TOXAEMIA OF PREGNANCY.

FLUID RETENTION AND ANTIDIURETIC HORMONE.

By

M. LINDSAY PATERSON,
B.Sc., M.B. Ch.B., M.R.C.O.G.

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PREFACE.

In the work of which this Thesis is the culmination, I am beholden to many people for the supply of material and specimens.

To Drs. Arneil and Wilson who first introduced me to this subject, I wish to express my gratitude.

To Professor Ian Donald, for his encouragement and assistance, I am deeply grateful. To the same gentleman, Emeritus Professor R.A. Lennie, Professor D.F. Anderson and Dr. J. Hewitt of the Glasgow Royal Maternity and Women's Hospital, who allowed me access to the patients under their care, I wish to express my thanks.

To Dr. A.D.T. Govan in whose Laboratory the work was mainly carried out I am indebted, not only for bench space but for his constant encouragement, criticism and assistance during the five years this work has at present continued.

I also wish to express my thanks to the Royal College of Obstetricians and Gynaecologists, one of whose Leverhulme Research Scholarships I held during the years 1954, 1955 and 1956.

A preliminary paper on the subject of this Thesis was published in the Journal of Obstetrics and Gynaecology of the British Empire in April, 1954.

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CLINICAL SECTION.

GENERAL INTRODUCTION.

About one in ten of all pregnant women are found to suffer from one or other of that group of disorders peculiar to the gravid, and referred to as the "toxaemias of pregnancy".

Kellar (1955) in British Obstetric Practice has subdivided this group as follows:-

(1) Acute toxaemias of pregnancy.
Eclampsia.
Pre-eclampsia. *

(2) Chronic hypertensive vascular disease.
Essential Hypertension.

(3) Renal Disease.

Acute and chronic glomerular nephritis.
Chronic Pyelonephritis.
Acute nephrosis (including bilateral cortical necrosis).

(4) Vomiting in Pregnancy.

(5) Liver necrosis in pregnancy, and it is to the acute toxaemias of pregnancy and their aetiology - in particular the retention of fluid therein - that this work is directed.

* Pre-eclampsia may be defined as a condition peculiar to the pregnant woman and characterised by hypertension, oedema and albuminuria, all three being present in many cases but any two of these signs in combination being sufficient for the diagnosis. Where, as may occur, convulsions follow, the condition is known as eclampsia.

The aetiology of pre-eclampsia has been the subject of endless research; most text-books of obstetrics devote at least one chapter to a discussion of the various theories which have been advanced. Amongst others Dieckmann (1952) and Sophian (1953) have published monographs on the subject.

The role of the posterior pituitary gland in the aetiology of the acute toxaemias of pregnancy was first advanced by Hofbauer (1918) when he suggested that pregnancy toxaemia was a sequel to disturbance of the normal neuro-hypophysio-adrenal equilibrium. As no experimental evidence could be produced in support of this theory little more was heard of it during the next ten years until it was

once again taken up by Rosenbeck (1927) and Kustner (1928).

Kustner claimed to have demonstrated, in the blood of eclamptic patients, a substance, which when injected into the frog resulted in stimulation of the melanophores. As the only substance thought to produce a definite melanophore response in these animals was posterior pituitary extract, Kustner claimed that his findings were in support of the theory of Hofbauer, and considered that the place of the posterior pituitary gland in the aetiology of eclampsia had been definitely established.

It is now known that the melanophore expanding hormone is produced by the intermediate lobe of the posterior pituitary gland. In the human these cells have migrated to the anterior lobe, and are associated with anterior lobe excretion. This being so, the proof

adduced by Kustner can not be accepted as evidence of overactivity of the posterior pituitary gland in toxaemia of pregnancy.

Before describing the further development of this theory in the ensuing years, it would seem that a summary of the pharmacology of the posterior pituitary gland is indicated. That the posterior pituitary is an active secretory gland was under investigation at the beginning of the present century, the activity of extracts being studied.

Oliver and Shafer (1895) showed that injections of an extract of the posterior pituitary gland had a pressor action and this was confirmed by Howell (1898). That such an extract had a diuretic action in anaesthetized animals was demonstrated by Magnus and Shafer (1901) and the first reference to the antidiuretic and chloruretic activity

in unanaesthetized animals was contained in a paper by von den Velden (1913).

Various other activities of this posterior pituitary extract were also described, a hyperglycaemic action (Borchardt, 1908) a stimulation of intestinal peristalsis (Bell, 1909), an oxytocic action (Dale, 1909) and a galactogogue activity (Ott and Scott, 1910).

That one active principle could be responsible for such a multiplicity of actions was doubted from the first but it was not until 1928 that Kamm and his co-workers were able to separate in relatively pure state the two active principles. The one "the pressor fraction" embraced the pressor, antidiuretic, chloruretic, stimulation of intestinal muscle and hyperglycaemic activities previously described; while the other "the oxytocic fraction" comprised the oxytocic and galactogogue

activities.

With these two relatively pure fractions as a starting point purification was continued in an effort to isolate the active principles, and from these to derive the chemical structure. This culminated in the publication by du Vigneaud et al. in 1953 of the chemical structure of oxytocin as an octopeptide containing aspartic acid, cystine, glutamic acid, glycine, proline, tyrosine, leucine and isoleucine. Du Vigneaud, Lawler and Poperoe (1953) have also described the chemical structure of vasopressin. This is also an octopeptide containing the same first six amino acids as oxytocin but in addition phenylalanine. The eighth amino acid varies as to the source of the vasopressin and is arginine in beef vasopressin and lysine in vasopressin from the hog.

These workers have given details

of the synthesis of both oxytocin and vasopressin, and shown that the two vasopressins may, as would be expected, result in different anti-diuretic potency consequent upon which vasopressin occurs naturally in the group of animals under test.

The above work was carried out largely by counter current distribution using large quantities of relatively crude posterior pituitary extract as a starting point, and is obviously unsuitable for clinical application.

Likewise whilst it seemed relatively simple to test for the presence of posterior pituitary substance by the melanophore expansion which followed injection of the extract into a frog, this activity is now known to be due to a hormone elaborated in the pars intermedia and not the presence of actual posterior pituitary substance in the extract.

The measurement of anti-diuretic

activity must be carried out by bioassay. This is both an inexact and time-consuming method of measurement and is not, except as a research procedure, suitable for clinical use.

A simpler approach to this problem has been described by Arneil and Wilson (1953) and Valeri, Zacco and Perrini (1953). These workers claimed the isolation from the urine of patients suffering from nephritis or nephrosis in the one case, and pre-menstrual tension in the other, of active pituitary polypeptides. This was carried out by the use of two dimensional chromatography on paper. While there were certain differences in technique, material under study and conclusions between these authors the results were essentially similar and the method employed seemed suitable for application to other conditions where the posterior pituitary gland may be involved.

As these pharmacological and technical advances were being made, it was only natural that further attention should be paid to the theory that the posterior pituitary was involved in the aetiology of pre-eclamptic toxaemia, particularly following the separation of posterior pituitary extract into two active fractions by Kamm and his co-workers.

Anselmino, Hoffman and Kennedy (1931) using ultrafiltrates of blood from eclamptic and pre-eclamptic patients claimed to have demonstrated both a pressor and an antidiuretic principle which "in their chemical and physical properties were found to be identical with those of the autocoid of the posterior lobe of the pituitary gland". They further claimed that there was full agreement between the most important individual symptoms and experimentally reproducible actions of

the autocoid, including water retention through inhibition of diuresis, increase in blood pressure, capillary spasm, coma and fits. Quantitative estimations they claimed ran parallel with the severity of the symptoms.

The next contribution to the posterior pituitary theory was anatomical and histological. Cushing (1933) in the course of an anatomical investigation into the functional activity of the neuro-hypophysis demonstrated that when the normal activity of the pars intermedia was exaggerated in pathologic states, there occurred marked hyperplasia of the basophilic elements. A few fatal cases of eclampsia were examined and in these cases a massive basophilia of the pars nervosa was noted. Cushing (1934) extended these observations and reported the findings in nine fatal cases of eclampsia. It appeared that the basophilia was related to the degree of

hypertension. In six typical cases of eclampsia the basophilia was excessive, whilst in one case where the blood pressure was only 130/90 mm. Hg. there was slight basophilic infiltration only. Ahlstrom (1935) continued the work of Cushing and reported the examination of the posterior pituitary glands from 61 patients who had died of eclampsia. Of these 36 were without any hypertension and 18 of this group showed basophilic infiltration of the pars nervosa, whilst of the 25 in the group showing hypertension 24 had marked basophilic infiltration. In view of these results Ahlstrom was not able to support completely the findings of Cushing. At the same time however, he concluded that there was some relationship between the posterior pituitary gland and the toxaemias of pregnancy.

Reassessment of this work reveals an interesting fact, in the 36 patients

in whom hypertension was not a feature, oedema was very marked. It is now known that the pressor and antidiuretic hormones of the posterior pituitary gland are in fact one and the same, and it would appear in the light of this that the results of Cushing and Ahlstrom are in fact compatible.

Following this anatomical work attention once again turned to the detection of the pituitary hormones by means of bioassay. The existence of a posterior pituitary pressor substance as claimed by Anselmino, Hoffman and Kennedy was denied in turn by de Wesselow and Griffiths (1934) and Hurwitz and Bullock (1935). However Mukherjee (1941) using methods similar to those of Anselmino, Hoffman and Kennedy has shown that the blood of severely toxaemic patients does in fact contain a pressor substance, which he claims to be of pituitary origin.

The antidiuretic activity of the posterior pituitary gland has attracted even more attention. Coester (1935) claimed to have found an antidiuretic substance in the urine of toxaemic patients but Byrom and Wilson (1934), Theobald (1934) and Levitt (1936) were unable to detect any such activity.

Teel and Reid (1939) once again reported the presence of a urinary antidiuretic substance in toxaemic patients and claimed that it was of posterior pituitary origin. Ham (1941) repeated this work, but, while confirming their findings of an antidiuretic substance declared that the substance differed in some respects from posterior pituitary hormone.

Krieger and Kilvington (1940) claimed that the antidiuretic factor could be extracted from urine of normal pregnant and even non-pregnant women as well as that of toxaemic patients.

In 1946 these authors reviewed their work and claimed that significant amounts were only found in the urine from toxaemic patients, but the position was further confused by Krieger, Butler and Kilvington (1951) who reported that certain strains of Bacterium coli produce an antidiuretic substance. This led them to state that after due consideration they could not exclude the possibility that their previous results were due to bacterial contamination of the urine. They concluded that "it is unlikely that antidiuretic substances of pituitary origin can be regularly detected in the urine of toxaemic patients". With this conclusion Willson, Carrington, Hadd and Bontwell (1954) are in agreement.

Meanwhile attention was directed towards the placenta as a possible source of antidiuretic and pressor factors in these patients, and in 1942 Ham and Landis claimed to have detected the presence of

these factors in saline extracts of that organ. This substance was said to be of a colloid nature but was certainly not posterior pituitary extract substance. This work was repeated by Byrom (1951) but he was unable to find significant difference in the antidiuretic titre between pre-eclamptic and normal placentae and further suggested that the antidiuretic activity was largely, if not wholly, accounted for by bacterial contamination.

Despite this controversy and the failure to find specific factors responsible for oliguria and hypertension the search continues, and a considerable body of opinion supports the idea of their existence. This idea has been sustained particularly in view of the repeated observation that toxaemic patients show a marked sensitivity to posterior pituitary extract (Dieckmann and Michel 1937, Schockaert and

Lambillon 1937, de Valera and Kellar 1938) and this increased sensitivity has been variously attributed to the action of the gonadotroptic hormones either of chorionic or pituitary origin (Byrom, 1938; Browne 1946; Govan and Mukherjee 1950).

In addition the recent work on renal function and haemodynamic changes in pregnancy toxaemia indicates that these changes are functional and postulates some humoral mechanism (Kenny, Lawrence & Miller 1950 a.b.)

In view of the wide divergence of opinion which has been briefly reviewed above it was decided to re-investigate the place of the antidiuretic hormone of the posterior pituitary gland in the causation of the acute toxaemias of pregnancy. The methods chosen were originally those of Arneil and Wilson, as mentioned previously, and from this starting point was developed the

investigation of which this thesis is the result.

THE EXTRACTION OF A "POLYPEPTIDE" FROM THE URINE OF PATIENTS SUFFERING FROM ACUTE TOXAEMIA OF PREGNANCY.

THE EXTRACTION OF A "POLYPEPTIDE" FROM THE URINE OF PATIENTS SUFFERING FROM ACUTE TOXAEMIA OF PREGNANCY.

The use of partition filter-paper chromatography in the separation of amino-acids and polypeptides in mixtures was first suggested by Gordon et al. (1943) and greatly improved by Consden et al (1944). This method of separation depends upon the different relative solubilities of each amino-acid or polypeptide between the water, which is invariably held in the cellulose fibres of ordinary filter-paper, and a solvent, not miscible with water, which is allowed to creep slowly along the filter-paper past a spot which contains the mixture under test. The amino-acids or polypeptides are drawn along at definite speeds behind the moving solvent and thus arrange themselves in a characteristic order.

In their original paper Consden et al. took care to avoid the presence

of inorganic salts in their mixtures as these they stated resulted in unsatisfactory chromatograms. This has not been the experience of later workers, and Dent (1946) successfully applied the method to urine, protein-free blood filtrates and other biological fluids, where the ratio of inorganic salts to amino-acids did not exceed 15 to 1.

The methods which have been applied in the present investigation are largely those of Dent, and will be described in some detail in the present section so that repetition may be avoided later.

APPARATUS.

The cabinets used in the two dimensional chromatography were adapted from Dent (1948). They consisted of large wooden boxes of inside dimension 32" x 32" x 14" and required to be completely air-tight. Into each side

was let a glass window 28" x 28" so that the running of the chromatograms could be observed. The lid was 32" x 14" and was flanged to secure an air-tight fit. As a further precaution a cork gasket was placed over the flange and the lid secured in place by six wing-nuts, the bolts of which were affixed to the outside of the box, and over them fitted iron tongues on the upper surface of the lid. Three inches below the lid on either end wall of the tank was fixed a projecting wooden batten on which the solvent troughs were supported.

These solvent troughs were constructed of aluminium and had a flange on either end which rested upon the supporting wooden battens. Along either side of the trough, but not touching the trough, was a glass rod. These rods were held in place by clips mounted on the upper surface of the flange at either end. The purpose of

these rods, over which the paper when in position passed, was to prevent capillary syphoning between the outside of the trough and the paper.

A heavy glass slide ran perpendicularly along the middle of each trough and was held in place by grooves at either end. This glass slide was used to maintain the filter-paper in position.

Three of these solvent troughs could be fitted inside each chromatography tank, and as a filter-paper could be mounted on each side of the trough, this allowed six papers to be processed at any one time.

Prior to use, the inside of each chromatography tank and lid was thickly painted with beeswax. In all, four of these tanks were constructed and utilised, each tank being retained for one particular solvent.

SOLVENTS.

The solvents employed were two in number, phenol-ammonia and n-butanol-acetic acid. The phenol employed throughout was phenol liquefactum B.P. Care has to be exercised that no decomposition has taken place and should this have occurred, the phenol must be discarded as satisfactory chromatograms cannot be obtained. The phenol was saturated with water and stored in the same room in which the chromatographic tanks were kept in order that there should be no temperature difference.

n-Butanol-acetic acid, hereinafter referred to as butanol-acetic acid is obtained by mixing in a separation funnel four parts of n-butanol with one part of glacial acetic acid and five parts of water. This mixture is thoroughly shaken and allowed to settle. The lower layer is discarded and the upper layer used as the solvent -

Partridge 1946.

DRYING CABINET.

The drying cabinet was constructed from an old wardrobe. Notched battens were fitted to the inside to hold glass rods on which the filter-papers could be dried. A tubular heater was mounted in the floor of the cabinet and the lower two inches of the door removed and replaced by a fine wire mesh. Holes were bored in the roof, and when the tubular heater was switched on, convection resulted in a current of air passing through the cabinet. This apparatus proved most satisfactory.

DEVELOPMENT OF THE CHROMATOGRAMS.

Ninhydrin 1% in n-butanol was used for the development of the chromatograms. Each batch of Ninhydrin was tested separately with pure solutions of amino acids before being passed for use, as it was found that various batches did not always give a satisfactory colour

reaction.

The Ninhydrin dissolved in n-butanol was sprayed on the papers and the colour developed by heating to 80° C. for 5 minutes - Copley 1941.

The colour reaction tended to fade over a period of time and for this reason pencil outlines of the stained portions were drawn on the chromatograms immediately after development.

PAPER.

The paper used throughout was Whatman No. 1 filter-paper and was supplied in sheets 22½ by 18 inches specially prepared for chromatography.

RUNNING CONDITIONS.

The chromatographic tanks and solvents were kept in a draught free room in which temperature fluctuation was minimal.

Prior to running any chromatograms in the tanks these were first tried out with standard solutions of amino-acids

to ensure that neither the position of the paper in the tank, nor the small temperature fluctuations that occurred in the room, prevented the repetition of the results.

In the tank where phenol was the solvent, a tray placed in the foot was filled with water and phenol so that the atmosphere inside the tank would be saturated with both substances. Once the tank had been set up and before the lid was closed, 1 millilitre of concentrated ammonia (Sp.Gr. 0.88) was run in, and a few crystals of potassium cyanide dropped in to the aqueous liquid at the bottom of the tank. This was done to suppress the catalytic oxidation of the solvent due to the presence of copper in the filter-paper - Partridge.

In the base of the tank used for butanol-acetic acid runs a tray containing water, and butanol was placed to maintain a saturated atmosphere inside

the tank.

The urine to be examined was collected either as a clean swab specimen or by catheter and was applied to the paper within six hours of being voided. (A clean swab specimen of urine is voided after the vulva has been cleaned, and the vagina temporarily tamponnaded to prevent contamination of the urine with vaginal discharge). In the interval the urine was stored in a refrigerator. These precautions were taken to avoid the possibility advanced by Krieger, Butler and Kilvington that anti-diuretic activity in the urine was the result of organismal activity.

The urine was measured, acidified with dilute acetic acid, and then heated in a boiling water bath to precipitate any protein present. After filtration the volume was restored to the original by the addition of distilled water.

Initially the "polypeptide" was

precipitated with Tungstic acid as suggested by Arneil and Wilson (1955), centrifuged and the precipitate dissolved in 0.3% ammonia. Once the presence of the "polypeptide" in the urine of patients with hypertensive toxaemia of pregnancy was established, this method was abandoned and only thereafter used where larger quantities of the "polypeptide" were required for further investigation.

Various volumes of deproteinised urine were run as two-dimensional chromatograms, and finally by trial and error 0.05 ml. of urine was fixed upon as giving the most satisfactory results.

The urine was applied as a spot some three inches from the edge and three inches from the top of the filter-paper, which was arranged so that the phenol-ammonia run took place in the shorter length of the paper. The area of the spot of application was kept as small as possible by successive applications and

dryings. An electrical hair-dryer was found to be of great assistance. The paper was then folded, placed in the trough with the upper edge in the phenol solvent, and the chromatograph tank arranged as has been described. A run of some ten to twelve inches was allowed and under the conditions applying this took approximately eighteen hours.

The paper was then removed, hung in the drying cabinet in a current of warm air, and thoroughly dried. On average two days were allowed for complete drying.

The chromatogram was then turned at right angles and run in the second solvent - butanol-acetic acid. The paper was always so arranged that the point of application of the urine was uppermost, i.e. nearest the solvent trough. A run of approximately twelve to fourteen inches was allowed in the butanol-acetic acid, and this took some

twelve to fifteen hours.

The paper was removed from the tank, dried over a period of 24 hours, sprayed with Ninhydrin and developed as has been described.

RESULTS.

The developed chromatograms showed little variation in the amino-acid pattern, but considerable difference in the intensity of staining of the same amino-acid between urine and urine. This was especially noticeable between chromatograms developed from the urine of pregnant and non-pregnant patients.

The following amino-acids were frequently identified in the chromatograms:- alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Of these amino-acids, alanine, arginine, aspartic acid, glutamic acid,

glycine, histidine and leucine have been almost constantly present.

The identification of these amino-acids was made by reference to the chart of Dent (1948), but in each case the identification was verified either by direct reference to known amino-acids, run under identical conditions, or by the use of added amino-acids. The details of these methods of identification are described in a later section of this thesis.

As has been previously mentioned the intensity of colour developed with Ninhydrin by the same amino-acid was noticeably increased in the pregnant urine.

Moore and Stein (1948) have stated that the intensity of the colour reaction developed with Ninhydrin may be taken as a rough indication of the quantity of the amino acid present. If this is so, then it would appear that there is an increased output of the individual amino-acids in

pregnancy, and that this increased output is particularly noticeable in the later months of pregnancy.

No significant difference in the individual amino-acids excreted, or in the apparent quantity, has been noted between the various groups studied, except for the changes already described as occuring in the pregnant.

Studies of the amino-acid output in pregnancy, both as regards the individual amino-acids and the quantitative output, have been reported by Smith (1949), Wallraft et al. (1950), Ruttinger et al. (1954) and Miller et al. (1954). These investigators have noted the increased amino-aciduria in pregnancy, and also agree that the amino-acids named previously are those commonly found in the pregnant urine.

Apart from these differences the main feature was the presence in a certain group of cases of the "polypeptide"

described by Arneil and Wilson.

This substance only moves in a linear direction under the influence of, and at the same rate as, phenol-ammonia and is not affected in position by subsequent running in butanol-acetic acid. The substance stains with Ninhydrin and is therefore easily recognised. The substance occupies the same position as the "nephrosis peptide", numbered 61 by Dent (1948), in his "Map of the Spots".

This position occupied by the "polypeptide" is constant and quite characteristic. It is sufficiently removed from the position occupied by all other Ninhydrin positive material so far discovered in the urine as to make confusion impossible.

The group of cases in which this substance could be detected in 0.05 ml. of urine were almost without exception cases of hypertensive toxaemia of pregnancy, and from this finding developed

the further work here described.

The full details of the distribution of this substance in the urine will be found in the clinical section of this thesis. Figure I is of a chromatogram showing the presence of the "polypeptide" while Figure II is a chromatogram from the urine of a normal pregnant patient where the "polypeptide" was not detected.

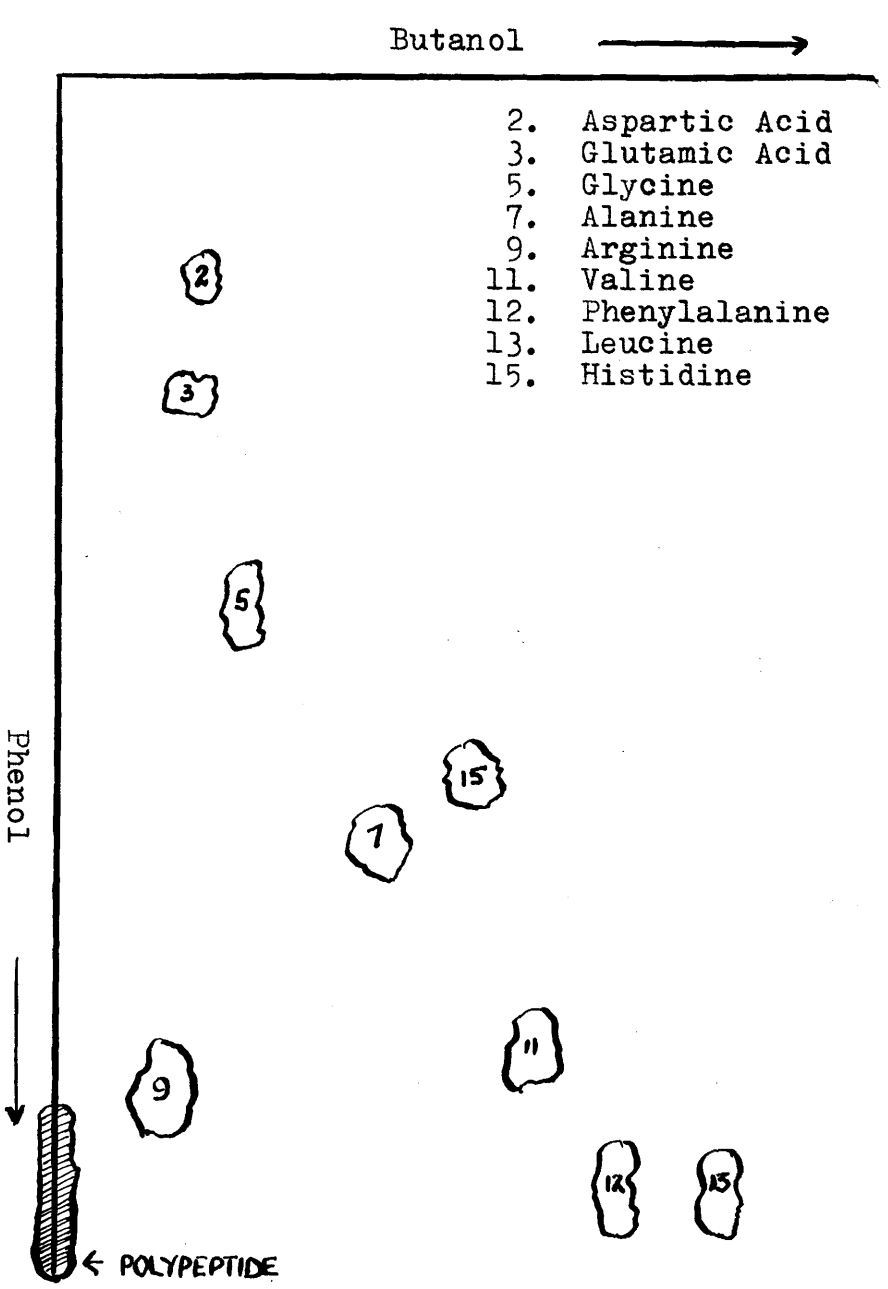


Fig. I. Chromatogram - Urine from patient with Acute Toxaemia of Pregnancy to show presence of Polypeptide.

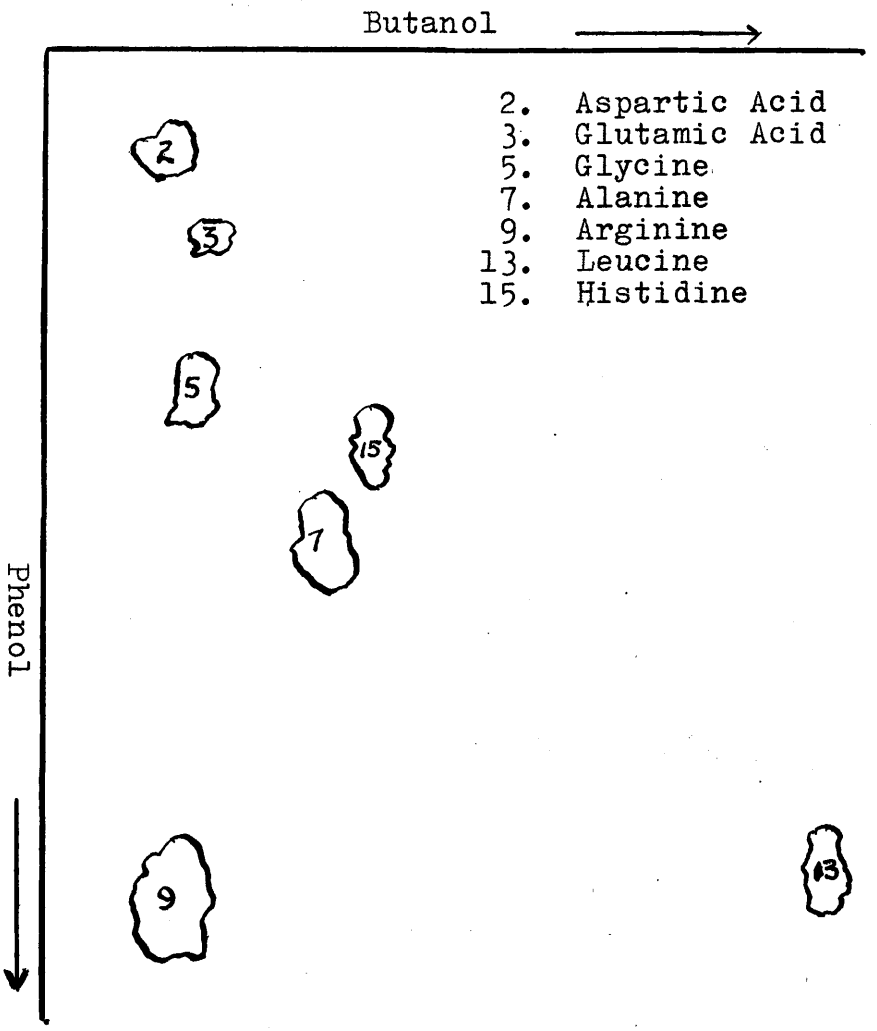


Fig. II. Chromatogram. Normal Pregnant Urine.

THE ISOLATION OF THE "POLYPEPTIDE" FROM THE BLOOD.

THE ISOLATION OF THE "POLYPEPTIDE" FROM THE BLOOD.

Following the isolation of a "polypeptide" from the urine of cases of acute toxaemia of pregnancy, it now became important to determine whether this substance could be detected in the blood-stream.

No technique for this isolation has been reported, but Giri et al. (1952) described a method for amino-acid analysis of blood by paper chromatography. It was decided to adopt this method to the present investigation in the following manner.

Venous blood - approximately 5 ml. - was withdrawn by syringe, with due precautions to prevent haemolysis, and was immediately transferred to a dry, chemically clean tube containing 75 international units of Heparin B.P. Mixing was obtained by gentle agitation of the tube which was then centrifuged

at 3000 r.p.m. for five minutes. One millilitre of the supernatant plasma was transferred immediately to a capped centrifuge tube containing 3 ml. of absolute ethyl alcohol A.R. This resulted in precipitation of the proteins in the plasma, and after further centrifugation, the protein free alcoholic extract was mixed with three times its own volume of chloroform A.R. in a stoppered centrifuge tube. This mixing with chloroform results in the extraction of the alcohol leaving an aqueous layer at the top of the chloroform alcohol mixture. After centrifugation this aqueous layer was carefully removed with a pipette, transferred to a special measuring cylinder, and the volume adjusted to 0.5 ml. either with double distilled water or evaporation in vacuo whichever was necessary.

This aqueous extract of blood was

now ready for application to a sheet of filter paper. For this purpose the volume selected was 0.05 ml. and the size of the spot of application was kept as small as possible by successive applications and dryings.

The paper was now transferred to a chromatography tank, and after running in phenol-ammonia and butanol-acetic acid in the form of a two-directional chromatogram, as previously described, was dried and developed with Ninhydrin.

The developed chromatograms revealed considerable variation in the amino-acid pattern but a consistent finding in all cases of acute toxaemia of pregnancy was a Ninhydrin staining material situated in the characteristic position occupied by the "polypeptide". Figures III and IV are reproductions of two typical chromatograms prepared from aqueous extracts of venous blood withdrawn from cases of acute toxaemia

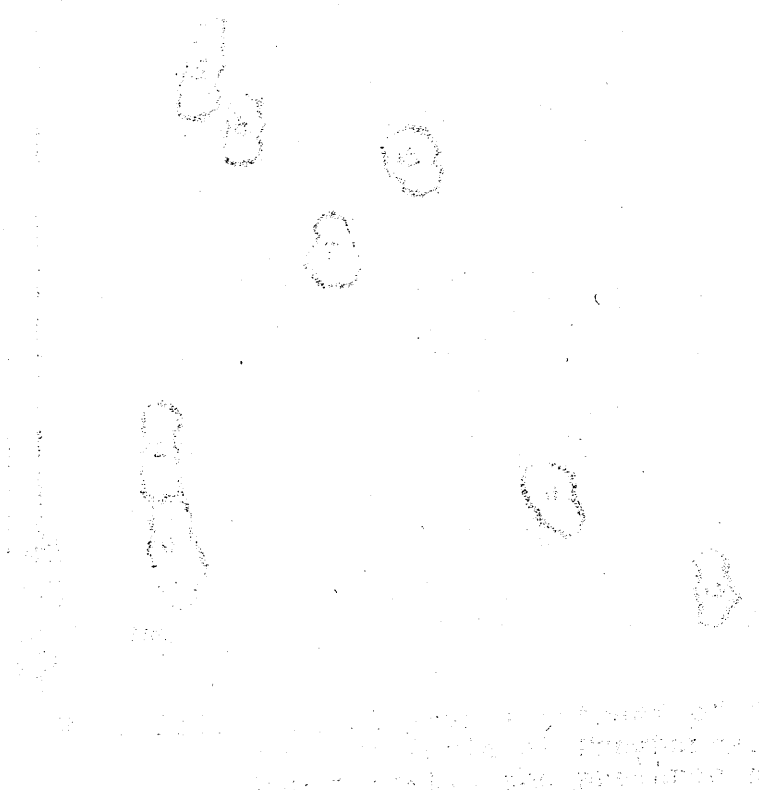
of pregnancy.

To eliminate the possibility that the polypeptide might be derived from the Heparin, solutions of Heparin B.P. 5000 and 25,000 international units per millilitre were examined. One millilitre of these solutions was treated as has been described for plasma, and an aqueous extract prepared. This was applied as a spot to filter paper and two dimensional chromatograms developed. In no case was it possible to demonstrate any Ninhydrin staining material in the position characteristically occupied by the "polypeptide".

It would thus appear that the "polypeptide" was in fact derived from the venous blood, and not due to any contaminant present in the Heparin.

The presence or absence of this "polypeptide" in the blood of the various groups of patients investigated will be described and discussed later in the

clinical section of the thesis.



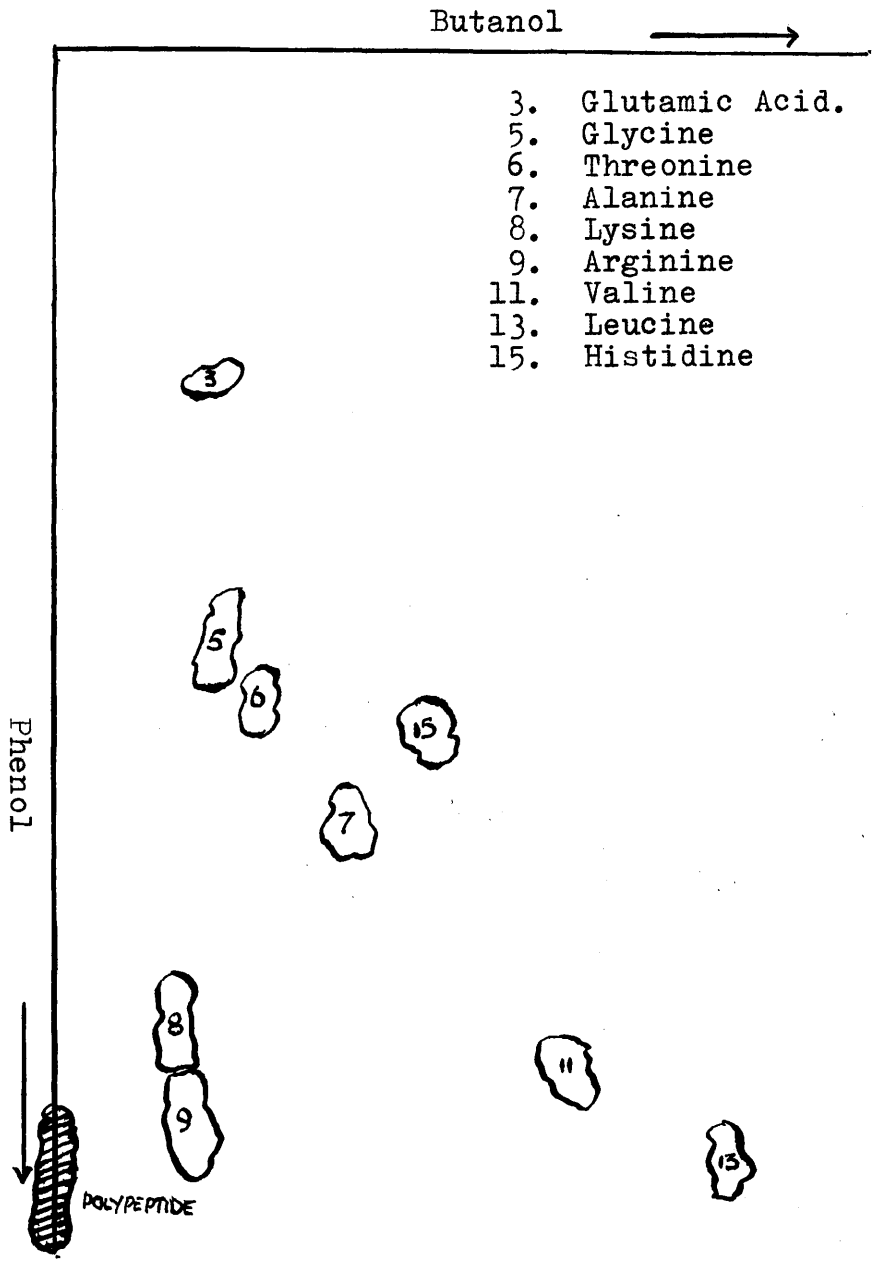


Fig. III. Chromatogram - Extract of Plasma from Toxaemia of Pregnancy. Demonstrates the presence of the Polypeptide.

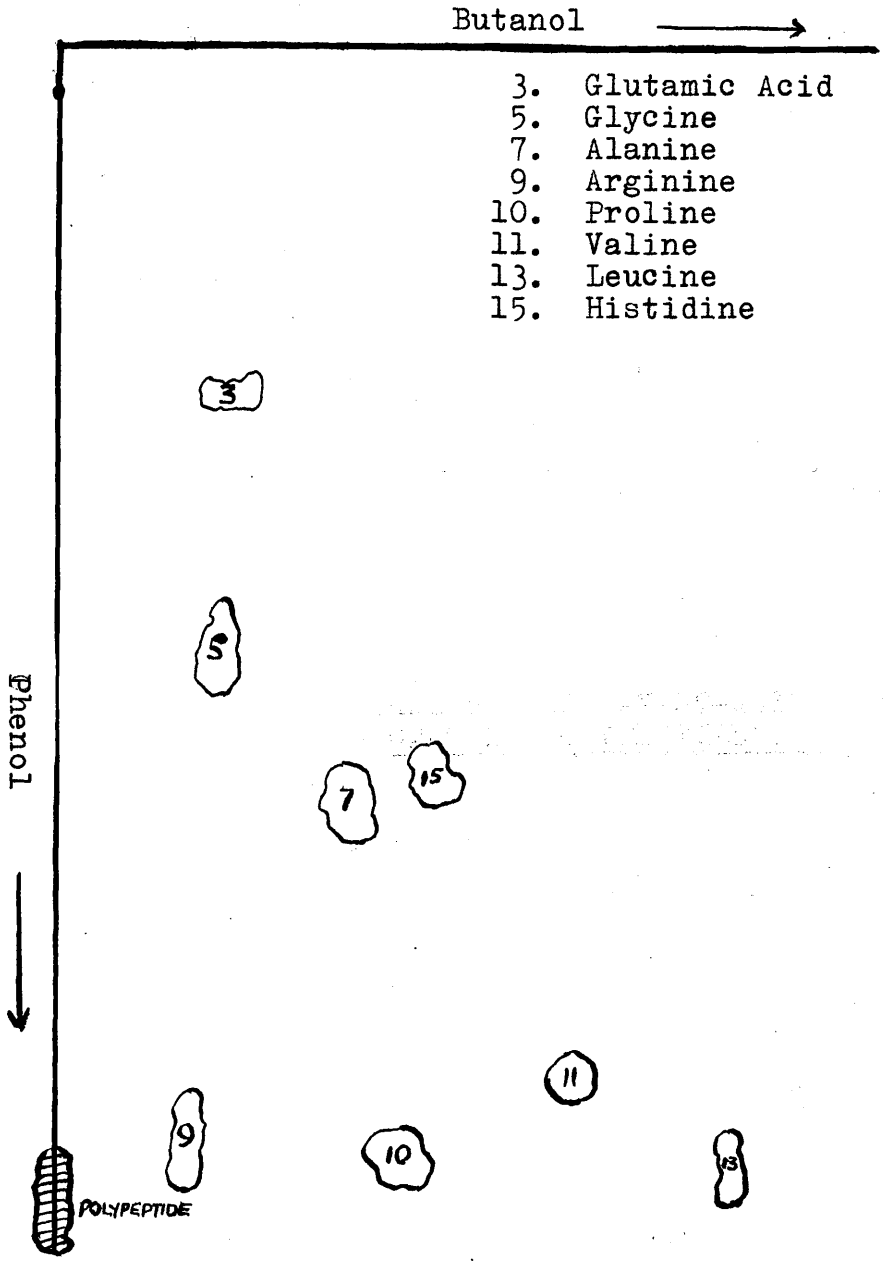


Fig. IV. Chromatogram. Extract of Plasma from Toxaemia of Pregnancy. To show the presence of the Polypeptide.

ELUTION, HYDROLYSIS AND AMINO-ACID ANALYSIS OF THE "POLYPEPTIDE".

ELUTION, HYDROLYSIS AND AMINO-ACID ANALYSIS OF THE "POLYPEPTIDE".

As has been described in the two previous sections, a Ninhydrin staining substance has been demonstrated in a consistent position following two directional paper chromatography using phenol-ammonia and butanol-acetic acid as solvents. It now became necessary to obtain larger quantities of this material that further investigations could be carried out.

This was possible as a result of the differential rate of flow of the substance in the two solvents. The following was the method employed.

The substance to be extracted - either a tungstic acid extract of protein free urine or an aqueous extract of blood - was applied as a line along the length of a sheet of Whatmann filter paper, about 3 inches from the edge. By successive application and drying it

was found that approximately 1 millilitre could be applied to each sheet of filter paper. These were now placed in the chromatographic tank and run with phenol-ammonia as the solvent. A run of about 15 inches was allowed and the paper was removed and dried in a current of air. Prior to drying a pencil mark was placed on each side of the sheet to indicate the lowermost level to which the solvent had run. After thorough drying, the paper was turned through 180°, and run again; this time the solvent used was butanol-acetic acid. It was not necessary to limit this second run and generally the solvent was allowed to fall off the lower edge of the paper for some time before the run was discontinued, and the paper once again removed and dried.

If one looks at the chromatograms reproduced previously the isolation of the "polypeptide" by the above method becomes obvious in that this substance

flows at the same rate as the phenol-ammonia solvent and is not moved at all by the butanol-acetic acid solvent, while contaminating amino acids such as arginine and lysine which would otherwise have been present are moved away from the termination of the phenol-ammonia run by the use of the second solvent. Any acetylated amino-acids which might be present are similarly removed.

The terminal inch of the phenol run was measured, using the pencil marks as the lower border, and this strip was cut from the dried sheet and eluted. The elution fluid used was originally 0.25% acetic acid but this proved unnecessary and double distilled water was substituted. Elution was carried out in a draught free glass cabinet. The strips of filter paper containing the material to be eluted were allowed to hang vertically downwards. The lower end of the strip was cut to a point

so that the eluate could be collected in a small beaker.

The upper end was held between two glass slides which rested in a Petri dish containing the eluting fluid. Elution was allowed to proceed until approximately 5 millilitres had collected in the receiving vessel. This eluate was now concentrated by evaporation in vacuo to approximately 1 millilitre and then split into two portions. The first was applied to paper, run in two directions as already described, and developed with Ninhydrin. This served two purposes. The one to prove that the substance under investigation was in fact present, and the other to show that no contaminant amino-acids remained.

Following this the second portion of the concentrated eluate was hydrolysed with 6 N. A.R. hydrochloric acid and heat in a sealed container. After evaporation to almost dryness on several occasions to reduce the acidity the

hydrolysate was applied as a spot to a sheet of Whatman filter paper and run as a two directional chromatogram. The chromatogram was dried, developed with Ninhydrin, and the individual amino acids identified tentatively by reference to the chart of relative rates of flow in the solvents employed. Figures V and VI are reproductions of typical hydrolysate chromatograms, and the legend indicates the various amino acids present. Figure V is a hydrolysate of the polypeptide from urine, and Figure VI an example of a hydrolysate of the polypeptide from venous blood.

The identity of the individual amino-acids was confirmed by the following experiments.

Specimens of pure amino-acids, which were thought to be involved, were obtained and an approximately 0.01 molar solution in 75 per cent v/v. ethyl alcohol A.R. prepared. A small

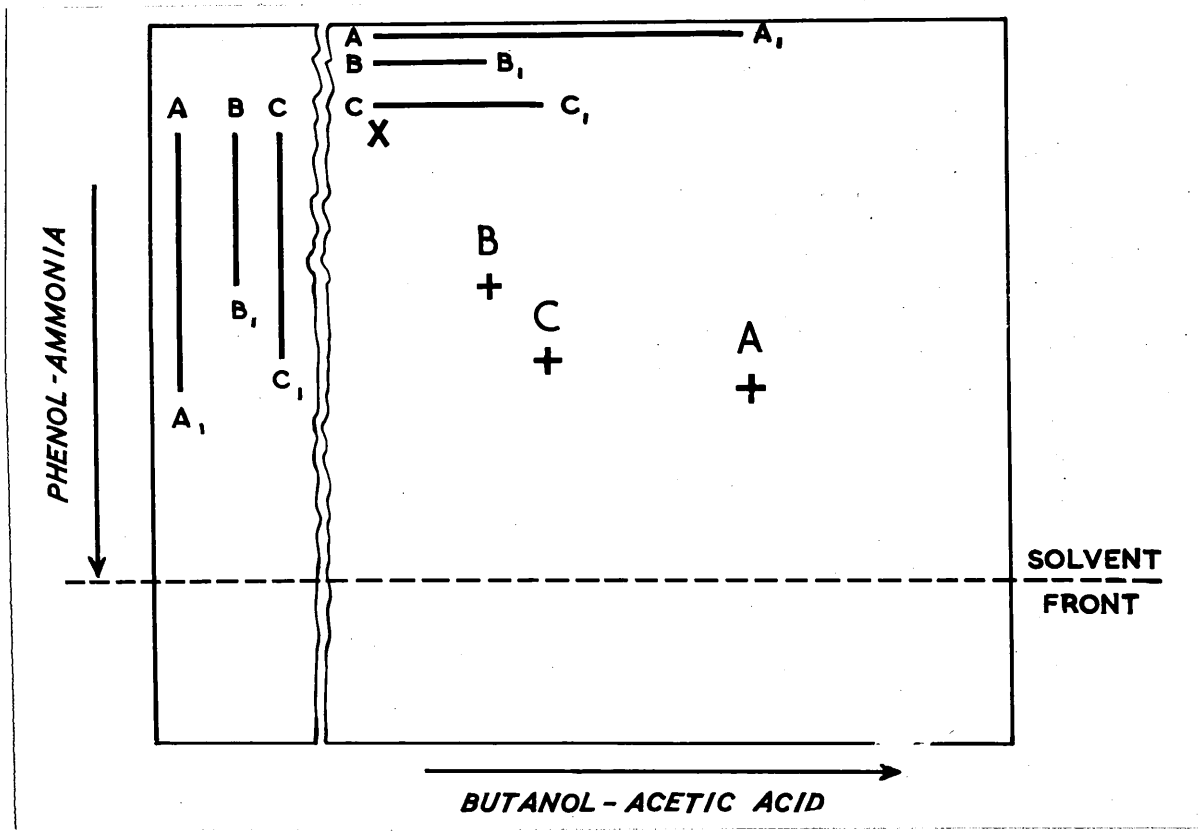


DIAGRAM I. TO ILLUSTRATE IDENTIFICATION OF AMINO ACIDS BY DIRECT MENSURATION.
X - POINT OF APPLICATION OF UNKNOWN MIXTURE.
A,B,C - POINTS OF APPLICATION OF KNOWN AMINO-ACIDS.

amount of concentrated hydrochloric acid was occasionally necessary to dissolve the less soluble amino-acids. These standard solutions, once prepared, will keep almost indefinitely.

A "spot" of the hydrolysate was applied to a sheet of Whatman filter paper some eight inches from the edge and three "spots" of standard solutions of amino-acids, each of approximately 0.01 millilitre, at the same distance from the top of the sheet but progressively nearer the edge. The prepared paper was now run in phenol-ammonia and dried. The edge strip, on which the amino-acids had been run, was cut off and preserved, and "spots" of the three amino-acids applied to the paper below the solvent front on a line parallel to the cut edge and passing through the site of application of the hydrolysate. The paper was now turned at right angles and run in butanol-acetic acid. A run of about 12 inches

was allowed, the paper removed, dried, and developed with Ninhydrin as was the edge strip previously removed.

It was now possible to use the edge strips, run under identical conditions as the hydrolysate, for direct measurement and to ascertain the presence or absence of the individual amino-acids employed. By using standard solutions of various amino-acids in turn, it was possible to identify accurately each amino-acid present in the hydrolysates.

Diagram I illustrates the method.

The second method of identification employed was to divide a hydrolysate into two equal portions, and to the one add a very small quantity of the standard solution of a selected amino-acid. Two directional chromatograms were now run using the original hydrolysate and the hydrolysate plus the added amino-acid. After drying and development with

Ninhydrin the chromatograms were compared. If the chromatogram prepared from the hydrolysate plus the amino-acid contained an extra Ninhydrin stained spot when compared with the chromatogram from the original hydrolysate, then obviously the added amino-acid was not one of those originally present. If, on the other hand, the chromatograms were identical, then the amino-acid added was amongst those contained in the polypeptide, and could be easily recognised by the deeper intensity of the Ninhydrin reaction.

Once the amino-acids had been individually identified, the pattern of the chromatogram was found to be sufficient for identification, since day to day variables always affect the Rf values in the same proportions, and do not therefore alter the pattern (Dent 1948).

Table I enumerates the amino-acids

in the hydrolysates obtained from urine and blood of patients suffering from acute toxaemia of pregnancy.

Amino Acid.	Source of Polypeptide.	
	Urine.	Blood.
Alanine.	+	+
Arginine.	+	+
Aspartic Acid.	+	+
Cystine.	+	+
Glutamic Acid.	+	+
Glycine.	+	+
Leucine.	+	+
Lysine.	+	+
Phenylalanine.	+	+
Proline.	+	+
Serine.	+	+
Threonine.	+	+
Tyrosine.	?	?
Valine.	+	+

Table I.

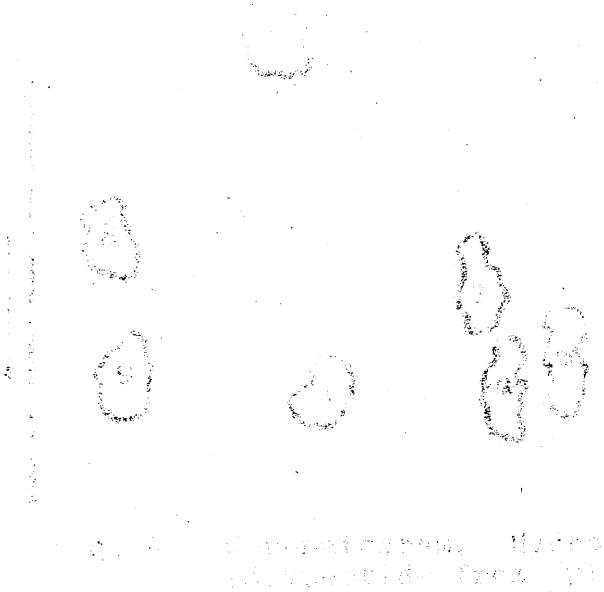
The above table requires one explanation, and that is the doubtful presence of tyrosine. This amino-acid was found in some hydrolysates, not in others, and in yet a further group was

doubtfully present. These anomalous findings can almost certainly be explained by the partial or complete destruction of tyrosine by acid hydrolysis. Attention has been drawn to this destruction of tyrosine on acid hydrolysis by Pierce and du Vigneaud (1950).

Figure VII is a reproduction of a chromatogram in which tyrosine was present for comparison with Figures V and VI where this substance was not recognised.

It had now become obvious by the characteristic position taken up by the polypeptide on two directional chromatography and the identical amino-acid structure that this polypeptide, whether derived from the urine or blood of patients with acute toxaemia of pregnancy, was in fact one and the same substance. This contention is further supported by the identical biological

activity of the polypeptide whatever the source. These results are reported later in the section dealing with the biological activity.



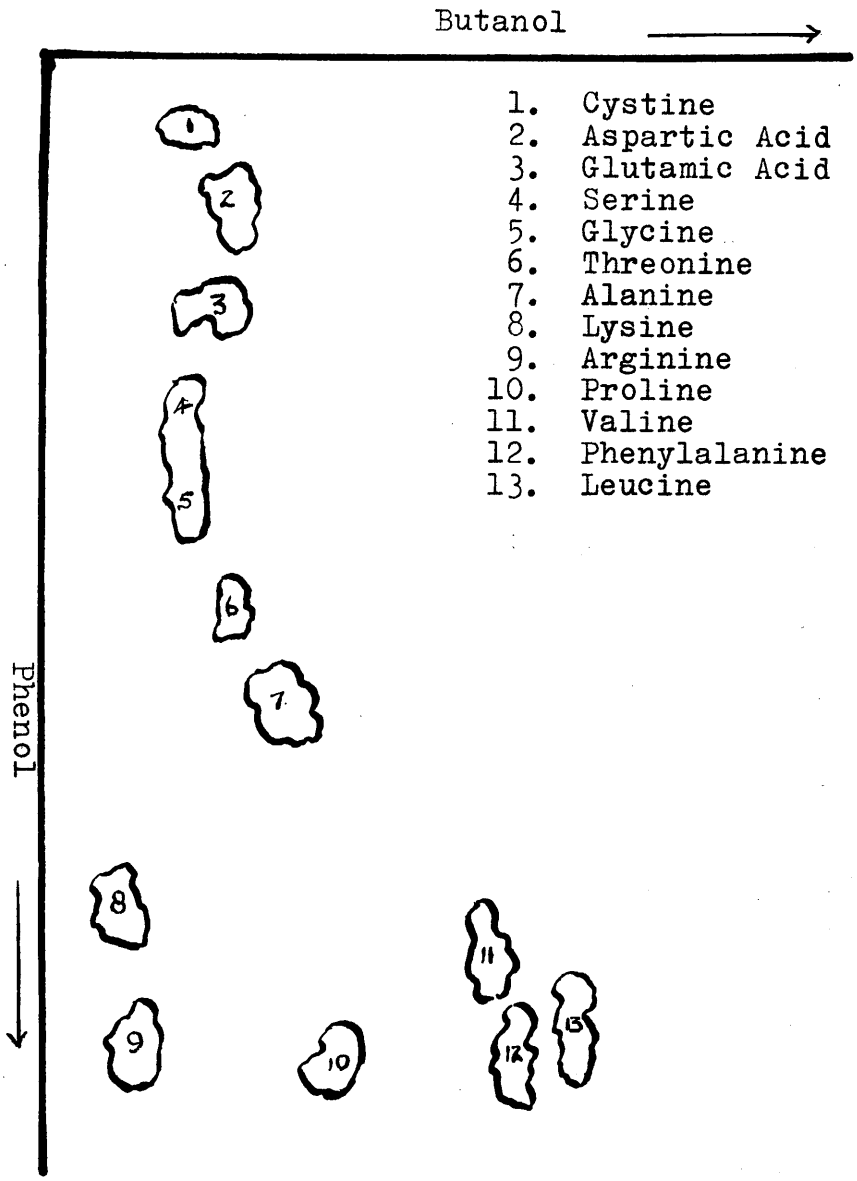


Fig. V. Chromatogram. Hydrolysate of Polypeptide from Urine.

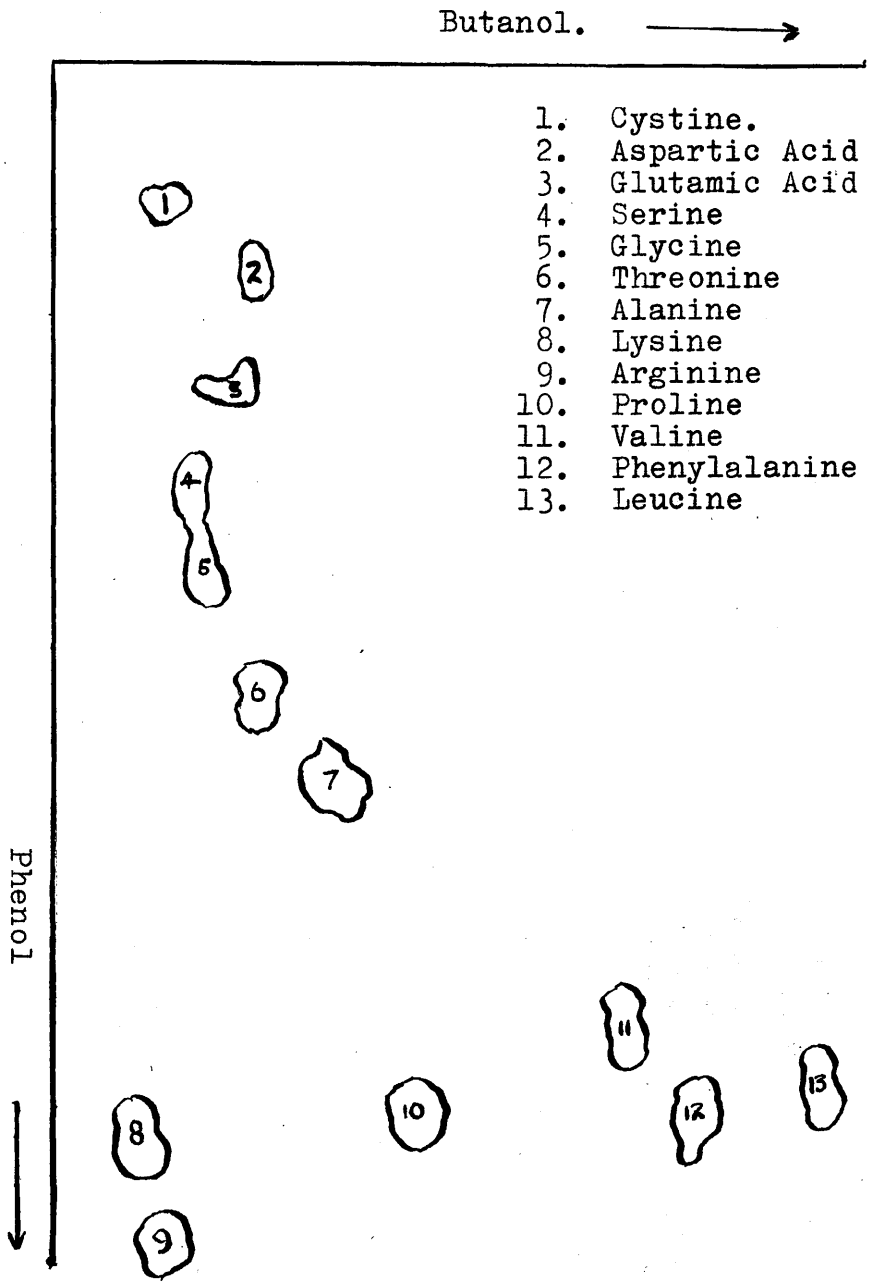


Fig. VI. Chromatogram. Hydrolysate of Polypeptide from Blood.

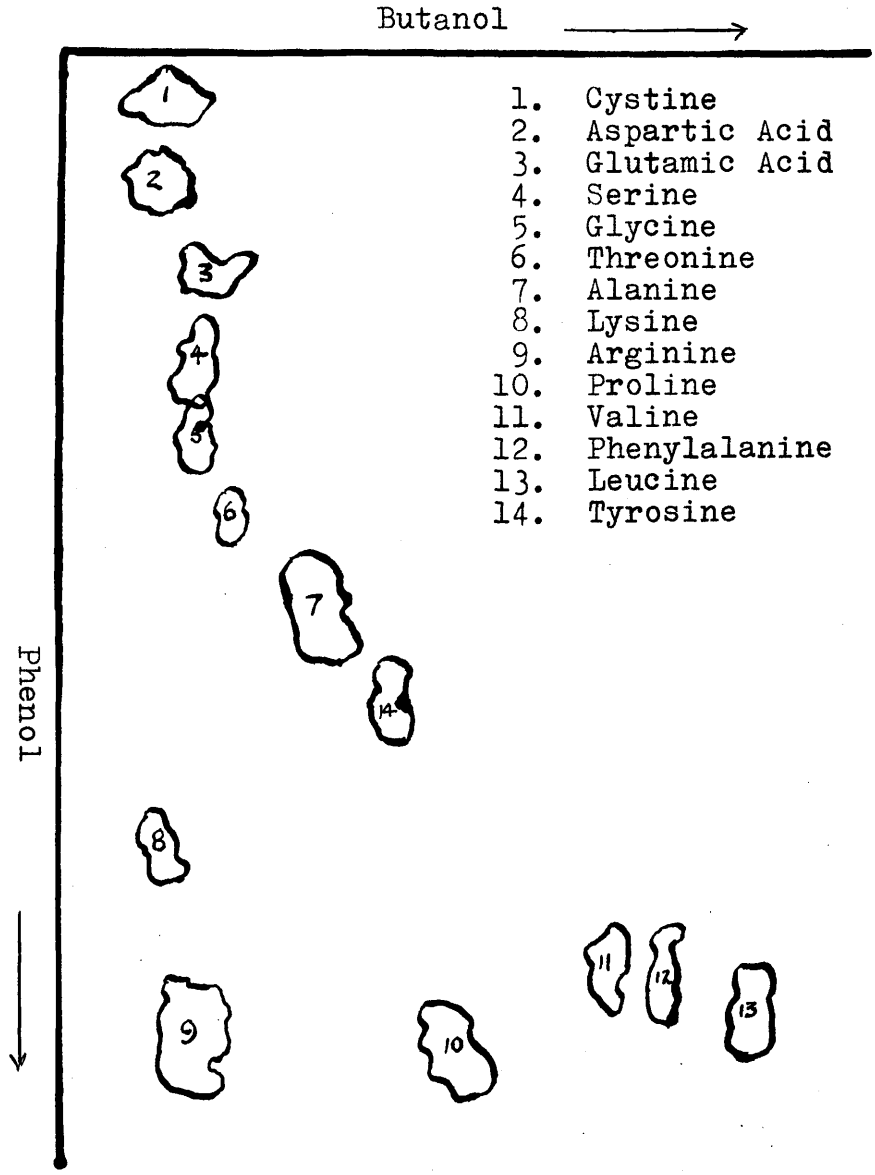


Fig. VII. Chromatogram. Hydrolysate of Polypeptide from urine to show the occasional presence of Tyrosine.

THE ANALYSIS OF COMMERCIAL PITUITARY EXTRACTS.

This investigation, in so far as it had proceeded, had only demonstrated the presence of a polypeptide in the blood and urine of certain patients. This polypeptide had been hydrolysed and its amino-acid content determined, but this analysis of amino-acid content agreed neither with that reported by Arneil & Wilson (1953), nor that reported by Valeri, Zacco and Perrini (1953). It therefore became necessary to repeat some of the work reported by the above authors, and at the same time determine the source of the present material and its biological activity if any. As the presumption was that the material was of pituitary origin, it was decided as a first step in the investigation of the source to submit commercial pituitary extracts to analysis by two dimensional chromatography.

EXTRACTION AND AMINO-ACID ANALYSIS OF THE POLYPEPTIDE FROM COMMERCIAL PITUITARY EXTRACTS.

A total of nine commercial preparations of posterior pituitary extract, either the extract of the entire posterior pituitary gland, or these preparations claimed to be the vasopressic fraction and including the three preparations Refisal, Piton and Postipon analysed by Valeri, Zacco and Perrini were submitted to two-directional paper chromatography by the technique already described. A tenth posterior pituitary extract was also analysed. This was the extract prepared from the standard pituitary (posterior lobe) powder by the method described in the British Pharmacopoeia (1953).

When the chromatograms were developed, a constant finding was the presence of a Ninhydrin staining material in the typical position of the polypeptide. Figure VIII illustrates one of these chromatograms.

This Ninhydrin staining substance was now obtained in greater quantity by applying the posterior pituitary extract as a line along the length of a sheet of filter paper, running in phenol-ammonia, drying the paper, and then turning the paper through 180° and running again in butanol-acetic acid. After drying the paper, the terminal inch of the phenol-ammonia run was cut out as previously described and eluted with distilled water. The eluate was concentrated by evaporation in vacuo, a little applied as a spot and run chromatographically to determine the presence and purity of the polypeptide, while the remainder was hydrolysed with 6 N hydrochloric acid A.R. and heat. The resulting hydrolysate was applied to paper, run as a two dimensional chromatogram and developed with Ninhydrin.

The chromatograms developed from the hydrolysates of polypeptides obtained from all ten posterior pituitary extracts

were almost exactly identical. Figures IX and X are representative of this group. When the amino-acids were identified by the methods already described, the polypeptides from the various commercial sources proved to contain the same amino-acids, and these amino-acids were also identical with the amino-acids derived from the polypeptides from blood and urine of patients with acute toxaemia of pregnancy.

Various commercial hormonal preparations, amongst others adreno-corticotrophic hormone, and several preparations purporting to be the gonadotrophic hormones of the anterior pituitary gland, were submitted to two dimensional chromatography, as were samples of a renin containing kidney extract and a sample of purified angiotonin. None of these substances contained a Ninhydrin staining substance in the characteristic position and were

therefore considered not to contain the polypeptide.

The foregoing was considered to be suggestive of a posterior pituitary source for the polypeptide but did this material represent the active portion of these extracts or not?

The answer to this question was obtained by the procedure now reported. The extract prepared from standard pituitary (posterior lobe) powder, a commercial pituitary extract of British origin, and one of the three posterior pituitary extracts employed by Valeri, Zacco and Perrini, were selected at random. These were each in turn submitted to the following examination.

The preparation under test was applied as a line along a sheet of filter paper and run in phenol-ammonia. The paper was removed and dried. A thin strip was cut from one edge and developed with Ninhydrin. This proved

the presence of Ninhydrin staining material, presumably including the polypeptide, at the solvent front.

The distance from the point of application to the solvent front was measured and the paper then divided into eight equal strips parallel to the solvent front. These strips were numbered one to eight, number one lying at the point of application and progressing to number eight which included the solvent front.

These strips were separately eluted with distilled water, and the eluates then concentrated to 1 ml. by evaporation in vacuo. A small portion of each of these eluates was run as a two-dimensional chromatogram to confirm the presence or absence of the polypeptide, and the remainder examined for biological activity.

Strip number eight - that nearest the solvent front - in each of the three

preparations tested proved to contain the polypeptide and showed anti-diuretic activity on bioassay. Strip number seven also proved to contain the polypeptide, but in lesser quantity as judged by the Ninhydrin staining reaction. The presence of the polypeptide was confirmed by anti-diuretic assay. (See Section F.) Strips number one to six in no case could be shown to contain the polypeptide and antidiuretic activity could not be demonstrated. Table II summarises these results.

This procedure was repeated on the three selected pituitary extracts, but this time the solvent employed was butanol-acetic acid. As has been shown the polypeptide does not flow in this solvent and the edge strip when stained with Ninhydrin showed the presumptive presence of the polypeptide at the point of origin.

Eight equal strips were again

Strip Number.	Standard Post.Pit.Powder.		Post.Pit.Extract of British Origin.		Post.Pit.Extract of Italian Origin.	
	Poly-peptide.	Anti-diuretic Activity.	Poly-peptide.	Anti-diuretic Activity.	Poly-peptide.	Anti-diuretic Activity.
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	+	+	+	+	+	+
8	++	++	++	++	++	++

ACTIVITY OF ELUATES FROM ONE DIRECTIONAL CHROMATOGRAM RUN IN PHENOL-AMMONIA.

TABLE II.

measured, separated, eluted, and the eluates concentrated. When these eluates were assayed and run as two-dimensional chromatograms, the results showed the presence of the polypeptide and anti-diuretic activity in strip one only. That is in the strip nearest to and including the point of application. In no other strip could the polypeptide be demonstrated, nor could antidiuretic activity be demonstrated. Table III summarises these findings.

These results, obtained with posterior pituitary extract of varied origin have shown that the polypeptide is present in all the extracts, and that the position occupied on two-dimensional chromatography is consistent. The amino-acid content is identical with similarly running polypeptides obtained from the blood and the urine of patients with acute toxaemia of pregnancy.

That this polypeptide "runs" with,

Strip Number.	Standard Post.Pit.Powder.		Post.Pit.Extract of British Origin.		Post.Pit.Extract of Italian Origin.	
	Poly-peptide.	Anti-diuretic Activity.	Poly-peptide.	Anti-diuretic Activity.	Poly-peptide.	Anti-diuretic Activity.
1	+	+	+	+	+	+
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-

ACTIVITY OF ELUATES FROM SIMILAR CHROMATOGRAM RUN IN BUTANOL-ACETIC ACID.

TABLE III.

and at the same rate as, phenol-ammonia and is unmoved by butanol-acetic acid has been demonstrated. Lastly, it has been shown that activity of posterior pituitary extracts is either directly or indirectly associated with the presence of the polypeptide, and that where this substance is absent, so is any demonstrable anti-diuretic activity.

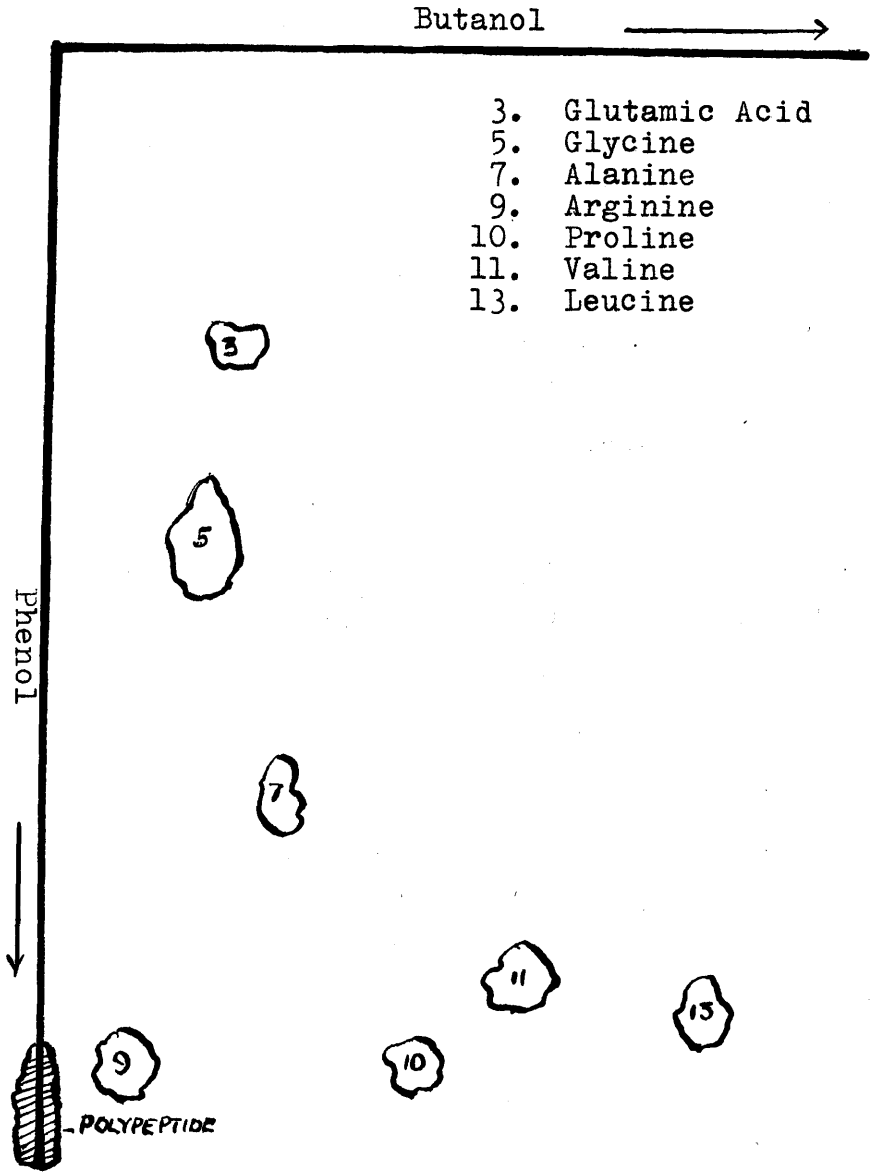


Fig. VIII. Chromatogram. Commercial Posterior Pituitary Extract to show presence of Polypeptide.

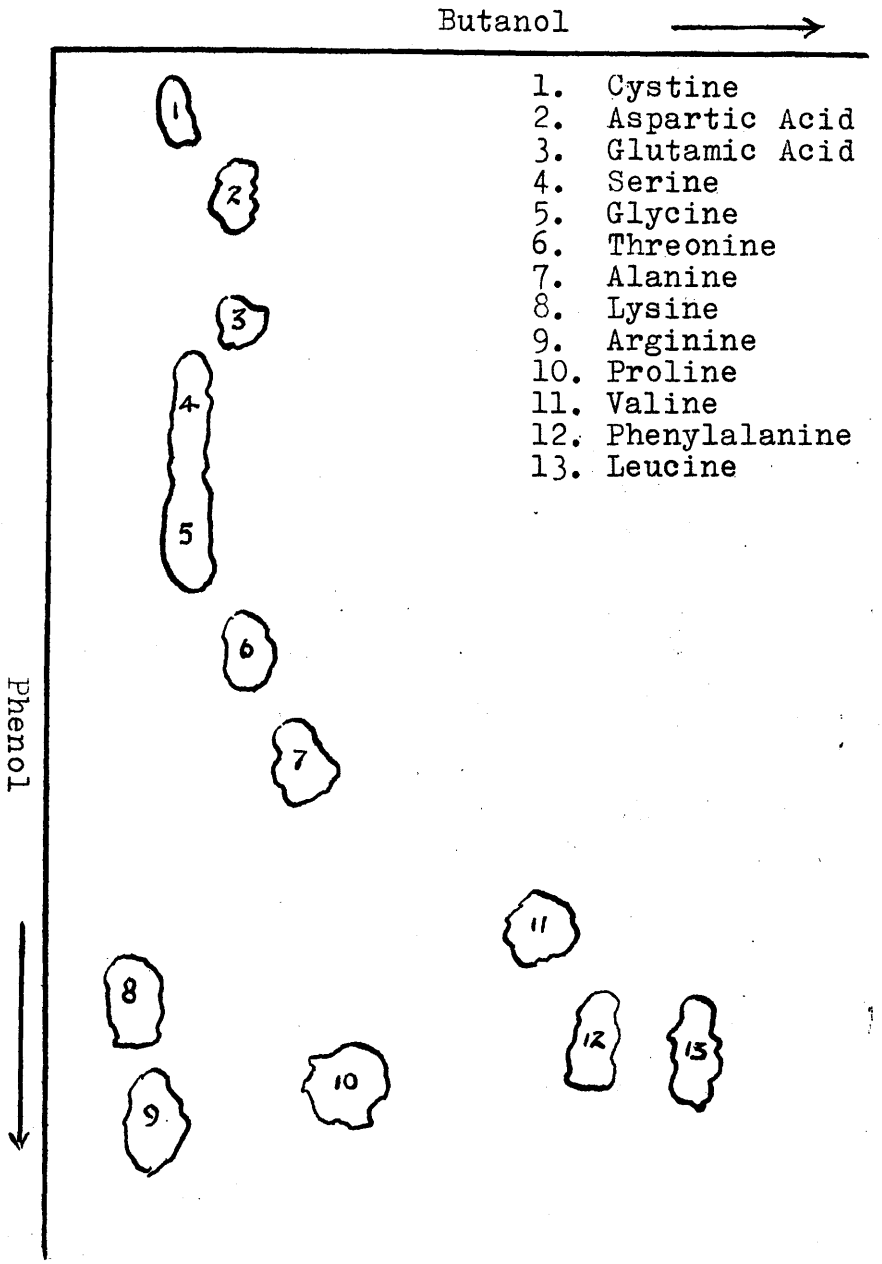


Fig. IX. Chromatogram - Hydrolysate of Polypeptide from Commercial Posterior Pituitary Extract.

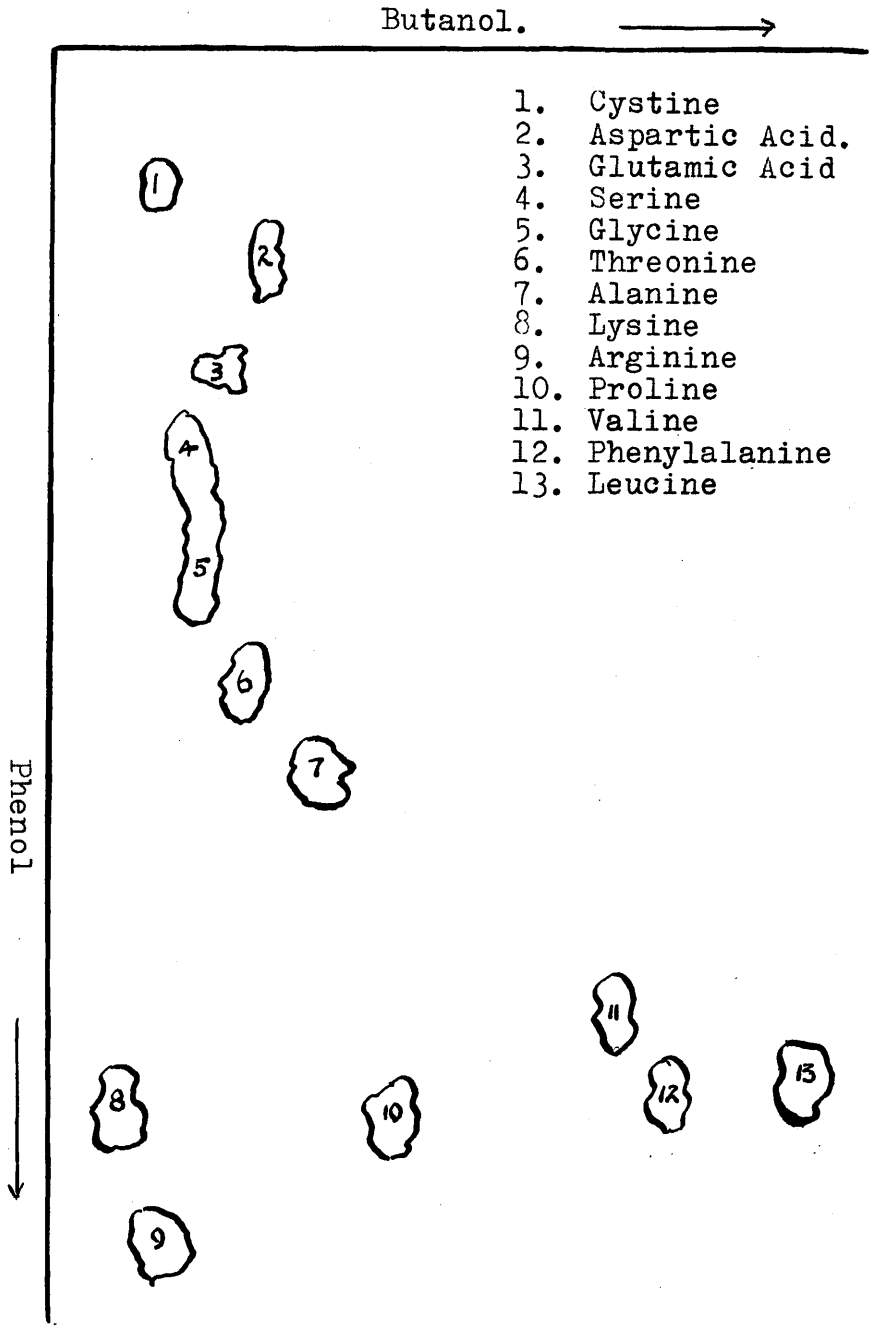


Fig. X. Chromatogram. Hydrolysate of Polypeptide from Commercial Posterior Pituitary Extract.

PROOF OF THE PRESENCE OF THE SAME POLYPEPTIDE IN THE HUMAN POSTERIOR PITUITARY GLAND AND ITS ABSENCE IN OTHER ORGANS INCLUDING THE PLACENTA.

PROOF OF THE PRESENCE OF THE SAME POLYPEPTIDE IN THE HUMAN POSTERIOR PITUITARY GLAND AND ITS ABSENCE IN OTHER ORGANS INCLUDING THE PLACENTA.

The pituitary gland was obtained within two hours of death from five patients who had died in the Glasgow Royal Maternity and Women's Hospital. The posterior lobe was dissected free from other tissue, and after sub-division was extracted in a glass tissue grinder - modified Griffith's tube - with double distilled water acidified to a pH of between 3 and 4 with glacial acetic acid. The temperature at which this was carried out was between 70-80° C. so that the proteins might be coagulated and any autolytic enzymes present destroyed (British Pharmacopoeia-1948). The extract was now filtered, sterilised by heat, and concentrated by evaporation in vacuo. Thereafter the extract was stored in a refrigerator until required for use.

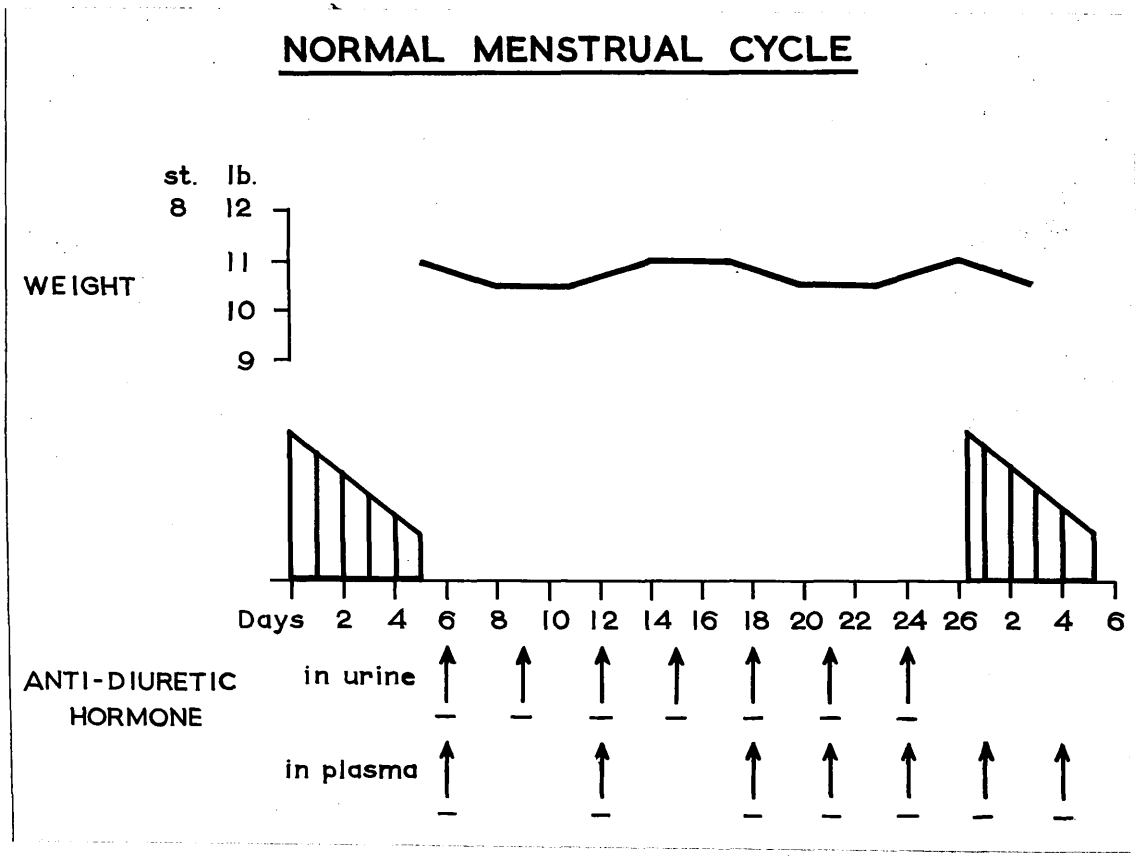


FIGURE I. NORMAL MENSTRUAL CYCLE.

NOTE ABSENCE OF ANTIDIURETIC HORMONE IN URINE AND PLASMA THROUGHOUT THE CYCLE.

days preceeding menstruation. The details of these two cases are shown in Figures II and III.

That the normal non-pregnant female does not exhibit the anti-diuretic hormone in 0.05 ml. of urine is shown by the above investigation. Equally significant is, that where pre-menstrual tension is present - a syndrome known to be due to water retention, even to the extent of localised or generalised oedema - Atkinson and Ivy (1936), Thorn et al.(1938) - resulting in weight gain in the pre-menstrual phase of the cycle - the anti-diuretic hormone of the posterior pituitary gland can be detected in the urine.

That the syndrome is indeed due to water retention is confirmed by the clinical cure obtained by diuretics.

Robinson and Farr (1940), Lloyd and Lobotsky (1949, 1950) have also shown this high anti-diuretic titre in

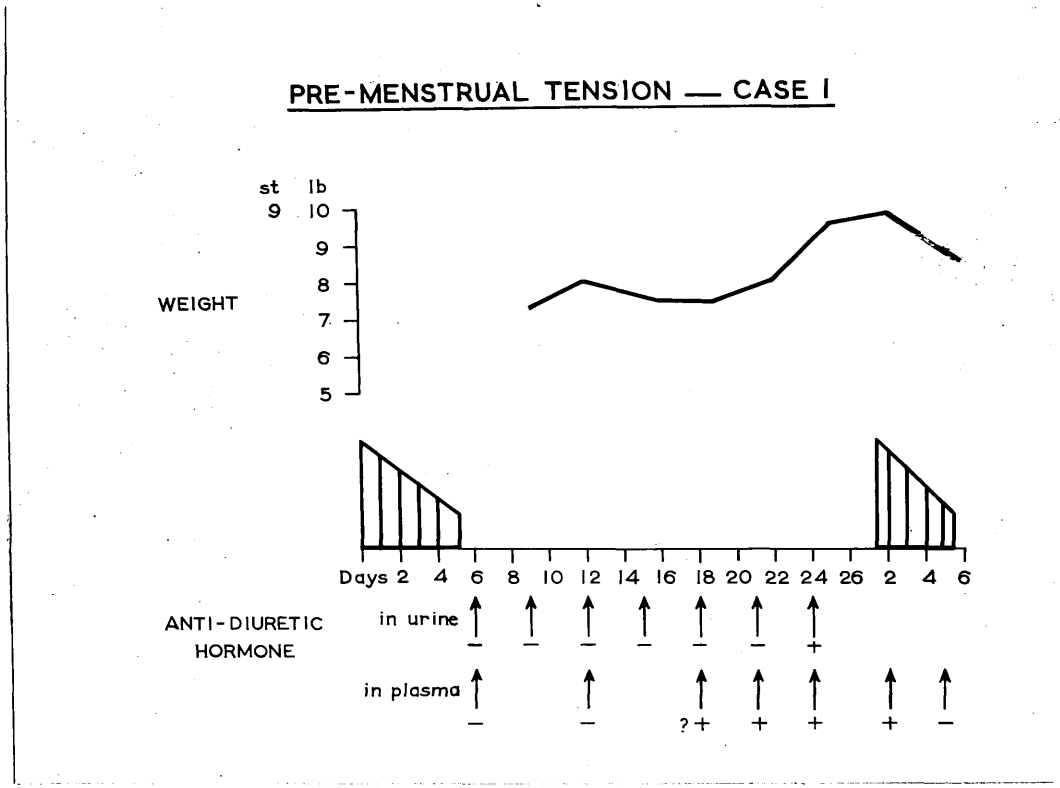


FIGURE II. - PREMENSTRUAL TENSION.

NOTE THAT APPEARANCE OF ANTIDIURETIC HORMONE IN PLASMA PRECEDES INCREASE IN WEIGHT AND APPEARANCE OF ANTIDIURETIC HORMONE IN URINE.

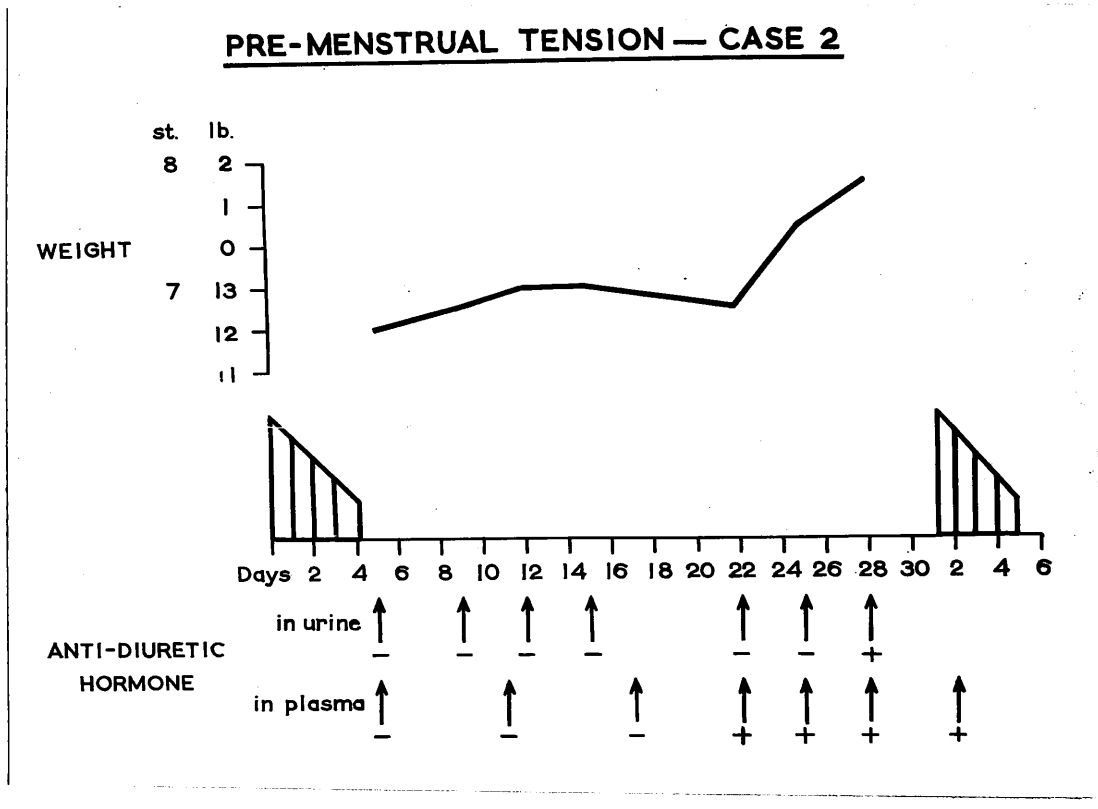


FIGURE III. PREMENSTRUAL TENSION.

APPEARANCE OF ANTIDIURETIC HORMONE AND WEIGHT GAIN COINCIDE. BUT BOTH PRECEDE APPEARANCE OF ANTIDIURETIC HORMONE IN URINE.

association with clinical oedema in the pre-menstrual phase of the cycle.

Males.

The first morning specimen of urine from eight healthy male subjects, all non-smokers, all normotensive and none exhibiting albuminuria, was examined on twenty-two occasions in all.

In none of these specimens (0.05 ml.) was it possible to demonstrate the anti-diuretic hormone.

Normal Pregnant Females.

The urine of 94 normal pregnant patients was examined. These patients had been admitted to hospital for some reason other than hypertensive toxaemia, essential hypertension or chronic nephritis in pregnancy. On clinical examination these patients proved to be normotensive, without clinical oedema, and albumen was absent from the urine.

The patients had been admitted to hospital for a variety of reasons, and

were representative of almost all durations of pregnancy. They fell into four groups, twenty patients were less than 14 weeks pregnant, twenty between 14 and 28 weeks pregnant, thirty between 28 and 40 weeks and 26 were, by expected date of confinement, post-mature (minimum of 8 days).

In all, the urine of these 94 patients was examined on some 140 occasions and only twice was the anti-diuretic hormone detected in 0.05 ml. of urine. The two cases where anti-diuretic hormone was detected were as follows.

Case 1.

This patient, a primigravida was admitted at term with a diagnosis of contracted pelvis and slight cephalo-pelvic disproportion. The blood pressure was 120/70 millimetres of mercury. There was no oedema and the urine was free of albumen. A positive

finding of anti-diuretic hormone was obtained with the first urinary specimen examined, but this finding was not repeated in a further specimen examined prior to labour, nor in two specimens examined in the immediate puerperium.

As this first specimen was one of several collected and processed that day, the possibility of error cannot be excluded. It was not possible to repeat the examination as some five days must elapse between the collection of the urine and the establishment of the presence of the posterior pituitary anti-diuretic hormone by the methods employed.

It did prove possible however to examine a further ante-natal specimen from this patient and the result was negative. In the six days of the patient's stay in hospital before the onset of labour, no evidence

of hypertensive toxaemia was detected, and no oedema was noted.

Case 2.

This patient, a para 4 + 0, was admitted at 32 weeks maturity of pregnancy because of an iron deficiency anaemia. The blood pressure was 110/70 millimetres of mercury and there was no oedema or albuminuria. The specimen of urine tested on the morning after admission showed the presence of anti-diuretic hormone in 0.05 millilitre.

Premature labour ensued two days after admission and, as the patient's haemoglobin level was 7.8 grams%, transfusion of blood was required.

A further specimen of urine was tested on the fourth day post-partum with negative results.

As Hawker (1952) had suggested that the onset of lactation and the titre of an anti-diuretic substance

No. of Cases in Group.	Duration of Pregnancy.	Anti-diuretic Hormone.	
		Negative	Positive.
20	Less than 14 weeks.	20	0
20	14-28 weeks.	20	0
30	28-40 weeks.	28	2*
26	40+ weeks.	26	0
7	3rd. day Post-Partum.	7	0
6	5th. day Post-Partum.	6	0
8	7th. day Post-Partum.	8	0

TABLE XIII.

* Details of these two patients are given in the text of the thesis.

Urinary findings in Non-Toxaemic Pregnancy.

were interconnected, the opportunity was taken to examine the urine in the puerperium. This was done at three different times, the third, fifth and seventh days post-partum. These days were selected as being prior to the establishment, of lactation, about the time when the milk flow is becoming established and lastly when lactation should be well established.

The specimens of urine had to be withdrawn by catheter to prevent contamination with lochial discharge. Seven specimens were obtained on the third day post-partum, six on the fifth day and eight on the seventh day. In none of these specimens was the anti-diuretic hormone detected.

Table XIII gives details of the results obtained in the normal pregnant patient.

Acute Toxaemias of Pregnancy.

The acute toxaemias of pregnancy have already been defined in the introduction to the thesis.

The criteria necessary to establish the diagnosis were:-

(1) That the patient be 28 or more weeks pregnant.

(2) That the blood pressure be 140/80 millimetres of mercury or greater.

(3) That albuminuria, oedema, or both be present.

Where convulsions were not present the results are given under the heading of pre-eclamptic toxaemia and under eclampsia where convulsions occurred.

Pre-eclamptic toxaemia.

The urine of 86 patients who satisfied these criteria was examined. Where protein was present this was first removed by coagulation with heat, filtration, and the urine volume

restored to the original by the addition of distilled water.

In none of the cases examined was the urinary output less than thirty-four ounces in any 24 hours.

The anti-diuretic hormone of the posterior pituitary gland was present in 79 of the 86 urines examined. Table XIV gives details of five of these cases selected at random.

Of the seven cases where anti-diuretic hormone could not be detected in the urine it is noteworthy that oedema was absent in two, very slight in four, and moderate in one. Details of these seven cases with negative findings are given in Table XV.

For comparison in the 79 cases where anti-diuretic hormone was present in the urine, oedema was marked in 14, moderate in 63, and slight in two. In no case where the anti-diuretic hormone was present in the urine was oedema

Maturity in weeks.	Blood Pressure Mms. of Hg.	Oedema.	Albumin-uria G/Litre.	Anti-diuretic Hormone. Poly-peptide.	Amino Acid Analysis.	Bio-Assay.
32	150/100	++	3	Present	Characteristic	Positive.
33	210/135	+++	3	Present	"	Positive.
38	150/100	+	1½	Present	"	Positive.
40	145/90	++	0	Present	"	Positive.
40+	160/95	++	2	Present	"	Positive.

TABLE XIV.

Clinical details and Urinary findings in five Pre-eclamptic Patients selected at random.

Maturity weeks.	Blood Pressure. Mm. of Hg.	Oedema.	Albumin-uria. G/Litre.	Anti-diuretic Hormone.
40	170/100	±	¼	Not detected.
36	150/105	±	1	" "
39	145/85	+	0	" "
40+	150/90	+	1	" "
40	155/85	0	½	" "
37	210/120	0	2	" "
40+	150/90	±	0	" "

TABLE XV.

Clinical details of the seven Pre-eclamptic Patients in whose urine no Anti-diuretic Hormone was found.

Each of these human posterior pituitary extracts was examined for its anti-diuretic activity by bioassay, and then run as a two dimensional chromatogram. In each case the extract was proved to possess anti-diuretic activity and to contain a Ninhydrin staining substance in the characteristic position of the polypeptide on the developed chromatogram.

The polypeptide was then extracted for further examination by the method already described. Each of these "polypeptide extracts" was tested for the presence of the polypeptide and this was confirmed in all cases. Each of these polypeptide extracts should ideally have been tested for biological activity but due to the scarcity of the material this proved possible in two cases only. The two extracts tested proved to be anti-diuretic in action.

Each of the polypeptide extracts was hydrolysed and two dimensional

chromatograms prepared. The amino-acid constitution of the polypeptide proved to be identical with that previously demonstrated in the case of the polypeptide derived from blood and urine of toxaemic pregnant women and that derived from the various commercial posterior pituitary extracts.

Figure XI is of a chromatogram showing the presence of the polypeptide in an extract from the human posterior pituitary lobe. Figure XII is a chromatogram of the amino-acid constitution, while Table IV summarises the findings in the human posterior pituitary gland.

As a control portions of liver, kidney and ovary were extracted by the same method. In none of these could the polypeptide be demonstrated.

In view of the claims of Ham and Landis (1942) that an anti-diuretic substance could be extracted from the

Case Number.	Original Extract.		Polypeptide Extract.		
	Presence of Polypeptide.	Anti-diuretic Activity.	Presence of Polypeptide.	Anti-diuretic Activity. % reduction in urine flow.	Characteristic Amino-Acid Analysis.
Female.					
1	+	+	+	34%	+
2	+	+	+	Not tested	+
3	+	+	+	23%	+
4	+	+	+	Not tested	+
5	+	+	+	Not tested	+
Male.					
1	+	+	+	16%	+
2	+	+	+	Not tested	+
3	+	+	+	Not tested	+

THE RESULTS OF EXTRACTION OF THE HUMAN POSTERIOR PITUITARY GLAND.

TABLE IV.

placenta, portions of this organ both from cases of normal pregnancy and of hypertensive toxaemia of pregnancy, were examined.

These placentae, three in number from each group, were obtained within two hours of delivery and immediately processed. This was done in order to meet the objection of Byrom (1951) that the anti-diuretic activity of placental extracts was largely, if not wholly, accounted for by bacterial contamination.

As blood in cases of acute toxaemia of pregnancy has been shown to contain the polypeptide, the portions of placenta selected were washed and perfused with distilled water to render them as free from blood as possible.

Small portions of these relatively blood free placentae, approximately five grams in weight, were now extracted as has been described for

the human posterior pituitary gland, and the extracts after filtration, sterilisation and concentration immediately applied to paper and run as two directional chromatograms. In none of the six placental extracts was it possible to demonstrate a Ninhydrin staining substance in the characteristic position of the polypeptide. This was taken as proof that the placenta did not store the polypeptide, and in all probability was not concerned in its elaboration either in normal or toxaemic pregnancy.

The possibility that this material might be exclusive to the female was next considered. To resolve this point the posterior pituitary glands obtained from three fresh male post-mortems were examined. These pituitary glands were extracted by the method already described. The extracts proved to be anti-diuretic. On two dimensional paper chromatography a

characteristically situated polypeptide was noted. The polypeptide was now extracted, only one of the three extracts was assayed for anti-diuretic potency and this proved positive. All three extracts were hydrolysed and the amino-acid content determined. This proved to be in all respects similar to that of the polypeptide obtained from posterior pituitary glands of female origin. It was also identical to that already determined for the polypeptide from the various other sources.

In view of the above findings it was held that the elaboration or storage of this active polypeptide in the human pituitary gland - whether of male or female origin - had been definitely established.

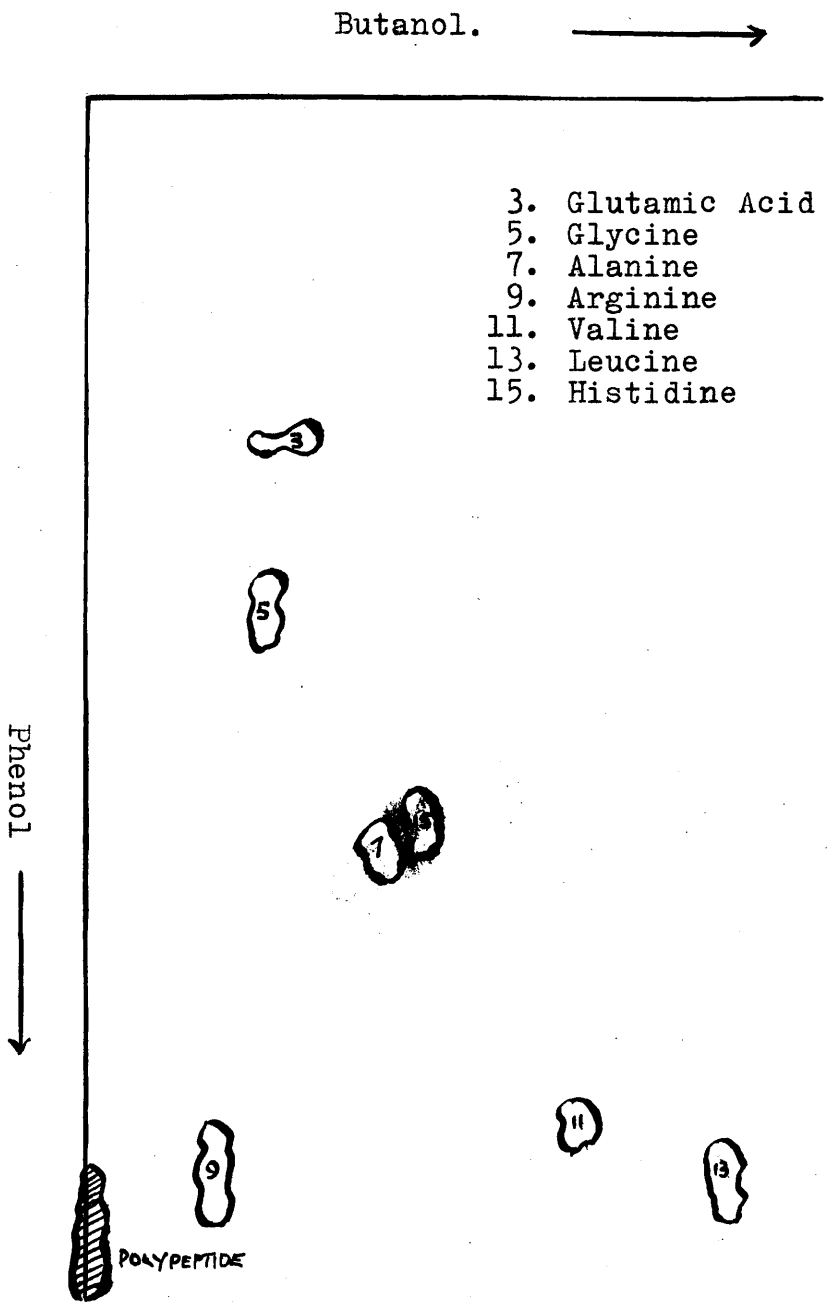


Fig.XI. Chromatogram. Extract of Posterior Pituitary Gland. (Human). To show presence of the Polypeptide.

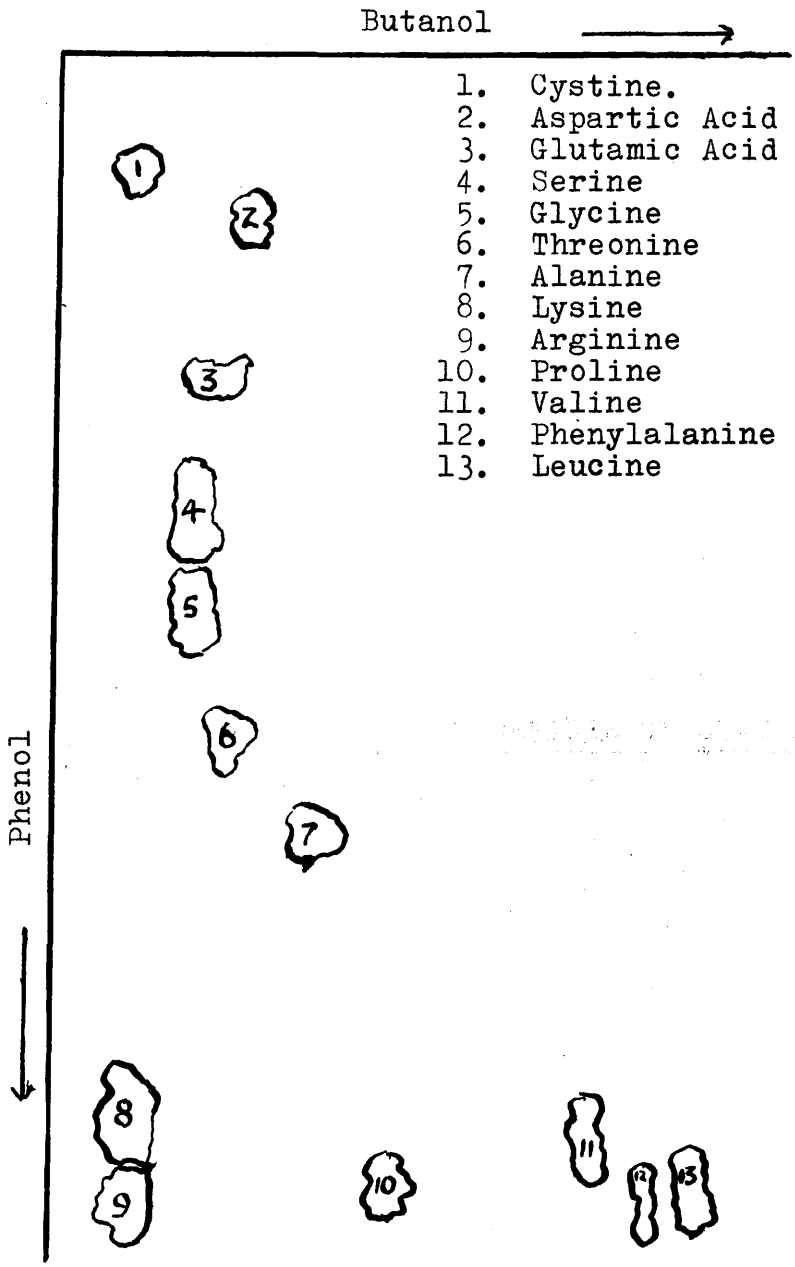


Fig. XII. Chromatogram. Hydrolysate of Polypeptide derived from Human Posterior Pituitary Gland.

POSTERIOR PITUITARY DEFICIENCY STATES.

POSTERIOR PITUITARY DEFICIENCY STATES.

The distribution of the polypeptide in blood and urine is described in Section II of this thesis; suffice it to summarise here that the substance can not normally be detected in the blood and is normally absent from urine in detectable quantity. As further proof of the pituitary origin it was considered worth while to investigate cases where non function of the posterior pituitary gland was known to be present.

The principle example of this type of disease is diabetes insipidus of central origin, where due to lack of excretion of the anti-diuretic hormone of the posterior pituitary gland, excretion of urine is markedly increased. The condition can be relieved by the administration of posterior pituitary extract.

Samples of blood and urine were kindly supplied from two proven cases

of this condition both before and after treatment.

In neither blood nor urine of the untreated cases was it possible to demonstrate the presence of the polypeptide, while after treatment with posterior pituitary extract (Pitressin tannate in oil injected intramuscularly), resulting in clinical cure of the polyuria, it was possible to demonstrate the polypeptide in both the blood and urine.

A case of pan-hypopituitarism following massive post-partum haemorrhage was also investigated. This patient showed the typical clinical condition of Simmond's disease and in association developed diabetes insipidus. The clinical and bio-chemical findings in this case have been reported by McGillivray and Adams (1954).

In this case the repeated examination of extracts of blood and urine failed to

reveal the presence of the polypeptide, but once the polyuria of diabetes insipidus was brought under control with injections of pitressin tannate in oil, it became possible to demonstrate the polypeptide in both the blood and urine by the methods already detailed.

The absence of the polypeptide from both the blood and urine in known cases of posterior pituitary non-function, while the clinical manifestations of the conditions persist, and the anti-diuresis produced in association with the presence of the substance in the blood, suggests that the polypeptide is anti-diuretic in the human, and of posterior pituitary origin.

THE EFFECT OF SMOKING AND INJECTION OF VOLUNTEERS.

That nicotine is anti-diuretic in action has been recognised for a considerable time. That this anti-diuretic action is mediated through the anti-diuretic hormone of the posterior pituitary gland was established by Chalmers and Lewis (1951).

These facts suggested the following experiment. Six volunteers, all non-smokers, co-operated in the test. As a first step the bladder was emptied, this urine was collected individually and labelled Specimen I. They then drank a litre of water. The ingestion of this water resulted in a well marked diuresis and when this was established, some thirty minutes from the consumption of the water, the bladder was again emptied. All urine passed since Specimen I was pooled and labelled Specimen II. Each of these volunteers now smoked a cigarette, inhaling as much as was possible. This

led to one person being actively sick and taking no further part in the test. It was noticeable that a well marked anti-diuretic response was produced. Approximately one hour after smoking the cigarette the bladder was again emptied. This urine constituted Specimen III and concluded the experiment.

The volume of each individual urine was measured and 0.05 ml. run as a two-dimensional chromatogram. In case the polypeptide was found to be present, a tungstic acid extract of the urine was prepared. This was run in phenol-ammonia and butanol-acetic acid and a sufficient quantity of the polypeptide obtained for hydrolysis and the preparation of amino-acid analysis chromatograms. By a similar technique two urines in each group were extracted and the polypeptide extract assayed for anti-diuretic activity.

The results of these investigations

are summarised in Table V.

From this Table it becomes obvious that in Specimen I the polypeptide was not found in any of the urines, and that in neither of the two urines tested was any anti-diuretic activity present.

The findings, following the ingestion of water, Specimen II, are similar and the diuresis is shown by the volume of urine excreted.

Specimen III, that obtained after the inhalation of a cigarette and therefore in all probability due to the action of nicotine, shows the presence of the polypeptide in all the specimens examined, and that this is the same polypeptide is demonstrated by the identical amino-acid analysis obtained. In two cases the polypeptide was submitted to anti-diuretic assay and in both cases anti-diuretic activity was demonstrated. That anti-diuresis had occurred is abundantly evident when the volumes of

urine excreted as Specimen III are studied.

Table V summarises the results obtained and the anti-diuretic assays are reported in the section dealing with bioassay.

The same six volunteers co-operated in a further experiment.

In this experiment the urine was tested for the presence of the polypeptide at the commencement and in no urine was the polypeptide detected. Five units of "Pitressin" were then given by intramuscular injection and the next urinary specimen voided examined for the presence of the polypeptide. This was found to be present in each case and in two cases where the amino-acid constitution was investigated, the characteristic amino-acids were found.

Anti-diuretic assay was not carried out on any of these urines.

Volunteer Number.	Specimen I. Volume of Urine.	Presence of Polypeptide.	Typical Amino Acid Analysis.	Anti-diuretic Activity.	Ingestion of 1 Litre of Water.	Specimen II. (approx. ½ hr. after Spec.I.) Volume of Urine. *	Presence of Polypeptide.	Typical Amino Acid Analysis.	Anti-diuretic Activity.	Inhalation of a cigarette.	Specimen III. (approx. 1 hr. after Spec. II.) Volume of Urine. *	Presence of Polypeptide.	Typical Amino Acid Analysis.	Anti-diuretic Activity.
1	196 ml.	-	-	NT		342 ml.	-	-	NT		308 ml.	+	+	NT
2	55 ml;	-	-	-		358 ml.	-	-	NT		242 ml.	+	+	+
3	88 ml.	-	-	NT		357 ml.	-	-	-		Actively sick. Discontinued.			
4	123 ml.	-	-	NT		286 ml.	-	-	NT		296 ml.	+	+	NT
5	306 ml.	-	-	-		327 ml.	-	-	-		195 ml.	+	+	+
6	160 ml.	-	-	NT		370 ml.	-	-	NT		318 ml.	+	+	NT

TABLE V. The presence of the Polypeptide was assessed in 0.05 ml. of urine.

* The healthy male should excrete a litre of ingested water within a period of 2 hours.

The two experiments here reported give further evidence of the posterior pituitary origin of the polypeptide.

In the first experiment the posterior pituitary gland was stimulated by nicotine. This resulted in the secretion of the anti-diuretic hormone with diminution in urine flow and the excretion of the polypeptide in the urine.

In the second experiment the same polypeptide appeared in the urine, presumably from an exogenous source i.e. following intramuscular injection of posterior pituitary extract.

BIOASSAY.

The methods of assay of anti-diuretic hormone are numerous and varied. The accuracy varies from method to method, and generally is directly proportional to the difficulty of the technique.

In the present investigation three methods have been used. In the earlier part of the work the original test described by Burns (1931), as modified by Gilman and Goodman (1937), was employed but after a short time was replaced by the modification of Birnie et al. (1949). This resulted in more consistent results being obtained but still required a considerable amount of the material under test. This was a disadvantage and led to the ultimate use of the method described by Eisen and Lewis (1954).

As a result of this progression of tests many of the assays of anti-diuretic potency have been repeatedly confirmed under different conditions.

Some indeed have been assayed by all three methods which will now be described briefly.

The Burns method of anti-diuretic assay depends upon the performance of standard groups of four male rats under standardised conditions.

The feeding of the animals was kept constant, and a period of 8 hours starvation preceeded each assay. Three hours before the commencement of the test a dose of water equal to 2½ per cent of body weight was given by stomach tube. At the time of the test a further dose of water - 5 per cent of body weight - was given and the material under test injected subcutaneously. The volume of the material under test was made up to 1 cc. per 100 grams of body weight prior to injection.

The animals, in groups of four, were placed in cages over a large funnel beneath which a measuring cylinder was

positioned. A wire mesh in the mouth of the funnel was so arranged as to hold back the faeces.

The end point of the test was taken as the time of excretion of 50 per cent of the total second water dose.

The animals were not used for active assay until such time as dummy assays resulted in consistent results, and in each group of assays a control group was inserted.

In the method devised by Birnie et al, male rats of approximately 200 grams body weight are used. These animals are given water but no food for the eighteen hours prior to the test. The animals are used in groups of three, a control group being included with each series of assays. The animals were placed individually in cages arranged for urine collection as already described. 3 ml. of distilled water per 100 sq. cm. of body surface were

given by stomach tube and this hydration repeated one hour later. The water dosage can be calculated from a formula (Dosage equals 6.27 x Weight $^{0.73}$ ml.) and for a 200 gram. animal is 9 millilitres.

The volumes of urine excreted in the two hours following the initial gavage are compared, and any animal showing a 50 per cent deviation from the mean is discarded. The animals are now arranged in groups of three, a third dose of water given, and the substance under test injected intra-peritoneally. An equal volume of distilled water was injected in the control animals.

The urinary output was then noted at 30 minute intervals for 90 minutes.

The result is expressed as a percentage water excreted and is obtained thus:-

$$\frac{\text{Urine excreted during 90 mins. of test}}{\text{Water load (3 doses) - Urinary output in initial 2 hours}} \times 100$$

Animals were used for this test on a maximum of three occasions only and at intervals of not less than 7 days.

The third and final method of assay employed was that described by Eisen and Lewis (1954). This method is more sensitive than the other two, more specific for posterior pituitary anti-diuretic hormone, requires smaller quantities of the test material, and is more directly adaptable to quantitative estimation. This, then, was the method employed latterly and was used for all quantitative blood estimations.

In the method, male rats of approximately 200 grams body weight, are given by stomach tube, 5 ml. of 10 per cent ethyl alcohol in water per 100 grams, and 30 minutes later a further 3 ml. of water per 100 grams are given.. A catheter is tied into the bladder and a vein cannulated with an indwelling needle. The needle is kept patent and

hydration maintained by injecting 0.5 ml. of a glucose electrolyte solution every 5 minutes.

The urinary output per 5 minutes is measured, and after some little time becomes consistent within the range of 100-200 micro-litres per minute. The sensitivity of the animal is tested by the intravenous injection of 10 micro-units of 'Pitressin", and if satisfactory anti-diuresis resulted, the animal was considered suitable.

The anti-diuretic response was expressed as a percentage of the urine excreted in the 10 minutes following injection, as compared with the 10 minutes preceeding.

The intake of fluid to the animal could be kept constant by adjusting the amount of glucose-electrolyte solution given and quantitative assays carried out by direct comparison with known standard solutions of "Pitressin".

An "active" injection could be given every 20 to 25 minutes, generally over a period of about 3 hours, before the assays had to be abandoned.

In the results, to be reported here, no attempt was made to undertake a quantitative assessment of any anti-diuretic hormone present. The maximum amount of the material under test which was available was used. This was done so that a definite positive or negative result could be determined. The methods of assay as will be seen largely employed the less sensitive subcutaneous and intraperitoneal injection, and in only a few cases was the intravenous route employed. Quantitative determinations, where these have been carried out, will be reported in the appropriate clinical section.

RESULTS.

(1) The polypeptide extracted from urine.

(a) Cases of hypertensive toxaemia of pregnancy.

Twelve samples of the polypeptide derived from the urine of cases of hypertensive toxaemia of pregnancy were assayed for anti-diuretic activity. In each and every case, a well marked anti-diuretic effect was demonstrated. Table VI gives the details of these cases.

(b) The urines passed during the "smoking experiment" were assayed for anti-diuretic activity. In all, six assays were carried out, the urines being selected at random.

The two specimens tested at the commencement of the experiment were negative, as were two urines from different persons after the ingestion of 1 litre of water. Two urines tested during the anti-diuretic phase induced by nicotine both proved to have an anti-diuretic effect.

Table VII gives the details of these tests.

Patient No.	Method. Gilman & Goodman. Time of 50% excretion.		Birnie et al. % water excreted at 90 mins.		Eisen & Lewis. % reduction in urine flow.
	Polypeptide.	Control.	Polypeptide.	Control.	Polypeptide.
1318	165 min.	90 min.	-	-	-
1266	145 "	85 "	-	-	-
1424	120 "	90 "	51%	64%	-
842	235 "	80 "	-	-	-
1542	160 "	95 "	-	-	-
1540	205 "	75 "	28%	70%	-
1473	130 "	90 "	47%	63%	-
1520	125 "	80 "	-	-	-
1157	125 "	75 "	43%	68%	-
1839	190 "	75 "	-	-	-
2062	220 "	85 "	-	-	58%
1795	170 "	80 "	-	-	35%

Table VI.

Anti-diuretic Assay of Polypeptide derived from urine of Toxaemic Patients.

Time of Sample.	Spec. No.	Method of Assay. Gilman & Goodman. Time of 50% Excretion. Test.	Control.	Birnie et al. % Water excreted at 90 mins. Test.	Control.
Before Expt.	1	-	-	68%	67%
	2	75 mins.	85 mins.	-	-
After Water.	1	-	-	63%	69%
	2	80 mins.	75 mins.	-	-
After Smoking.	1	140 mins.	75 mins.	51%	64%
	2	-	-	46%	67%

Table VII.

Anti-diuretic Assay of urines passed during smoking experiment.

(11) The polypeptide extracted from the blood.

Seven samples of polypeptide extracted from the blood were assayed. The results are given in Table VIII.

It will be noted that anti-diuretic activity was present in all samples.

(111) The polypeptide extracted from commercial pituitary extracts.

The samples of the polypeptide obtained from commercial pituitary extracts, as detailed in a previous section, were submitted to assay. All proved anti-diuretic.

Anti-diuretic assay was also used to demonstrate that the activity of posterior pituitary extract was confined to that portion which contained the polypeptide. These results have been given in Tables II and III.

(IV) The polypeptide extracted from eight human posterior pituitary glands.

This was assayed at two stages.

No.	Method of Assay.		
	Birnie et al.		Eisen and Lewis.
	% Water excreted at 90 mins.		% Reduction in Urine flow.
	Polypeptide.	Control.	Polypeptide.
1	29%	63%	-
2	54%	70%	-
3	42% *	68%	32%
4	39% *	65%	47%
5	-	-	18%
6	-	-	63%
7	-	-	44%

Table VIII.

* Two animals were used instead of the usual three, due to lack of material under test.

Anti-diuretic Assay of Polypeptide derived from Blood.

The first, the crude extract which was assayed in all eight cases, the second, the polypeptide extracted therefrom. The polypeptide was assayed on three occasions, twice when derived from the female posterior pituitary gland and once when derived from the male gland.

The results of the crude extract assays are given in Table IV.

The three polypeptide extracts when tested by the method of Eisen and Lewis showed reductions in urine flow of 16 per cent, 23 per cent and 34 per cent.

Destruction of anti-diuretic activity by Sodium Thioglycollate.

It is known that anti-diuretic activity of posterior pituitary origin can be destroyed by incubation with 0.01 M. sodium thioglycollate - Van Dyke et al. (1942).

To be effective the sodium thioglycollate must be fresh and was,

in the present instance, prepared immediately before use from thioglycollic acid.

Two samples of the polypeptide derived from urine, and two samples from the blood, were treated with sodium thioglycollate, and it was found that anti-diuretic activity was abolished in each case.

Table IX gives details of these tests.

Source of Polypeptide.	Method of Assay - Eisen and Lewis. Treatment with Sodium Thioglycollate. Before.	After.
Urine.	58%	0
Urine.	35%	0
Blood.	63%	0
Blood.	44%	0

Table IX.

The figures refer to percentage reduction in urinary flow.

Anti-diuretic Assay of Polypeptide before and after treatment with Sodium Thioglycollate.

DISCUSSION.

DISCUSSION.

The proof that the polypeptide is of pituitary origin seems overwhelming. That this polypeptide can be extracted from the human posterior pituitary gland and from none of the other organs tested would seem to indicate an origin, or storage, in the posterior pituitary, and the fact that biological assay of the polypeptide shows the pharmacological activity expected from the posterior pituitary gland seems to confirm the belief that this polypeptide does in fact, contain the active principle of the gland.

Further proof is to be found in the cases of pituitary disease investigated. In the two untreated cases of diabetes insipidus, the polypeptide could not be detected in either the blood or urine, but after treatment with posterior pituitary extract, and when clinical improvement

was manifest, the polypeptide could be found in the morning urine specimens examined.

Similar findings were present in the case of panhypopituitarism following post-partum haemorrhage, which was reported by Adams and McGillivray (1954). A criticism of this proof could be the suggestion put forward by Gilman and Goodman (1937) that "the body handles the foreign pharmaceutical preparations differently than the natural anti-diuretic hormone", and this criticism would be equally valid against the demonstration of the polypeptide, after the injection of 5 units of "Pitressin", in the urine of volunteers whose urine had previously not contained this substance. However, it in no way invalidates that proof which depends on the appearance of this polypeptide in the urine of non-smokers, after the inhalation of a cigarette. It is

known that nicotine injected intravenously may cause inhibition of water diuresis in the dog, due to release of anti-diuretic substance from the neuro-hypophysis (Burn et al. 1945). A similar mechanism has been shown to operate in the human (Chalmers & Lewis, 1951).

When one turns to that portion of the work which deals with the commercial posterior pituitary extracts it is noteworthy that the polypeptide was present in all specimens examined. This would appear highly coincidental were the material to be purely a contaminant, and when it is noted that only that portion of the extract which contains the polypeptide, and no other portion, demonstrates anti-diuretic activity, it would seem that coincidence can be excluded.

In summary, it would appear that this polypeptide contains the active

anti-diuretic principle of the posterior pituitary gland. Table X summarises these findings.

Similar work to the present has been carried out by Arneil and Wilson, who have shown the presence of an anti-diuretic polypeptide in the urine of children suffering from acute haemorrhagic nephritis or nephrosis; and Valeri et al.who isolated an anti-diuretic polypeptide from the urine of patients with pre-menstrual tension, and who compared this polypeptide with two anti-diuretic fractions obtained from named commercial posterior pituitary extracts. There is a discrepancy, one with the other, between the amino-acid analysis of these workers, and the results of neither of these previous groups of workers agrees with the present findings. Table XI gives a comparison of the amino-acid analyses of the three series. Turning first

Source.		Presence of Polypeptide.	Characteristic Amino Acid Analysis.	Biological Activity.
Human Post. Pit. Gland.		+	+	+
Commercial Post. Pit. Extracts.		+	+	+
Acute Toxaemia of Pregnancy.	Blood.	+	+	+
	Urine.	+	+	+
Diabetes Insipidus (Untreated).	Blood.	-		
	Urine.	-		
Diabetes Insipidus (Treated).	Blood.	+	+	N.T.
	Urine.	+	+	N.T.
Volunteers.	Urine.	-		
" (after 10 Units Pit. Extract).	Urine.	+	+	+
Non-smokers.	Urine.	-		
" " (After Cigarette).	Urine.	+	+	+

TABLE X.

Amino Acid.	Present Communication.	Arneil & Wilson.	Valeri et al.	
			Fraction E.	Fraction F.
Arginine	+	+	+	
Alanine	+	+	+	+
Aspartic Acid	+	+	+	+
Cystine	+	+	+	+
Glycine	+	+	+	+
Tyrosine	? (+)		+	+
Proline	+		+	+
Phenylalanine	+		+	+
Leucine	+	+	+	+
Valine	+	+	+	+
Lysine	+	+		
Threonine	+	+	+	
Serine	+	+	+	+
Isoleucine		+		
Glutamic Acid	+	+	+	+
? Tryptophane			+	

TABLE XI.

to the difference between the amino-acid analysis of Arneil and Wilson and that of the present series, this difference is more apparent than real, as in more recent work Arneil and Wilson agree with the amino-acid composition as reported here (Arneil and Wilson - personal communication - 1954).

The difference in the amino-acid analysis as reported by Valeri et al. is more interesting. These authors isolated from the urine of patients with pre-menstrual tension and from three named commercial posterior pituitary extracts, two anti-diuretic fractions - E and F. Of these fraction F was the more active and contained fewer amino-acids than fraction E - see Table XII. In all but one particular, that lysine was absent and tryptophane possibly present, the amino-acid analysis of fraction E agreed with that of the present

Amino Acids.	Present Series.	Turner et al. Fract. 1	Turner et al. Fract. 6	du Vigneaud et al. Vasopressin Beef.	du Vigneaud et al. Vasopressin Hog.	du Vigneaud et al. Oxytocin.
Alanine	+	+				
Arginine	+	+	+	+		
Aspartic Acid	+	+	+	+	+	+
Cystine	+	+	+	+	+	+
Glutamic Acid	+	+	+	+	+	+
Glycine	+	+	+	+	+	+
Leucine	+	+				+
Lysine	+	+			+	
Phenylalanine	+	+	+	+	+	+
Proline	+	+	+	+	+	+
Serine	+	+				
Threonine	+	+				
Tyrosine	? (+)	+	+	+	+	+
Valine	+	+				
Isoleucine						+

TABLE XII.

polypeptide. These authors remark "that it is questionable however if one would expect to see tryptophane after acid hydrolysis". The positions occupied by lysine and tryptophane on chromatography are similar and with this in mind, supplies of the three posterior pituitary extracts used by Valeri et al.were obtained,and analysed as reported previously. Except that lysine was consistently present and tryptophane absent,the findings agree entirely with those of fraction E.

The present method of descending chromatography,as against ascending chromatography used by Valeri, Zacco and Perrini,does not isolate fractions E and F separately. It is worthy of comment however that fraction F which is claimed to possess greater anti-diuretic activity than fraction E is said to contain neither arginine nor lysine.

As the formula for pure vasopressin has recently been proved by du Vigneaud et al.(1953) to be an octopeptide, in which arginine and lysine are interchangeable as to species, arginine occurring in beef vasopressin and lysine in hog vasopressin, it is interesting to compare the present polypeptide with the pure substance. Table XII gives this comparison, and also the amino-acid structure of fractions 1 and 6 of Turner, Pierce and du Vigneaud (1951). Turner et al. used dried posterior pituitary gland as a starting material, and after initial extraction by the procedure of Kamm et al.(1928), obtained by counter current distribution six fractions, numbered one to six, which showed increasing anti-diuretic activity per unit weight. Fraction 1 (potency 200 units per mgm.) proved on analysis to have an identical amino-acid structure with the present polypeptide -

see Table XII. Fraction 6 (potency 400 units per mgm.) contained only eight amino-acids, all of which have been identified in the present substance. The amino-acids present in fraction 6 were present in molar ratio, and this analysis agrees in all respects with the formula for vasopressin determined by du Vigneaud et al.

From the foregoing, two possibilities arise. The first, as suggested by van Dyke (1950) and Croxatto, Rojas and Barnasi (1951), is that the posterior pituitary gland liberates a large protein molecule which by fragmentation gives rise to the various active principles of the gland. This theory would seem to find support in the very similar amino-acid structure of oxytocin and vasopressin. In view of the purification method of Turner et al; however, it would appear more likely

that the present substance is in fact a mixture of polypeptides which behave similarly on chromatography, and that the active anti-diuretic substance is in all probability the same as the eight amino-acid polypeptide - vasopressin.

It is felt, that on the evidence presented, the material isolated has been proved to be the anti-diuretic hormone of the posterior pituitary gland.

CLINICAL SECTION.

INTRODUCTION.

In the first part of this thesis, the presence of a polypeptide occupying a constant position in chromatograms has been demonstrated, and this has been proved to be the anti-diuretic hormone of the posterior pituitary gland.

Before proceeding to the study of the relationship between the presence of anti-diuretic hormone and the symptomatology of hypertensive toxaemia of pregnancy, it was necessary to investigate the blood and urine of the non-pregnant female. This investigation of the non-pregnant female entailed following a group through a normal menstrual cycle, and this introduced the complication of the pre-menstrual tension syndrome. This condition, of which the basis is water retention, is dealt with more fully later.

For completeness a small group of normal males were also investigated.

The results obtained in this non-pregnant group have served as a control, and allowed comparison with the results obtained in cases of acute toxaemia of pregnancy - both pre-eclamptic and eclamptic.

The findings obtained in several cases of pregnancy complicating chronic nephritis have also been reported.

In view of the clear cut results reported in the first section, it was obviously unnecessary to carry out a full identification of the polypeptide in each case. Nevertheless it was decided to undertake a complete biochemical and biological investigation in a number of instances in order to leave no possible doubt that the polypeptides isolated were identical in nature.

A difficulty arose in that while plasma could be stored in the frozen state, the urines required to be

examined almost immediately. This immediate examination was necessary as the majority of the specimens of urine were "clean", and not catheter specimens. Organismal contamination was therefore likely, and as has already been mentioned, has been held to result in anti-diuretic activity.

To meet any possible objection to the significance of the findings, the urines were run as two-directional chromatograms, and at the same time a tungstic acid extract of the urine was prepared and further extracted by chromatography. The eluate from this tungstic acid extract was then available for hydrolysis and amino-acid analysis, and for bio-assay.

Thus, in the section dealing with the urinary findings, the presence of the anti-diuretic hormones means that the polypeptide was present in each and every case, and that either the

amino-acid analysis was confirmatory or that anti-diuretic activity was proven on bio-assay, or that both these investigations had been carried out.

The same is true of the section dealing with the results in the plasma but due to the ability to store this material it was possible to repeat and confirm the findings in any given case.

URINARY FINDINGS.

Non-pregnant Females.

Specimens of fasting morning urine were obtained from twelve non-pregnant female volunteers. These girls were all healthy and of age from twenty-two to thirty-two years. They were all normotensive, the maximum blood pressure recorded being 132/80 millimetres of mercury, and in no case did the urine contain albumen. They were all menstruating normally and regularly. Non-smokers only were selected so that any possible fallacy

which might have been introduced by nicotine was excluded.

Of the morning urines so obtained, 0.05 ml. was run as a two directional chromatogram. In eleven of the urine specimens no anti-diuretic hormone was detected, but the substance was definitely present in the twelfth specimen.

This specimen had been obtained from a girl aged twenty-eight years and was passed on the day before menstruation commenced. On questioning this girl further, it was found that she suffered from the typical symptoms of pre-menstrual tension e.g. swelling of the breasts, a sensation of abdominal distension, headache and irritability in the pre-menstrual phase of the cycle.

In view of this finding of anti-diuretic hormone in the urine of pre-menstrual tension, which agreed with the work of Valeri, Zacco and Perrini (1953), it was decided to follow

several volunteers through a complete menstrual cycle.

For this purpose six volunteers were selected, four of whom were normal and two of whom admitted to pre-menstrual tension, one being the case mentioned above.

Morning specimens of urine were obtained every third day, commencing with a specimen on the day immediately following the cessation of menstruation, and continuing until the onset of the succeeding flow. It was not considered justifiable to ask these volunteers to submit to catheterisation during the menstrual flow.

Body weights were also recorded on the days on which urine specimens were collected. As far as possible these weighings were carried out under standard conditions.

The results of this investigation amply confirmed the results of Valeri,

Zacco and Perrini. In none of the urinary specimens from the group who did not suffer from pre-menstrual tension could the anti-diuretic hormone be identified on chromatography in 0.05 ml. of urine, and this was true irrespective of the phase of the menstrual cycle. It is also noteworthy that none of this group showed any significant weight gain in the pre-menstrual phase. Figure 1 is a diagrammatic representation of the results in this group.

On the other hand, the two patients in whom the symptoms of pre-menstrual tension developed, showed the presence of anti-diuretic hormone in the urine. In the one this substance first appeared between the sixth and the third day pre-menstrual, and in the other between the fifth and second day before menstruation. Both cases showed a weight increase in the

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absent.

Table XVI contrasts the clinical estimation of the oedema with the presence or absence of the anti-diuretic hormone.

Ten cases, where the anti-diuretic hormone was present on initial testing, have been followed with repeated examinations both before and after labour. In none of these cases did the anti-diuretic hormone disappear from the urine before labour, but in only one case was the substance still detectable on the third day post-partum, and in no case at any later time.

Figure IV represents the findings in these cases of acute (non-convulsive) toxaemia of pregnancy.

ECLAMPSIA.

The urinary findings in six cases of eclampsia have been investigated. In each patient a specimen of urine was obtained by catheter as soon after the

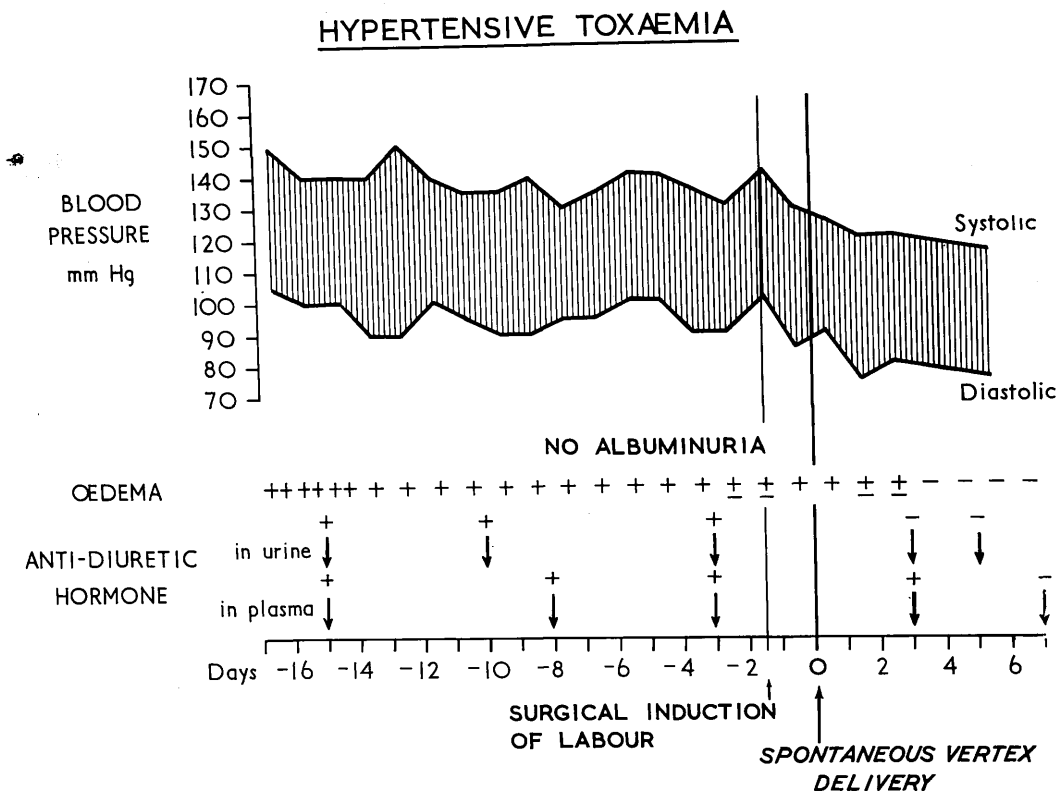

FIGURE IV. HYPERTENSIVE TOXAEMIA OF PREGNANCY.

TO ILLUSTRATE THE CORRELATION BETWEEN OEDEMA AND THE ANTIDIURETIC HORMONE IN THE URINE AND PLASMA. NOTE THAT WITH DISAPPEARANCE OF THE OEDEMA, THE URINE AND THEN THE PLASMA BECOME NEGATIVE.

Clinical Oedema.	No. of Cases Examined.	Anti-diuretic Hormone. Present	Absent.
Marked	14	14	0
Moderate	64	63	1
Slight	6	2	4
Absent	2	0	2

TABLE XVI.

Correlation of severity of Oedema with presence of Anti-diuretic Hormone in Pre-eclampsia.

first convulsion as was possible, and repeated examinations carried out at intervals while the convulsive phase lasted and for at least 72 hours thereafter. In all forty-two specimens of urine, from the six cases, were examined.

Unfortunately none of these patients were under observation prior to the onset of convulsions, and as none of the patients suffering from pre-eclampsia developed convulsions, we have no evidence regarding the relationship or changes in production of anti-diuretic hormone at the onset of convulsions.

The patients, here described, were all admitted to hospital following at least one convulsion, so that the first specimen of urine in all probability represents a mixture of that secreted before and following the convulsion.

All these cases were examples of

ante-partum eclampsia and were treated with rectal Bromethal and the induction of labour where necessary.

In each case the urine present in the bladder on admission was examined and specimens obtained at intervals thereafter, generally until two successive negative specimens had been obtained.

All the specimens obtained on admission contained anti-diuretic hormone, and the presence of this substance persisted for at least 48 hours after the onset of convulsions. In one case anti-diuretic hormone could still be detected some 160 hours after the first convulsion but was absent at 196 hours.

It was noteworthy that in those cases where clinical improvement was most rapid the anti-diuretic hormone disappeared from the urine most rapidly. Figure V illustrates the results obtained in a case of eclampsia where

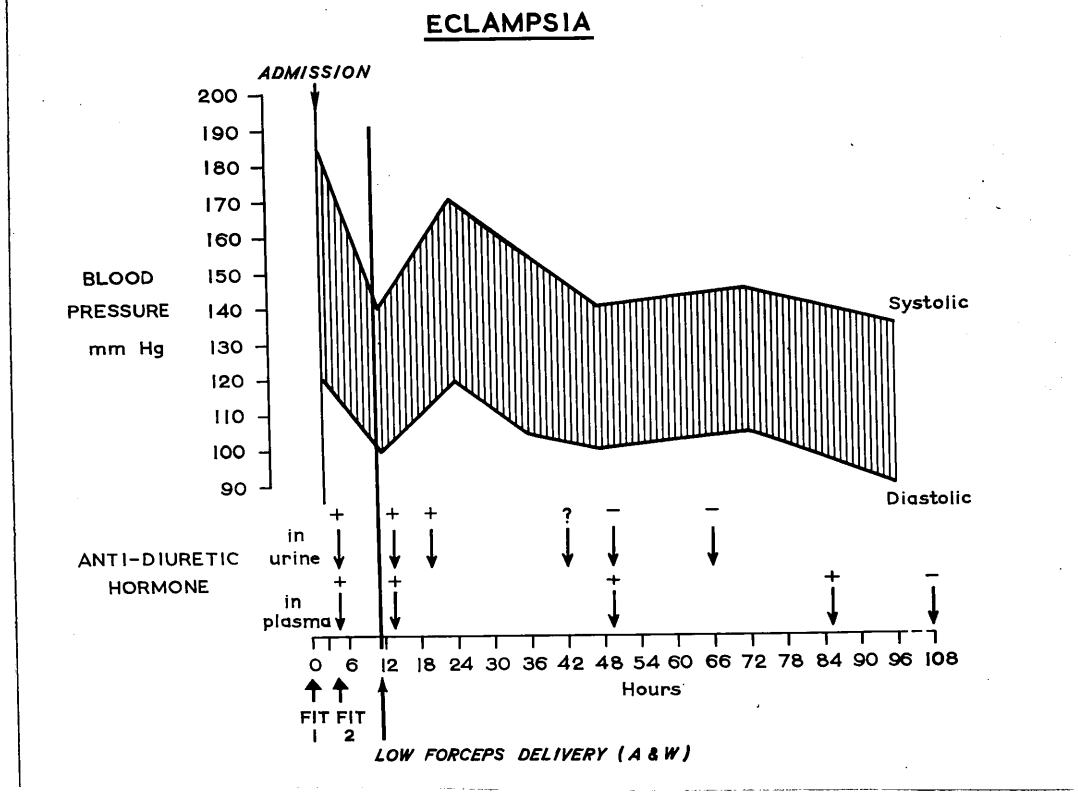

FIGURE V. ECLAMPSIA.

TO ILLUSTRATE THE RAPID DISAPPEARANCE OF THE ANTIDIURETIC HORMONE FROM FIRST THE URINE AND THEN THE PLASMA ASSOCIATED WITH CLINICAL IMPROVEMENT.

clinical improvement was rapid and the anti-diuretic hormone disappeared from the urine after 48 hours.

On the other hand, Figure VI. is of a much more severe case. In this case convulsions returned after a quiescent phase lasting 116 hours.

It is noteworthy that the anti-diuretic hormone persisted in the urine during the quiescent phase of apparent clinical improvement, and only disappeared from the urine some 60 hours after the final convulsion, when the patient was well on the way to recovery.

This disappearance of anti-diuretic hormone from the urine some 48 to 72 hours after the first convulsion has been a typical finding in five of the six cases here reported. This did not happen in the sixth case reported above, but anti-diuretic hormone was detected all through the

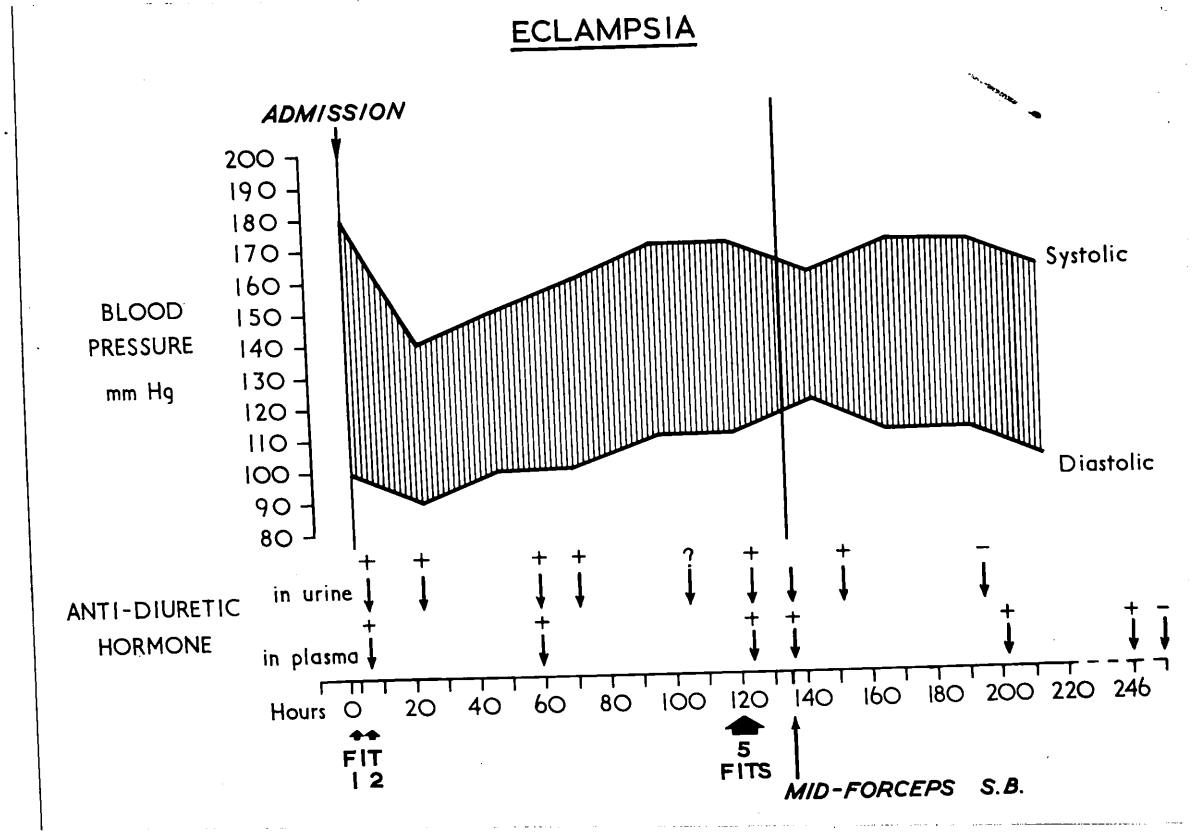

FIGURE VI. ECLAMPSIA.

TO ILLUSTRATE THE PERSISTENCE OF ANTIDIURETIC HORMONE IN THE PLASMA AND URINE WITH THE RECURRENCE OF FITS.

quiescent phase. It is felt that, had the significance of this finding been appreciated at the time, more active steps might have been taken in the treatment of the case, and the second series of eclamptic convulsions averted.

Table XVII gives the clinical details of these cases, and the time of disappearance of anti-diuretic hormone from the urine.

CHRONIC NEPHRITIS IN PREGNANCY.

What was apparently posterior pituitary anti-diuretic hormone was reported in passing by Dent (1948) under the title of "nephrotic-peptide", and this is apparently the same substance as Arneil and Wilson (1953) reported as an anti-diuretic polypeptide in the urine of children with acute nephritis and nephrosis. It therefore seemed worth while to investigate the urine of cases of chronic nephritis in pregnancy for the presence of the

Case Number.	Parity.	Maturity. (weeks).	Blood Pressure. (Maximum)	Albumen (Gm/Litre)	Oedema	Number of Convulsions.	Presence of Anti-diuretic Hormone.		Time of Disappearance of Anti-diuretic Hormone. (From first convulsion)	
							Plasma	Urine.	Plasma Between (Hours)	Urine. Between (Hours)
1	0+0	40	190/110	2	++	1	+ve	+ve	75 - 82	51 - 62
2	0+1	38	176/105	5	++	3	+ve	+ve	78 - 101	44 - 54
3	0+0	35	160/100	9	+++	2	+ve	+ve	97 - 115	57 - 69
4	0+0	36	185/120	1¼	++	2	+ve	+ve	85 - 108	42 - 50
5	1+0	34	160/110	3	++	1	+ve	+ve	92 - 114	48 - 56
6	0+0	40+	180/100	4	+++	7	+ve	+ve	246 - 262	152 - 195

TABLE XVII.

Eclampsia - Clinical details and time of disappearance of Anti-diuretic Hormone.

anti-diuretic hormone, both in view of the above work and the known susceptibility of these patients to develop pre-eclampsia during pregnancy.

Four pregnant patients with chronic nephritis were available for study. All gave a history of a previous attack of acute nephritis. In one a previous pregnancy had been terminated by abdominal hysterotomy.

The duration of pregnancy when first seen varied between sixteen and twenty-six weeks. Albuminuria was present in all four patients as was hypertension. Oedema was present in three from the time of admission, but in the fourth developed three weeks after admission.

The urine of these four patients was examined twenty-three times in all, and on every occasion but one the anti-diuretic hormone could be detected during pregnancy. The one occasion

when this substance was doubtfully present was in the patient without oedema on admission. The duration of pregnancy was then some 16 weeks. This examination was repeated 10 days later and anti-diuretic hormone was present.

Post abortion (one case) or delivery (three cases) - seven urinary examinations were carried out. These showed that the anti-diuretic hormone was present in the urine for an appreciably longer time post partum than in the pre-eclamptic patient. It could not be detected in one case after seven days, in two after fourteen days, but in the fourth case the substance was still present twenty-eight days post partum. This patient reported for post natal examination on the thirty-ninth day post partum. The urine was examined and no anti-diuretic activity could be detected.

ATTEMPTED QUANTITATIVE ESTIMATION OF ANTI-DIURETIC HORMONE ISOLATED FROM URINE.

Previous workers in this field - Teel and Reid (1939), Krieger and Kilvington (1940) have established increased anti-diuretic titres in the urine of patients with hypertensive toxaemia of pregnancy, but the proof that this anti-diuretic activity was of posterior pituitary origin has never been established with complete conviction.

It was felt that by isolation of the anti-diuretic hormone, identification and quantitative assay, the objection could be overcome.

With this in mind, known amounts of commercial posterior pituitary extract were submitted to extraction by two directional chromatography at 180°, and the area of the first solvent front eluted with distilled water and concentrated to 1 ml. by evaporation in vacuo. This 1 ml. eluate was then

treated with Ninhydrin by the method of Smith and Tompsett (1954). After development in a boiling water bath for 15 minutes, and cooling in the dark, the absorption of the purple colour which results was read in an Unicam spectrophotometer S.P. 600 at 570 mu. The results were read off a graph prepared from a standard solution of glycine.

The results obtained by this method using known amounts of commercial posterior pituitary extract were not reproducible, and it was not considered practicable to apply this method to such quantities of anti-diuretic hormone as might be detected in the urine.

As a second method the eluate once it had been reduced to standard volume was tested by bioassay. Once again the results were not reproducible for known quantities of commercial pituitary extract.

From the foregoing it would appear that the variation preceeded the actual estimation of quantity, and therefore lay in either the chromatographic extraction, the elution or the concentration. This variation persisted despite all attempts to correct it and hence the quantitative assay of anti-diuretic hormone in the urine was abandoned.

PLASMA FINDINGS.

The results reported here were obtained from blood withdrawn by venipuncture. Clotting was prevented by the use of Heparin, and the plasma separated immediately after the blood had been centrifuged. Plasma was normally extracted immediately, as has been described previously.

Heparinised plasma was preferred to serum in that any substance such as 5-hydroxy-tryptamine, produced by clotting, and which might have interfered with either the extraction or assay could be avoided. That this precaution was wise has been shown by the recent work of Hawker (1957) in which serum and plasma levels of anti-diuretic substances have been contrasted.

Some samples of plasma were stored in the frozen state and it was found that this storage did not interfere with the qualitative detection of the

anti-diuretic hormone.

NON-PREGNANT FEMALES.

The same volunteers who supplied the urinary specimens also submitted to venipuncture. The blood was withdrawn however somewhat later in the day, approximately 10 a.m., and was not taken in the fasting state.

The twelve samples of heparinised plasma were extracted as previously described, and in eleven no anti-diuretic hormone could be detected. This corresponded to the urinary findings. In the twelfth plasma however, anti-diuretic hormone was definitely present on the chromatogram, and was later confirmed by bioassay. This corresponded with the finding of anti-diuretic hormone in the urine of this case.

As has been stated previously, this girl suffered from pre-menstrual tension and was in the pre-menstrual phase of the cycle. It therefore became

necessary to carry out blood examinations during the course of the menstrual cycle.

The same six volunteers, four normal and two with pre-menstrual tension agreed to co-operate.

Samples of venous blood were withdrawn on the first day after cessation of the menstrual flow, six days later, a further six days later, and thereafter at intervals of three days up to and including menstruation.

In the four girls with normal cycles who did not exhibit any evidence of pre-menstrual tension, anti-diuretic hormone was not detected at any time.

In the two patients with pre-menstrual tension however, the anti-diuretic hormone was detected in the plasma in the pre-menstrual phase of the cycle.

In the one, the anti-diuretic

hormone was doubtfully present nine days before the onset of menstruation, and certainly present six days before. In this girl anti-diuretic hormone was present on the first day of the flow but was absent by the 4th. day. It will be noticed from Figure II that the appearance of anti-diuretic hormone anticipated the gain in weight as would be expected, since this weight gain is due to water retention.

In the second case of pre-menstrual tension, the anti-diuretic hormone was first detected six days prior to menstruation, and was still present on the second day of the flow. If one accepts the intensity of the colour reaction with Ninhydrin as a rough index of the quantity present (Moore and Stein, 1948), then this later result was of a very much reduced amount as compared with the earlier positive results. In this case

weight gain and the appearance of the anti-diuretic hormone could not be separated in time. See Figure III.

These results would suggest that, except in special circumstances, anti-diuretic hormone can not be detected in the plasma of the non-pregnant female, and where present is of limited duration only and associated with clinical water retention. These findings are in agreement with the recently published results of Hawker (1957).

NORMAL MALE.

Heparinised plasma was obtained from all eight of the male group whose urinary findings have been previously reported. Twelve samples in all were obtained. In none of these samples was it possible to detect any anti-diuretic hormone.

The plasma of three of the six persons who took part in the "smoking"

experiment reported earlier was also examined on two occasions, - the first after the ingestion of water, the second some thirty minutes after the inhalation of a cigarette. In none of the specimens examined after the ingestion of the water could anti-diuretic hormone be detected, but it was uniformly present in all three plasmas obtained after the inhalation of tobacco smoke.

This is as would be expected, in that the anti-diuretic effect of nicotine is mediated through the anti-diuretic hormone of the posterior pituitary gland - Chalmers and Lewis, 1957.

NORMAL PREGNANT FEMALES.

The majority of the cases of normal pregnancy, from whom urinary findings have been reported, were also submitted to investigation of the blood findings. In all some 76 samples of

venous blood from 55 patients were analysed.

In seven of the patients the gestation period was less than 14 weeks and in none of these was anti-diuretic hormone detected in the venous blood. Negative findings were also obtained on examination of the venous blood of eleven patients where the gestation period lay between 14 and 28 weeks.

A somewhat different picture emerged after the twenty-eighth week of pregnancy.

In the group of patients, 23 in all, where the gestation period was between twenty eight and forty weeks, the anti-diuretic hormone was detected on three occasions. This finding on each occasion was confirmed by examination of a further plasma sample and by bioassay.

In the group of patients where the maturity was more than 40 weeks,

anti-diuretic hormone was found in two out of fourteen patients. This finding was confirmed by repeat examination of a further sample of plasma in one case, in the other case delivery had taken place before the result was obtained.

These five cases where anti-diuretic hormone was present in the plasma showed no essential difference from other cases of normal pregnancy where the substance was absent. Table XVIII.

It is of interest, that in none of these five cases where anti-diuretic hormone was present in the blood was the substance detected in the urine. Of the two cases of normal pregnancy with anti-diuretic hormone in the urine, it proved possible to examine the blood in Case I only. This blood examination proved to be negative and the only explanation which can be advanced for

Anti-diuretic Hormone.	Age (Years).	Maturity (weeks)	Parity.	Indication for Hospital Admission.	Blood Pressure. (mms. of Hg.)	Oedema.	Albuminuria.
Positive.	25	34	0	Twin Pregnancy	110/65	-	0
Negative.	32	36	0+1	Twin Pregnancy	115/70	-	0
Positive.	26	38	0	Anaemia	130/80	-	0
Negative.	29	38	2+0	Anaemia	130/75	-	0
Positive.	36	40	3+1	A.P.H.	120/70	-	0
Negative.	33	39	2+0	Anaemia	120/70	-	0
Positive.	22	40+	0+0	Post Maturity	115/70	-	0
Positive.	19	40+	0+0	Post Maturity	120/70	-	0
Negative.	20	40+	0+0	Post Maturity	115/65	-	0

TABLE XVIII.

Comparison of Patients with Anti-diuretic Hormone in the Plasma with selected patients with similar clinical findings but in whose plasma anti-diuretic hormone was not detected.

the positive urinary finding is that some confusion in the labelling of specimens took place.

Six patients, three with negative and three with positive blood findings, were followed post partum. In none of the patients where anti-diuretic hormone had been absent from the plasma in pregnancy did the substance appear in the puerperium. Of the three patients with positive findings in pregnancy, in two of these the substance was still present on the third day of the puerperium, but in none could anti-diuretic hormone be detected on the fifth day post partum.

Table XIX gives the summarised results of the blood findings in normal pregnancy.

ACUTE TOXAEMIAS OF PREGNANCY.

Pre-eclampsia.

Samples of plasma taken from fifty-three patients diagnosed as

Duration of Pregnancy.	No. of Patients.	No. of Primary Examinations.	Anti-diuretic Hormone		Confirmatory Examinations.
			Positive.	Negative.	
0-14 weeks.	7	7	0	7	-
14-28 weeks.	11	11	0	11	-
28-40 weeks.	23	23	3	20	5
40+ weeks.	14	14	2	12	4
3rd. day Post-partum.	6	6	2	4	-
5th. day Post-partum.	6	6	0	6	-

TABLE XIX.

Non-Toxaemic Pregnancy - To show absence of Anti-diuretic Hormone in Plasma.

suffering from pre-eclamptic toxaemia were examined.

In 48 of these patients the presence of the anti-diuretic hormone was established, in three the findings were doubtful, and in two no anti-diuretic hormone could be detected.

As all these samples of plasma had been obtained from patients whose urinary status was known, it was considered interesting to contrast the blood findings with those obtained from the urine.

In the 48 cases where anti-diuretic hormone had been detected in the urine, it was also present in the plasma in 47. In the remaining case the findings must be considered of doubtful significance.

Of the seven pre-eclamptic patients where anti-diuretic hormone was absent from the urine, the plasma findings were available in five and

these showed considerable variation. The presence of the anti-diuretic hormone was definitely established in one case. In two cases the findings must be classed as doubtful in that while the polypeptide was faintly present on two directional chromatography, bioassay of eluates gave negative results. In the remaining two cases the anti-diuretic hormone was not detected.

When these findings are related to the clinical assessment of oedema in the patient, it will be noted that where oedema was marked, the anti-diuretic hormone could be detected in all samples of urine and plasma examined. Table XXI.

Where the oedema was considered to be moderate anti-diuretic hormone was identified in the urine of all but one case. The plasma findings were in agreement but for one case where the result was doubtful. It should be noted that this was the patient where

anti-diuretic hormone was not detected in the urine.

In only three cases where the oedema was slight were both urinary and plasma findings available. This group is too small to draw any valid conclusions,but it should be noted that in one patient where anti-diuretic hormone was absent in the urine the plasma findings were doubtfully positive.

In the last group, two cases in number, where oedema was absent, neither the urine nor the plasma could be stated to contain anti-diuretic hormone,although one plasma specimen was doubtfully positive.

The interpretation of these results would suggest two possibilities:

a) The first that the oedema of pre-eclampsia is related to the presence of anti-diuretic hormone,and that the severity of the oedema varies directly with the amount of anti-diuretic

Clinical Estimate of Oedema.	Number of Cases.	Presence of Anti-diuretic Hormone in Urine.	Number of Cases.	Presence of Anti-diuretic Hormone in the Plasma.		
				Positive.	Doubtful.	Negative.
Marked	11	Positive	11	11	0	0
		Negative	0	-	-	-
Moderate	37	Positive	36	36	0	0
		Negative	1	0	1	0
Slight	3	Positive	1	1	0	0
		Negative	2	0	1	1
No oedema	2	Positive	0	-	-	-
		Negative	2	0	1	1

TABLE XXI.

Pre-eclamptic Toxaemia - To show the relationship between Oedema, Plasma and Urinary Anti-diuretic Hormone.

hormone present.

b) Secondly, there is the possibility, that the oedema only occurs in the presence of anti-diuretic hormone, and that the severity of the oedema is related to the duration of action.

There also would appear to be in the correlation of the urinary and plasma results some suggestion that anti-diuretic hormone may be a threshold substance in so far as the kidney is concerned.

The suggested relationship of anti-diuretic hormone to the oedema present, and the hypothesis that anti-diuretic hormone and lactation were connected, made it necessary to examine the plasma of puerperal patients delivered following a pre-eclamptic pregnancy.

This was carried out on four patients. Plasma samples were collected on the third, fifth and seventh days

post partum. All these patients had been assessed to have moderate oedema, and in all four this oedema was no longer manifest by the fourth day post partum.

In each of the four patients anti-diuretic hormone which had been detected ante-natally was still present on the third day post partum, in one of the four the substance persisted up to the fifth day, but in none could anti-diuretic hormone be demonstrated on the seventh day post partum.

Table XX relates the findings in the plasma with the clinical state of the oedema, while Table XXI is included to show the relationship between the urinary and plasma findings in the presence of various degrees of oedema.

Eclampsia.

The same six cases of eclampsia, from which urinary findings have

Clinical Estimate of Oedema.	Number of Cases.	Presence of Anti-diuretic Hormone.		
		Positive.	Doubtful.	Negative.
Marked	11	11	0	0
Moderate	37	36	1	0
Slight	3	1	2	0
No oedema	2	0	0	2

TABLE XX.

Pre-eclamptic Toxaemia - To show presence of Anti-diuretic Hormone in the Plasma and the relation to the severity of Oedema.

previously been reported, had repeated blood examinations carried out.

In each case a sample of plasma was obtained as soon after the first seizure as was compatible with treatment, and at intervals thereafter further specimens were withdrawn. In each case, examination of the plasma was continued until such time as at least one negative result had been obtained.

A total of thirty-four specimens in all have been examined.

The result of these examinations was to confirm the presence of the anti-diuretic hormone in the blood of all eclamptic patients examined during the convulsive phase of the disease. The anti-diuretic hormone was persistently present in the blood for a considerable period. In no case was the substance absent from the blood by the third day post partum,but only in the case previously described with

recurrent eclamptic seizures was anti-diuretic hormone still present in the blood on the fifth day post partum. It was not detected in a plasma sample withdrawn on the morning of the sixth post partum day. These findings are in keeping with the results obtained in the pre-eclamptic patients. Two points seem worthy of comment:

a) The first, the persistence of anti-diuretic hormone in detectable quantity in the blood for a considerable period after the urinary findings have become negative.

b) The second, that in the six cases of eclampsia examined, there did not seem to be any prolongation of the action of anti-diuretic hormone in the puerperium when compared with the pre-eclamptic patient. This is in keeping with the clinical picture of the two disease conditions, in each of

which rapid improvement in the puerperium is a characteristic feature. Figures V and VI show the urinary and plasma findings in two of these cases of eclampsia with the clinical findings. Figure V is of a typical case while Figure VI gives the details of the case where convulsions recurred.

Table XVII gives clinical details of the six cases examined, and also the duration of persistence of anti-diuretic hormone in the blood and urine.

CHRONIC NEPHRITIS IN PREGNANCY.

The presence or absence of posterior pituitary anti-diuretic hormone in the blood was investigated in four cases of chronic nephritis in pregnancy.

These cases were all first seen in the second trimester of pregnancy. At this time the blood in each case was shown to contain the anti-diuretic hormone.

Fourteen subsequent examinations were made in these cases prior to the onset of labour or abortion, which occurred in one case, and in each case the anti-diuretic hormone was detected.

Further examinations of the blood were carried out after abortion in one case and delivery in the other three. Anti-diuretic hormone was still detected in the blood in all four cases ten days after the termination of pregnancy. In two cases anti-diuretic hormone was still present in the plasma eighteen days after delivery. In the one case where the substance was still present on the twenty-eighth day post partum, anti-diuretic hormone was no longer detectable on the thirty-ninth day after delivery.

THE QUANTITATIVE ESTIMATION OF ANTI-DIURETIC HORMONE IN THE BLOOD OF NORMAL AND TOXAEMIC PREGNANT WOMEN WITH SIMULTANEOUS DETERMINATIONS OF OSMOTIC PRESSURE AND SELECTED ELECTROLYTE CONCENTRATIONS.

THE QUANTITATIVE ESTIMATION OF ANTI-DIURETIC HORMONE IN THE BLOOD OF NORMAL AND TOXAEMIC PREGNANT WOMEN WITH SIMULTANEOUS DETERMINATIONS OF OSMOTIC PRESSURE AND SELECTED ELECTROLYTE CONCENTRATIONS.

The presence of the anti-diuretic hormone of the posterior pituitary gland in the blood and urine of pre-eclamptic pregnant women has been established. This substance is found in the vast majority of these patients as distinct from the small minority of normal pregnant women, and it was therefore decided to carry out quantitative estimations of the anti-diuretic hormone in the blood, in an effort to relate the findings to the severity of the disease.

It is known that the osmotic pressure of the blood acting on osmoreceptors in the hypothalamus controls the output of anti-diuretic hormone (Verney - 1947). Further it is known that certain of the solutes which

contribute to this osmotic pressure are more evocative of anti-diuretic hormone than are others. This latter fact is due to the relative permeability or otherwise of the osmoreceptors to different solutes.

In view of the foregoing it was decided to carry out the following experiment in which two groups of pregnant women, the one normal, the other composed of cases of acute toxaemia of pregnancy, co-operated.

Arterial blood was withdrawn by femoral artery puncture. This arterial puncture was conducted under similar conditions and at standard time 10-11 a.m. The decision to employ arterial blood was taken for two reasons. These were the known rapid inactivation or utilisation of the anti-diuretic hormone in vivo, and also in an effort to obtain a sample of blood as similar as possible to the blood in the carotid artery and hence

the blood supplying the osmoreceptors of the hypothalamus. The femoral artery at the inguinal ligament was selected for puncture, in view of its accessibility and also as the content of anti-diuretic hormone would be substantially the same as that in the renal arteries.

Approximately 20 millilitres of blood were withdrawn into a sterile siliconed syringe, and this blood immediately separated into two portions.

The first portion of arterial blood, approximately 10 millilitres, was transferred to a heparin tube and the plasma separated.

The plasma was then available for anti-diuretic assay and testing for the presence of the polypeptide.

Assay of the anti-diuretic hormone was carried out by the method of Eisen and Lewis, previously described. Pitressin (Parke, Davis and Co.) was

used as a standard. The rat preparation required by the method was carried out, and the animal known to be reactive and stable, before the blood under investigation was withdrawn. This allowed of almost immediate assay of the anti-diuretic hormone in the arterial plasma. That portion of the plasma not required for assay could be stored at 4° C. for chromatography.

The second portion of the arterial blood was transferred to a chemically clean tube and allowed to clot. The serum was separated and the osmotic pressure, electrical conductivity, and the sodium, potassium and chloride concentrations determined.

The osmotic pressure was assessed by the depression of the freezing point of the serum. For this determination a micro-Beckmann thermometer was employed. From the depression of the freezing point as compared with

distilled water the osmotic pressure of the serum could be calculated. I am indebted to Dr. Stotherd T.R.S. Mitchell of the Department of Chemistry, the University of Glasgow, for advice and instruction in this technique, and his assurance that the accuracy was such as to allow small variations in osmotic pressure to be recognised.

All the results of freezing point depression reported here are the mean of duplicates, and any two results between which the discrepancy was greater than 0.005° C. were repeated.

The electrical conductivity was estimated by means of a conductivity bridge operated from a 1000 cycles per second Cambridge-Reed Hummer. The conductivity cell was of the Kohlrausch pattern, and was found to have a cell constant of 0.4066 when standardised with the usual potassium chloride solutions. All measurements

were made in a water thermostat controlled at 25.00 ± 0.02° Centigrade. The water employed for making up the solutions had been redistilled over permanganate in an all-glass Pyrex distillation apparatus protected from the atmosphere. It had a specific conductivity of 1.68 x 10.6 reciprocal ohms.

The concentration of sodium and potassium in the serum was estimated by means of an E.E.L. flame photometer.

The chloride concentration was measured by the method of Schales and Schales (1941).

RESULTS.

Twelve patients with acute toxaemia of pregnancy, including one in the immediate post-convulsive phase make up the one group, while ten normotensive, non-toxaemic pregnant women are included in the control group.

Clinical details of these patients

and the findings obtained in this investigation are summarised in Tables XXII and XXIII.

From these Tables, it is immediately noticeable that as well as the clinical difference, the cases are almost completely divided into two groups by the content of anti-diuretic hormone present in the arterial plasma.

In two of the ten normal pregnant women, anti-diuretic activity was demonstrated, but the level of activity was considerably less than that found in the group of patients suffering from acute toxaemia of pregnancy. In only one of this later group was anti-diuretic hormone not detected. The range of activity, excluding this one case, lay between 0.16 and 0.025 milliunits of anti-diuretic hormone per millilitre of plasma. It is of interest that

Maturity (weeks).	Blood Pressure. Mms. of Hg.	Oedema.	Albuminuria Gm./Litre.	Depression of Freezing Point. °C.	Conductivity (Reciprocal ohms). 1/10 Dilution x 10^2	1/10 Dilution x 103	Sodium. Milli-Equiv. /Litre.	Potassium. Milli-Equiv. /Litre.	Chloride. Milli-Equiv. /Litre.	Anti-diuretic Hormone. Milliunits/ ml.	
39	140/90	+	0	0.560	-	-	130	4.2	108	0.120	-
39	165/110	+	2	0.595	-	-	135	4.5	106	0.065	+
28	180/120	++	5½	0.568	-	-	130	4.2	110	0.045	
40	160/115	+/-	0	0.582	-	0.167	128	4.5	104	0.105	
39	140/95	+	0	0.533	-	0.172	133	4.8	110	0.035	
32	160/105	++	12	0.564	-	0.174	133	4.8	111	0.160	
40	140/100	+/-	0	0.543	0.156	-	133	4.4	104.5	N.D.	*
40	150/90	++	0	0.542	-	-	142	4.9	109	0.045	
40	150/110	+	1	0.592	0.153	-	136	4.1	104	0.080	
37	170/110	++	0	0.552	0.158	-	128	3.7	100	0.070	-
40	140/90	+	0	0.548	-	0.168	130	3.9	101	0.060	
30	160/100	++	0	0.561	0.157	-	130	4.0	102	0.025	

TABLE XXII.

* N.D. = Not detected.

Pre-eclampsia: Details of Anti-diuretic Hormone levels and other Blood investigations.

Maturity (weeks).	Blood Pressure. Mms. of Hg.	Oedema.	Albuminuria Gm./Litre.	Depression of Freezing Point. °C.	Conductivity (Reciprocal ohms). 1/10 Dilution x 10^2	1/10 Dilution x 10^3	Sodium. Milli/Equiv. /Litre.	Potassium. Milli-Equiv. /Litre.	Chloride. Milli-Equiv. /Litre.	Anti-diuretic Hormone. Milliunits/ ml.
39	110/65	-	0	0.572	-	0.170	135	4.1	99.5	N.D.*
38	120/80	-	0	0.568	-	0.164	125	4.7	98.5	N.D.*
39	125/80	-	0	0.562	-	0.163	138	4.3	98.5	0.040
36	110/70	-	0	0.568	0.157	0.167	139	4.0	103	N.D.*
32	110/75	-	0	0.555	0.156	0.169	139	3.9	109	N.D.*
39	110/60	-	0	0.535	0.155	0.168	136	4.5	104	N.D.*
34	110/65	-	0	0.551	0.155	0.166	136	4.5	104	0.015
39	110/70	-	0	0.550	0.154	-	138	4.5	101	N.D.*
39	120/70	-	0	0.522	0.154	-	136	4.7	102	N.D.*
37	120/60	-	0	0.541	0.157	-	136	4.5	104	N.D.*

TABLE XXIII.

* N.D. = Not detected.

Normal Pregnancy: Details of Anti-diuretic Hormone levels and other Blood investigation.

the greatest concentration of anti-diuretic hormone was observed in a patient in the immediate post-convulsive phase of eclampsia. Apart from this one case, no relationship was apparent either between the degree of toxaemia and the concentration of anti-diuretic hormone, or between the extent of the oedema and the concentration of anti-diuretic hormone.

Two of the samples of plasma showing anti-diuretic activity were treated with sodium thioglycollate and in each case the anti-diuretic activity was abolished.

The increase in arterial plasma anti-diuretic hormone, now assayed in the group of patients with acute toxaemia of pregnancy, did not seem to be associated with changes in osmotic pressure. The mean depression of the freezing point in the toxaemic group was 0.562° C., while the mean depression

of the freezing point in the control group was 0.552° C.

On statistical analysis this difference was not significant ($0.2 < P < 0.3$).

The osmotic pressure findings were corroborated by the results of the electrical conductivity of the serum. This is a measure of the ionic concentration of the serum and hence an indirect measure of the osmotic pressure.

Only such substances as are ionisable in the serum will affect the electrical conductivity, and hence in this estimation the concentration of such substances as urea and glucose will not alter the result. These substances do, albeit to a small extent, depress the freezing point of the serum. As neither urea nor glucose, in physiological concentration, have any effect upon the osmoreceptors

of the hypothalamus (Verney, 1947), the electrical conductivity of the serum may,in fact,represent a more accurate measure of the forces acting upon the hypothalamus than does the depression of the freezing point.

In the two groups of cases no difference was found in the electrical conductivity, the mean of the results in the groups being almost identical. (0.155: 0.156 - 0.170: 0.167).

Although no significant difference had been detected in the osmotic pressure of the serum between the groups, it was thought that some difference might be found in the concentrations of the various solutes which effect the osmoreceptors in the hypotholamus.

The concentrations of sodium, potassium and chloride were selected for investigation. No significant difference was found between the two groups in the serum concentration of

sodium and potassium. The mean concentration of sodium was 132.33 milli-equivalents per litre in the toxaemic group and 135.2 milli-equivalents per litre in the control group. This difference is not statistically significant ($0.1 < P < 0.2$).

The mean concentration of potassium was 4.33 milli-equivalents per litre in the toxaemic group and 4.37 milli-equivalents per litre in the control group. This again is not statistically significant ($0.8 < P < 0.9$).

A significant difference was found in the chloride concentration. In the toxaemic group this averaged 105.79 milli-equivalents per litre and in the control group 102.35 milli-equivalents per litre. This is statistically significant ($0.02 < P < 0.05$).

The interpretation of this latter finding is somewhat obscure. The chloride ion is known to have an effect

on the osmoreceptors and in higher concentration it would be expected to produce an increased output of anti-diuretic hormone.

The mean difference in chloride concentration between the toxaemic and normal groups, is 3.44 milli-equivalents per litre. If now one applies to this difference the formula -

$$\pi \ (37^{\circ} \text{ C}) = \frac{10 \times C \times R \times T}{M.W.}$$ where π equals the osmotic pressure in atmospheres at 37° C, C equals the concentration of chloride in grams per 100 millilitres, R equals the gas constant of 0.08204, T is the absolute temperature, and M.W. represents the molecular weight, the difference in chloride concentration represents an osmotic pressure of 0.087 atmospheres at 37° C. This figure applies to the chloride ion existing alone and is therefore obviously fallacious.

If this chloride were in the form of sodium chloride, the equivalent figure for the binary electrolyte would be an osmotic pressure of 0.16 atmosphere at 37° C.

The osmotic pressure of the serum in the non-pregnant is approximately 7.6 atmospheres at 37° C.

This figure would in all probability be lower in the pregnant due to the hydraemia and the fall in proteins normal to pregnancy. Accepting the figure of 7.6 atmospheres, the difference in chloride concentration represents less than the 1.8% alteration in osmotic pressure which Verney found to be the minimal alteration in the carotid blood evocative of anti-diuretic hormone. He, however, suggested that "an increase in the osmotic pressure of the aortic blood that is a good deal less than that found effective in the carotid blood

viz. 1.8% would suffice to produce the same degree of inhibition of urine flow".

The results reported here are of femoral arterial blood and therefore more representative of the aorta than of the carotid artery. However, when the anti-diuretic titres are compared with the chloride concentration no relationship is apparent.

There is however a suggestion that the osmoreceptors can accommodate during a long period of exposure to a constant increase in the sodium chloride content of the carotid blood - Baldes and Smirk (1934). Perhaps therefore the explanation of the present findings may lie in an alteration of the sensitivity of the osmoreceptors.

In summary, it would appear from the results obtained in the present experiment that the increased plasma

content of anti-diuretic hormone in toxaemia of pregnancy is not due to increased secretion from the pituitary gland,called forth by stimulation of the osmoreceptors either in response to total osmotic pressure variations or to variations in the concentration of sodium or potassium. It may be possible that an explanation will be found in an alteration of the chloride concentration allied to an alteration of the osmoreceptor sensitivity.

DISCUSSION.

DISCUSSION.

The posterior pituitary hormones are in fact secretions of the hypothalamic nuclei from where they migrate along the axons of the supraoptico-hypophysial tract to be stored in the posterior pituitary lobe. (Bargmann and Hild (1949), Hild (1951) and Zetler (1953). In man however, only a very small fraction of the total hormones stored can be recovered from the hypothalamus, and some 99% of the total storage is in the posterior pituitary gland. (van Dyke et al. (1955). From this fact arose the earlier held opinions that these hormones originated in the posterior pituitary gland.

Two hormones can be differentiated; the one, the pressor or anti-diuretic hormone, the other the oxytocic hormone. These hormones exist side by side and in the posterior pituitary gland the ratio of pressor to oxytocic approaches

unity - van Dyke et al.

Both hormones are liberated in response to stimuli of which variation of the osmotic pressure of the blood (Verney 1947) and suckling (Cross 1951) are the more physiological. Other stimuli known to produce liberation of the posterior pituitary hormones in man include emotional stress (O'Connor and Verney - 1942), fainting, shock, and severe haemorrhage (Brun et al. 1945, Noble & Taylor, 1953), certain drugs such as Pethidine and anaesthetic agents (Le Quesne and Lewis - 1953) and nicotine (Chalmers and Lewis - 1951).

In response to these various stimuli the posterior pituitary gland secretes the two hormones in varying proportion - Abrahams and Pickford (1954), Bisset and Lee (1957). From this evidence it would appear that the actual hormones are separately stored

in the posterior pituitary gland rather than a common precursor as suggested by van Dyke (1950) and Croxatto et al. (1951).

The pure hormones have recently been isolated by du Vigneaud and his co-workers, and it is to the octopeptide vasopressin and its effect in the pregnant patient with acute toxaemia that this work has been directed.

However secreted, the anti-diuretic hormone circulates in the blood stream and acts on the kidney causing inhibition of water diuresis without change in the blood flow through the kidney (Cowan, Verney and Vogt, quoted by Verney (1946). The quantity of anti-diuretic hormone which produces this result is very small. In a dog weighing 10 to 15 kilograms, deprived of its posterior pituitary lobe, the intravenous injection of 1 to 5 milliunits per hour will inhibit water

diuresis. It is now accepted that the action of the extract on the kidney is to cause the reabsorption of water by the tubules, without great change in the excretion of other urinary constituents.

Anti-diuretic hormone is normally destroyed by the liver, although some hormone may be excreted in the urine. This accounts for the rise in the circulating anti-diuretic hormone found in chronic liver destruction. (Ralli, 1945).

Slessor (1951) has suggested that adrenal cortical hormones of cortisone type also play a part in the destruction of anti-diuretic hormone.

When the findings of the present investigation are scrutinised in the light of these known facts in the production, liberation and destruction of anti-diuretic hormone the first question which arises is whether the substance under investigation is in

fact the anti-diuretic hormone of the posterior pituitary gland. This question has already been discussed at the conclusion of the first section of the thesis and it is held that the proof is incontrovertible. This proof comprised the isolation by chromatography of a characteristically situated polypeptide which proved to be the only active portion of commercial vasopressin. This polypeptide was hydrolysed and its amino-acid content determined. An identically situated polypeptide, of identical amino-acid constitution was also isolated from the human pituitary gland, from human urine and blood after the action of nicotine, and these polypeptides were shown to have the biological activity of the posterior pituitary anti-diuretic hormone.

Equally significant was the absence of this polypeptide from the

blood and urine of untreated diabetes insipidus of central origin and its presence following clinical cure by replacement therapy.

From this arises the second question. Is anti-diuretic hormone present in excess in acute toxaemia of pregnancy?

It has been shown that anti-diuretic hormone cannot be detected in the plasma or urine of the normal pregnant woman. Conversely, under the same conditions in the urine and plasma of the pregnant women with acute toxaemia, the anti-diuretic hormone is almost consistently present. No quantitative estimations of the output of anti-diuretic hormone in the urine were carried out, but in the plasma of these patients with acute toxaemia of pregnancy a significant quantity of anti-diuretic hormone has been detected. This contrasts with the consistently

negative results in the plasma of the normal pregnant patient.

From this fact arises the next question as to the cause of this finding. Obviously an excess of anti-diuretic hormone may be due to either an increased production or a decreased destruction.

Taking the former first, is there any evidence of increased production? The stimuli known to produce an increased output of anti-diuretic hormone have been mentioned previously. Of these stimuli, suckling, fainting, shock, haemorrhage, anaesthetic agents and drugs including nicotine can be immediately discounted. These stimuli are not operative in the cases under consideration.

Emotional stress must also be discounted. Selye (1946) has suggested that acute toxaemia of pregnancy could possibly be grouped with the so-called

diseases of adaption but this theory is no longer tenable.

This leaves the variation of osmotic pressure as the stimulus to be discussed. In the two groups of patients - the one toxaemic, the other normal - where anti-diuretic assays in the plasma and osmotic pressures of the serum were carried out simultaneously, no evidence was found to support this theory. The total osmotic pressure as measured by the depression of the freezing point was not significantly altered between the two groups. This was supported by the similar electrical conductivity results, and any question of total osmotic variation must be discarded.

The osmotic pressure of the blood is the summation of the osmotic pressures of a number of solutes. Not all these solutes evoke an anti-diuretic hormone response. For example, urea and glucose

in physiological amounts do not produce a response, while the sodium, potassium and chloride ions are all active (Verney 1948). The concentration of these ions was assessed in the same cases where the osmotic pressure, electrical conductivity and anti-diuretic hormone were determined. No significant variation was found between the toxaemic and normal groups as regards the sodium or potassium concentration, but the chloride concentration was higher in the toxaemic group where the anti-diuretic hormone was present. As has already been discussed, this alteration in the chloride concentration is equivalent to an increase of 1.2 per cent in that portion of the osmotic pressure due to the chloride ion, while if the total increase in chloride concentration were in the form of sodium chloride the increase would be in the order of 2 per cent. In these figures,

as has already been suggested, may be found an explanation for the increased anti-diuretic levels in toxaemia of pregnancy. The absence of any correlation between the concentrations of sodium and chloride and the anti-diuretic hormone level however would seem to necessitate an alteration in the sensitivity of the hypothalamic osmoreceptors, were this explanation to be acceptable. That this may be possible is shown by the work of Baldes and Smirk (1934).

It therefore seems than an alternative explanation may well exist for the apparent increased anti-diuretic hormone in pregnancy toxaemia.

The destruction of the hormone is partly by urinary excretion and partly by metabolism in the liver (Ralli et al.- 1945).

That a failure of urinary excretion is not the solution is apparent in

light of the finding that the anti-diuretic hormone can be detected in 0.05 millilitre of urine from cases of acute toxaemia of pregnancy but is not detectable in an equal volume of urine from the normal gravida.

The possibility of a failure of liver metabolism of the anti-diuretic hormone must be borne in mind; particularly so when it is accepted that pre-eclamptic toxaemia alters hepatic function.

The ordinary liver function tests fail to reveal any pathological changes in response as compared with the normal pregnant patient.

The use of loading tests with bilirubin, however, demonstrated a deficiency of functional reserve - Lyon (1938), Philpott et al. (1948). Dieckmann et al. (1951) in an assessment of liver function found that pre-eclamptic patients invariably

gave a higher degree of abnormality than normal gravidae. It would seem possible therefore that the functional capacity of the liver to destroy anti-diuretic hormone may be altered, and in this way the excess of anti-diuretic hormone in the circulating blood explained. This explanation however seems remote particularly when one considers the increase of anti-diuretic hormone present in cases of premenstrual tension.

A further method of destruction of anti-diuretic hormone has been suggested by McCartney et al.(1952) and more recently Hawker (1956a). These workers have shown that the blood of the normal pregnant patient contains a substance capable of inactivating the anti-diuretic hormone of the posterior pituitary gland. Hawker was unable to demonstrate this inactivating substance either in the blood of the toxaemic

pregnant patient or in the blood of the non-pregnant female.

In a further paper Hawker (1956b) has suggested that this substance may be in the nature of an enzyme.

In view of the evidence it would seem, therefore, that the excess anti-diuretic hormone present in the pre-eclamptic patient may not in fact represent an excess production of the hormone in pregnancy, but rather that a destructive enzyme apparently peculiar to the gravid state is absent in cases of toxaemia of pregnancy, and hence a build-up of anti-diuretic hormone occurs in the blood. This would also account for the constant urinary excretion.

However this excess of anti-diuretic hormone is produced it will have the effect of stimulating resorbtion of water in the distal convoluted tubules. This would apparently lead to an increase in blood volume in the toxaemic pregnant

woman. In fact the blood volume in toxaemia of pregnancy is not significantly altered from that of the normal gravida - (White, 1950). Nevertheless, it has been shown that large quantities of anti-diuretic hormones are present in the urine and blood of these cases, and by animal experimentation it has been shown to be biologically active. There seems no reason to doubt that this anti-diuretic hormone is equally active in the patient from whom it was derived. An explanation of the apparently anomalous blood volume findings may be found in the association between the presence of the anti-diuretic hormone and the occurrence of oedema in toxaemia. In the intact subject anti-diuretic hormone would undoubtedly increase the blood volume but this does not necessarily follow in such a complex condition as toxaemia.

It would seem reasonable to suggest that the fluid retained by the action of anti-diuretic hormone is for some reason directed into the tissues. This would add point to the rough quantitative relationship established between the clinical assessment of the oedema and the presence of the hormone in the urine.

As has been previously noted the anti-diuretic hormone has no effect on the renal blood flow. This is in keeping with the work of Chesley et al. (1940) as confirmed by Kenney et al. (1950) on the renal blood flow in pre-eclamptic toxaemia and eclampsia. Trueta et al.(1947) however reported a renal vascular shunt and suggested that there was more than one pathway for blood circulating in the kidneys. Experimental work has tended to support this theory, Franklin & Sophian (1949), but Shorr et al.(1951) while agreeing

with the anatomical possibility have yet to be satisfied with the functional occurrence of this shunt. De Snoo (1934) has reported that after eclampsia there is impairment of the elimination of water and urea. That the impaired elimination of water can be explained as a function of excess anti-diuretic hormone is obvious, and the impaired elimination of urea can be explained by the slower tubular flow of glomerular filtrate and hence the increased back-diffusion of urea. This back-diffusion of urea is known to be inversely proportional to the tubular flow.

It would therefore seem not impossible that some of the accepted renal changes in pre-eclampsia can be explained by the presence of excess anti-diuretic hormone.

When one turns to the clinical aspect of the toxaemias of pregnancy, it has long been accepted and practised

that a salt-poor diet is beneficial and often results in diminution of the oedema present. Might this be explained by a reduction in the absorption of sodium and chloride, hence a lessened liability to stimulation of the hypothalamic osmoreceptors to produce anti-diuretic hormone?

In this discussion up to the present anti-diuretic hormone has been considered as a substance acting alone. These conditions may obtain in the experimental animal, but in a complex condition such as human pregnancy, other factors require consideration. Mention has been made already of a possible enzyme whose purpose is the destruction of anti-diuretic hormone, but other factors also exist, in particular the pituitary gonadotrophins and the secretions of the adrenal glands.

That the pregnant woman, and in

particular the pregnant woman with toxaemia, showed an increased susceptibility to posterior pituitary extract has been mentioned in the introduction. Browne (1946) showed that synapoidin, a mixture of pituitary and chorionic gonadotrophins, increased the sensitivity of the individual to posterior pituitary extract. Govan and Mukherjee (1950) showed that this sensitivity was in fact due to anterior pituitary gonadotrophins and not to the gonadotrophins of chorionic origin. Govan (1951) has described post-mortem changes, closely resembling the lesions found in human eclampsia, in the liver and kidneys of animals, sensitised with anterior pituitary gonadotrophins and then treated with posterior pituitary extract. It is also of interest that the animals became oliguric and convulsive before death. These changes could also be produced

if urine from toxaemic patients was substituted for the anterior pituitary gonadotrophins.

That the gonadotrophins are increased in pregnancy, and particularly in toxaemia of pregnancy, has been long recognised but whether in fact these gonadotrophins are of pituitary or chorionic origin is not yet settled. Byrom (1937) has shown a sensitising effect of oestrogens to posterior pituitary extracts but this in all probability is secondary and follows upon the increase in gonadotrophins.

These facts seem to lend point to the statement of Harris (1948) that there is normally a balance between the specific anti-diuretic action of the neurohypophysis and a diuretic influence of the adenohypophysis, and suggest that the fluid retention in pre-eclampsia may rest in an upset of this balance.

The place of the adrenals in water balance is well recognised; more specifically the action of anti-diuretic hormone is considerably modified in such conditions as Addison's disease,and Slessor (1952) suggests that the slow response in these patients to water ingestion is in fact due to circulating anti-diuretic hormone,and only when this hormone is destroyed naturally by time or by the administration of cortisone does diuresis occur. From this he concludes that cortisone mediates the destruction of posterior pituitary anti-diuretic hormone.

Birnie et al.(1949) suggested a somewhat similar mechanism by their observation that the anti-diuretic content of the blood increased after adrenalectomy, and further evidence is to be found in the work of Gaunt et al. (1949) and Lockett (1951) who showed an

increased sensitivity of adrenalectomised animals to posterior pituitary extracts.

All this work suggests that the adrenal secretions are antagonistic to the anti-diuretic hormone of the posterior pituitary gland, and that the anti-diuretic effect is greater where adrenal secretion is less.

The position in pregnancy however is that adrenal secretion is increased over that found in the non-pregnant female - Venning (1946), Heard et al. (1946). Again in toxaemia of pregnancy a further increase over the higher levels of normal pregnancy has been claimed - Tobian (1949), Parviainen et al. (1950), Tampan et al. (1956).

When one considers the two groups of adrenal hormones particularly implicated in water metabolism, that is the mineralo-corticoids and electrocortin, it would appear generally accepted that the mineralo-corticoids

are increased in toxaemia of pregnancy while the excretion of aldosterone is similar in toxaemic and normal pregnancy - Martin and Mills (1956).

The glucocorticoids, of which cortisone is a representative, are not increased in toxaemia of pregnancy - Mastboom (1952), Assali et al. (1955) - and thus it would appear that this method of destruction of anti-diuretic hormone is not increased in the toxaemias. What relationship exists between the adrenal steroids and pituitary anti-diuretic hormone has not yet been clarified. Silvette and Britten (1938) presented evidence of a physiological antagonism between the actions of adrenal cortical hormone and posterior pituitary anti-diuretic hormone. This antagonism was in fact between the mineralo-cortoids and anti-diuretic hormone. Lloyd and Lobotsky (1950) elaborated this concept. They found

that the anti-diuretic activity of the serum and the urinary corticosteroid level both bear a relationship to the state of water balance. The comparative proportions of these substances are of greater significance than the absolute values of either one of them. Regardless of the absolute values,if the anti-diuretic hormone is relatively greater than the corticosteroid,then water retention occurs. They also found the converse to apply in diuresis. In these findings of Lloyd and Lobotsky may lie the explanation of the failure to correlate the severity of toxaemia of pregnancy with the level of anti-diuretic hormone in the plasma. A parallel estimation of the mineralo-corticoids present might have allowed a ratio to be developed which would have correlated with the clinical findings. A second possibility which

must be considered is that the raised level of anti-diuretic hormone in the plasma in toxaemia of pregnancy is a result of the increase in the mineralo-corticoids present,and is necessary to the organism to maintain a water balance.

The foregoing suggestions do not detract from the importance of a constant finding of anti-diuretic hormone in the plasma of toxaemia of pregnancy. That the finding is constant would appear to eliminate coincidence.

The lack of any correlation between the plasma level of anti-diuretic hormone and the severity of the toxaemia does raise doubts as to whether the finding may only be of secondary aetiological importance. But does the blood level alone indicate the activity of secretion? Some degree of correlation has been found, as

previously described, between the clinical oedema present and the presence of anti-diuretic hormone in the urine. Might it not be possible that a renal threshold exists to anti-diuretic hormone, and that blood levels above a certain figure represent only the unexcreted portion of the anti-diuretic hormone? Then a true index of hypothalamic activity could only be gained by summation of the total amount of anti-diuretic hormone excreted over given time with the mean quantity present in the blood during the same period.

Such a concept would also offer some explanation for the apparent discrepancy between anti-diuretic levels in toxaemia of pregnancy in the presence of similar serum chloride concentrations. Certainly in the chloride concentration alone was any possibly significant variation in anti-diuretic stimulating

substance detected.

A correlation between the level of anti-diuretic hormone and the severity of the toxaemia of pregnancy would strongly suggest that the increase in anti-diuretic titre was of primary importance in the aetiology, and that by leading to fluid retention resulted in compensatory adrenal hyperactivity. Inviting as this concept is, and particularly as it would fit with the suggested lack of enzymatic destruction of anti-diuretic hormone in acute toxaemia of pregnancy, it becomes less attractive in the light of other evidence.

Nash (1946) and Dieckmann (1952), in his monograph on the Toxaemias of Pregnancy, have both reported the occurrence of acute toxaemia of pregnancy in patients with long-standing diabetes insipidus. It is true that neither author indicates whether the disease

was of central or end-organ type, but the fact that the patients were receiving substitution therapy would appear to indicate a central origin for the disease. The lack of the destructive enzyme of pregnancy or an increased sensitivity to anti-diuretic hormone remain as possibilities, and it would appear that investigation of such further cases in the future could elucidate the place of posterior pituitary anti-diuretic hormone in toxaemia of pregnancy. If on the other hand, the raised blood level of anti-diuretic hormone in toxaemia of pregnancy is a secondary manifestation may the explanation not lie in a primary adrenal excess with the posterior pituitary gland coming into play in an effort to maintain homeostasis.

Whatever the explanation of the present findings may be, two facts are

established. First that an increased blood level of anti-diuretic substance exists in toxaemia of pregnancy and secondly that this anti-diuretic substance is in fact the anti-diuretic hormone of the posterior pituitary gland. That further work on this subject is required seems obvious, and it would appear that the balance between the various adrenal steroids and anti-diuretic hormone and further investigation into the nature and distribution of the destructive enzyme of pregnancy, particularly in early pregnancy as to its absence or presence, may afford the most profitable lines for further research.

In conclusion it seems apt to quote from F.J. Browne, who of all people, in this country, has probably done most to elucidate the disease process known as acute toxaemia of pregnancy, I can do no better to

illustrate the present knowledge than quote the title of his most recent contribution, - "Aetiology of Pre-eclamptic Toxaemia and Eclampsia - Fact and Theory". (1958).

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