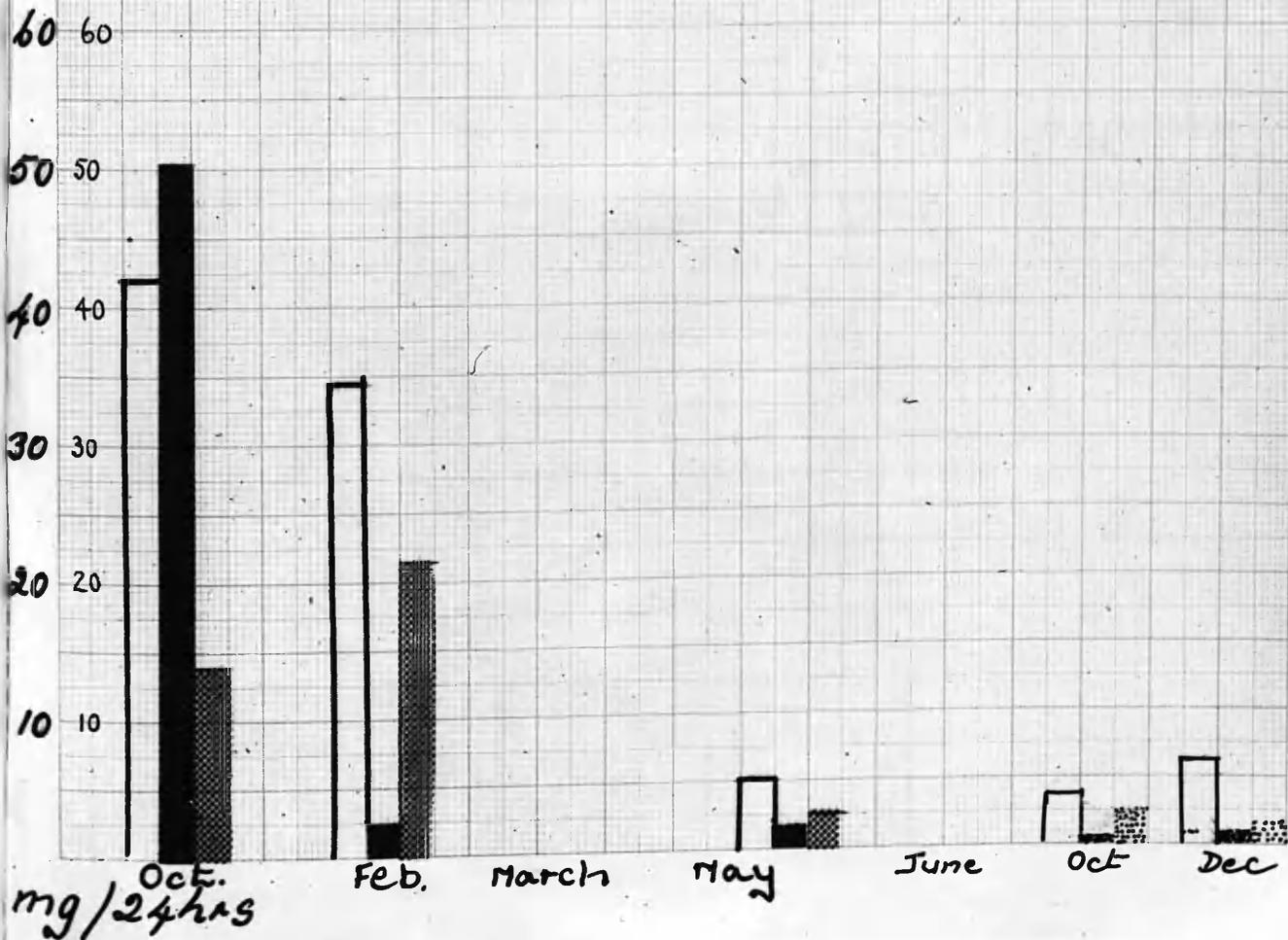
■ II Pregnanetriol
▨ III 11oxygenated 17OH.S.
▭ IY oxosteroids.
Corisone   Corisone
  75mg   100mg   Prednisolone 20mg   15mg
                       5mg qds   5mg tds.

mg/24hrs

<u>V.A.</u> <u>BORN 23.2.48</u>.

This boy is the only child of healthy
parents. He was first admitted to the Child-
ren'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 mainfest 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. (61⅛");
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 9∝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 concommitant fall in the potassium to 4 m.eq.
The blood pressure, however, did not rise.
Steroid excretion:

Date	17 keto-steroid mg/24hr.	17 ketogenic steroid mg./24 hr.		Ratio II/III
		II	III	
11-12/10/58	5.7	2	11	0.16
	4.8	0.5	12.2	0.04

His treatment was adjusted to 25 mg. cortisone
three times daily, and 0.25 9∝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.

At 11 years 3 months he was again reported to be lacking in energy. The steroid excretion was high:

Date	17 keto-steroid mg/24hr.	17 ketogenic steroid mg./24 hr.		Ratio II/III
		II	III	
10-11/5/59	12.6	10.7	29.7	0.36

The dose of cortisone was increased to 25 mg. four times daily with the same dose of 9 fluorohydrocortisone. 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 keto-steroid mg/24 hr.	17 ketogenic steroid mg./24 hr.		Ratio II/III
		II	III	
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 11½ years - weight 65.5 kg. (145 lbs.); height 164 cm. (64½"). 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 hyper-
plasia 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.

PAEGNANETRIOL FRACTION II

11 OXYGENATED 17OH STEROIDS

FRACTION III

17 KETOSTEROIDS

Oct. 1959.

Aug.

June.

May.

March.

Jan.

Oct. 1958

Oct. 1958

Oct.

mg/24 hrs.

30 28 26 24 22 20 18 16 14 12 10 8 6 4 2
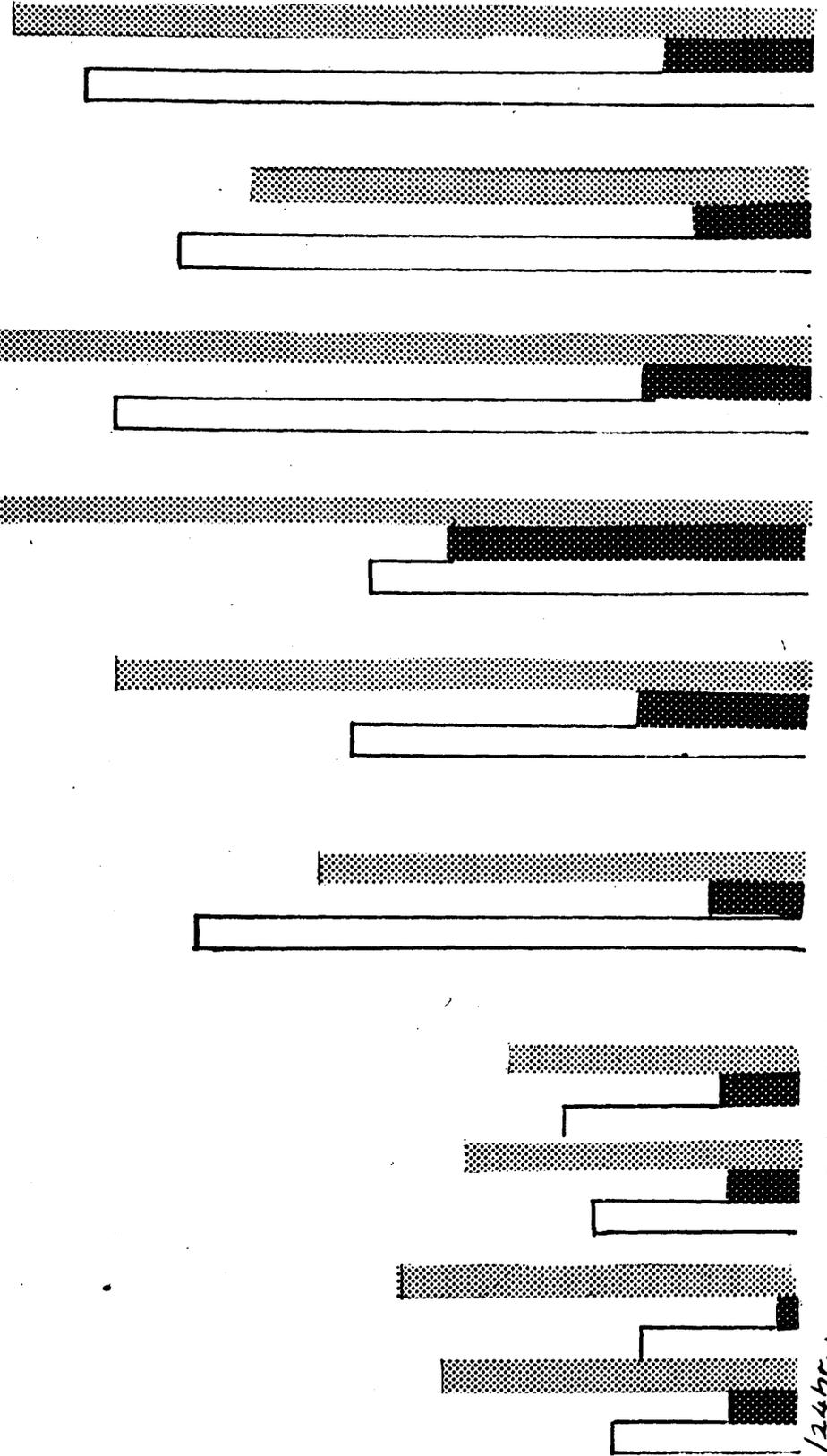
CONCLUSION

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 bismuthate. 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 hydroxypregnanolone and pregnanetriol. The second fraction estimated contains the more polar, 11 oxygenated 17 ketosteroids 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 17αhydroxy-
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.

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