Proteinuria in Pregnancy

By

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Preface

This study was undertaken in the Research Department, Royal Maternity and Women's Hospitals, Glasgow. The work was performed over a period of three years while the candidate was a Senior Registrar to the Glasgow Teaching hospitals attached to the Glasgow Royal Maternity Hospital. A research grant was awarded to the author by the Board of Management of the Royal Maternity Hospital to cover the cost of materials. The clinical material was obtained from patients attending this hospital.

I wish to express my thanks to the consultant staff of the Royal Maternity Hospital for access to their patients and to the nursing staff for their help.

I also wish to express my indebtedness to Dr. A. D. T. Govan, Director of Research, Glasgow Royal Maternity Hospital for his constant encouragement and helpful criticism throughout the course of the work.

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Introduction

Proteinuria of any significance is regarded as of serious importance in the non-pregnant individual indicating dangerous illness frequently progressive and chronic. While the ultimate aetiology may be in doubt, clinical diagnosis and prognostication can be made with a fair degree of confidence.

In pregnancy however, proteinuria is a baffling condition. Basically it is only manifest during pregnancy, is self limiting in its duration and does not appear to lead to chronic disease. Furthermore the degree of proteinuria does not appear to be closely related to the severity of the disease process and is not a reliable guide to prognosis. Even diagnosis is difficult and is made even more so by the fact that pre-existing but undiscovered renal or arterial disease may complicate the pregnancy.

Proteinuria arising de novo in pregnancy is commonly due to pre-eclampsia, a condition

which can only be defined vaguely by means of the signs; hypertension, oedema and proteinuria. This vagueness of definition and the variation in its mode of onset, development and outcome has given rise to endless speculation. Not least has been the controversy regarding the meaning of proteinuria in this syndrome.

Early medical literature on pre-eclampsia is concerned chiefly with eclampsia. The danger of convulsions in pregnancy is mentioned in ancient Chinese, Egyptian and Greek medicine. Hippocrates stated that a tendency to headaches, drowsiness and convulsions is of serious prognostic significance in pregnant women. The first appearance however of the word "eclampsia" was not apparently until 1619 in Varandaeus' treatise. In 1840 Rayer demonstrated proteinuria in normal pregnant women and three years later Lever in Guy's Hospital reports, reported its presence in nine out of ten eclamptics. This lead to speculation regarding

the role of the kidney in pre-eclampsia and eclampsia. Frerichs (1851), considered eclampsia a type of uraemia.and believed that oliguria and retention of urea were manifestations of a type of Bright's disease. Volhard (1918) and Fitzgibbons (1922) both claimed that eclampsia was of renal origin. Volhard stated that it was a form of uraemia. Fitzgibbons claimed that pre-eclampsia was a renal type of toxaemia. Paramore (1927) believed that the kidneys were always implicated in eclampsia. Eden and Holland (1931) commenting on nephritis in pregnancy also thought many supposed cases of pre-eclampsia were really chronic nephritis. The results of both Dieckmannet al (1958) and Pollak and Nettles (1960) have indicated that 10 - 20 per cent. of all patients with pre-eclampsia have a basic renal condition. Gibberd (1928) found an incidence of 14 per cent. chronic nephritis in patients with pre-eelampsia believed to be healthy before pregnancy. Kellog (1924) suggested that recurrent toxaemia

was a manifestation of faulty renal balance and that the load of pregnancy caused renal insufficiency. On the other hand, Schultz (1933) thought that chronic nephritis was an exceedingly rare sequel to pre-eclampsia and that the healing of the kidney lesion was the rule. Theobald (1933) reviewing the Registrar General's figures 1911 to 1920 to determine the relationship of albuminuria of pregnancy to chronic nephritis concluded that pregnancy had little causal association with Bright's disease, thus refuting the claims of a renal origin for pre-eclampsia.

Difficulty is experienced in demonstrating primary renal disease in pregnancy. An increase in glomerular filtration rate and renal blood flow in pregnancy was described by Sims and Krantz (1958) with a fall in blood urea below the non-pregnant level. Renal disease may therefore be present in pregnancy with a relatively normal blood. urea. According to Chesley et al (1939, 1940) renal blood flow

as measured by the diodrast clearance test was normal in normal pregnancy, pre-eclampsia and eclampsia. Corcoran and Page (1941) studied renal function in late toxaemia of pregnancy and found the filtration fraction may be normal, higher or low, whereas Assali et al. (1953) observed in pre-eclampsia a decrease in glomerular filtration. Elden et al. (1935, 1936) found the urea clearance test to be slightly lower in normal pregnancy than in the non-pregnant state but with no decrease in the clearance in pre-eclampsia, eclampsia and nephritis complicating pregnancy. Dieckmann (1935) also noted a slight fall in the values of urea clearance both in normal pregnancy and pregnancy associated with pre-eclampsia and eclampsia.

Morphological studies of the kidney in pregnancy are confused. Fahr (1924) emphasized the variation in the degree of glomerular changes in eclampsia. He considered the changes indicated a special type of glomerulonephritis and named them glomerulo-

nephrosis. Bell (1932) described changes in the kidney in pre-eclampsia resembling acute glomerulo-nephritis. Baird and Dunn (1933) found the common lesion in fatal eclampsia to be glomerular with a degree of this lesion constituting the anatomical basis of albuminuria of pregnancy. Percutaneous kidney biopsy in the differential diagnosis of pre-eclampsia had been claimed to be of value. Dieckmann Potter and McCartney (1957) did not confirm this, finding non-specific changes in pre-eclampsia. Pollock and Nettles (1960) reporting on 50 cases of kidney biopsy in pre-eclamptic women, found only sixty per cent. correlation between clinical and pathological diagnosis. Differentiation between proteinuria of pre-eclamptic origin and renal disease by means of kidney biopsy and light microscopy would appear impossible although the electron microscopic findings of Spargo et al. (1959), Altchek (1961), Ishikawa (1961) and Pirani and co workers (1963) have shown changes in the

glomerulus specific to pre-eclampsia. Despite this advance an important number of cases did not correspond with the pathological findings. Lopez-Llera et al. (1965) reporting percutaneous biopsy in 63 toxaemic women, felt that kidney biopsy studies had definite limitation for differential diagnosis of pre-eclampsia. Apart from the limitations of the technique itself, there was lack of correspondence between the clinical and pathological diagnosis and ultimate prognosis.

It is no wonder that in these circumstances it is difficult to make a competent diagnosis in many cases. Stacey (1946) stated that proteinuria must be present for the diagnosis of pre-eclampsia and Tenney and Parker (1940) judged it to be the most accurate sign of pre-eclampsia. The onset of pre-eclampsia may be heralded by any of the three signs and it becomes difficult to differentiate pre-eclampsia from essential hypertension on the one hand and nephritis on the other. Essential hypertension

may be complicated by a degree of proteinuria. Equally urinary infection may be accompanied by hypertension. Nephritis may remain undiagnosed in the non-pregnant state and only become overt during pregnancy. Lastly pre-eclampsia may complicate any of these conditions.

If diagnosis is difficult, prognosis is doubly so. Attempts to correlate the signs of pre-eclampsia with maternal and foetal prognosis have met with failure. According to Dexter and Weiss (1941) the course of pre-eclampsia is variable, "Spontaneously or as a result of therapy the process may subside completely and remain absent during the rest of the pregnancy or the condition may become aggravated".

Literature on the subsequent follow up of pre-eclampsia is conflicting. Harris, (1924) Acosta-Sison (1931) and Gibberd (1929) stated that pre-eclampsia could give rise to subsequent chronic nephritis but the vast majority of workers including Doderlein (1925)

McKelvey (1935) have shown that chronic glomerulonephritis is not only rare in pregnancy but is also not a late effect of pre-eclampsia. Assali (1958) demonstrated the rapid disappearance of proteinuria within a few weeks of delivery.

Dieckmann and Brown (1939) concluded that 25 per cent. of patients with pre-eclampsia and eclampsia develop permanent hypertensive disease after pregnancy. However Light (1948) and Gibson (1956) have presented evidence that the incidence of residual hypertension is related to the age of the patient rather than to the duration of the pre-eclampsia and Chesley (1956) who originally was of the opinion that prolonged pre-eclampsia caused chronic hypertension, now believes that this predisposition was present prior to pregnancy. Throughout the literature there has been the continuing theme of possible renal factors in the aetiology, diagnosis and prognosis of pre-eclampsia. Various attempts have been made

to study the urinary proteins to see if changes analagous to nephritis could be domonstrated. The first studies were chemical in nature and carried out by Wallis in (1921) who applying refractometric and nephelometric tests to urinary proteins found an albumin globulin ratio of 2 to 1 in toxaemia and 6 to 1 in nephritis. Eastman (1931) detected high urinary globulin levels in eclampsia. Parviainen (1951) in a comparative study of urine and serum protein reported in the mild pre-eclamptics a decreased amount of albumin and increased alpha globulin but in severe pre-eclampsia the albumin was increased and the alpha globulin reduced in the urine as compared to plasma. Mack (1955) observed similar results and stated that electrophoretic analysis of urine in toxaemia as well as in other disorders with proteinuria have demonstrated all protein fractions found in blood excepting fibrinogen agreeing with the suggestion of Parviainen et al. (1949) although albumin

seemed to predominate in the urine of pre-eclamptics, the commonly employed term, albuminuria is incorrect and therefore ought to be changed to proteinuria. Lorincz et al. (1961) using paper electrophoresis also compared serum and urine proteins and revealed differences in urine patterns suggesting that this could provide a means of differentiating the various entities associated with proteinuria in pregnancy. Pfau et al. (1960) found a relatively lower urinary excretion of gamma globulin in severe toxaemia than in mild toxaemia. Immunological procedures were then tried. Glass et al. (1963) measured a ratio of albumin to total protein concentration by haemagglutination inhibition technique and found a higher ratio in pre-eclamptics than in the normal pregnant adult, but thought that measurement of other urinary proteins might prove to be of greater importance from a diagnostic standpoint and recommended further study of protein excretion patterns in pregnancy.

From the above it can be seen that not only is the part played by the kidney in pre-eclampsia uncertain, but the significance of proteinuria in pregnancy remains extremely obscure. For this reason it was decided to re-investigate the problem of proteinuria by using immuno-electrophoresis. This technique would enable a more precise definition of the proteins in the urine and possibly might provide a more recognisable pattern of excretion which would be of diagnostic help. It would also give an indication of the permeability of the kidney, and possibly of the nature of the process at work.

METHODS AND MATERIAL.

METHODS.

Detection of Proteinuria:

Proteinuria is initially detected by means of Albustix reagent strips (Ames & Company Division of Miles Laboratory Stoke Poges,Slough).

ALBUSTIX REAGENT STRIPS

These reagent strips are quick, simple standardized colour tests for protein in urine based on the protein error of indicators.

Composition:

The strip is of stiff absorbent cellulose, one end of which is impregnated with the indicator tetrabromophenol blue and buffered to pH 3.

Principle:

Tetrabromophenol blue, at pH 3, is yellow in the absence of protein but changes to a shade of green when protein is present depending on its type and concentration.

Directions:

1. Dip test end of the reagent strip in urine and remove immediately.

2. Compare colour of test end closely with colour chart at once.

   Test end turns shade of green at once POSITIVE

   Test end remains yellow NEGATIVE

   A colour block system is present on the strip in proportion to the amount of protein present representing Trace : less than 30 mg. protein, Intermediate : 30 to 100 mg. protein, Heavy : over 100 mg. protein.

This method of primary investigation is preferred to the heat test which can give equivocal results and also to the sulphosalicylic method which is more sensitive. In their evaluation of clinical methods for detecting proteinuria Rennie and Keen (1967) asserted that the Albustix test could be accepted with confidence, the intermediate reaction being indicative of a significant degree of proteinuria. The albustix test is carried out on a fresh mid-stream urine sample. All urines are subjected to a bacteriological count by the streak plate method. This allows detection of bacteriuria and provides controls

for the urinary tract infection cases.

Concentration of Urine:

Various estimations have been made of the amount of protein present in normal urine. 2.2 to 7.8 mg. per cent. (Mörner 1895); .5 mg. per cent. (Kolmer et al. 1945); 6 mg. per cent. (Everett et al. 1946); 3.7 mg. per cent. (Gunton et al. 1947); 10 to 30 mg. in 12 hours (Addis 1948) and up to 15 mg. per cent. in pregnancy (Wearing 1957). These protein levels in the urine are too low for protein separation by electrophoresis and to do so concentration is required. In addition, even when proteinuria is demonstrated by the Albustix method, the concentration is frequently too low for adequate electrophoretic separation and it may be impossible to demonstrate certain proteins. For these reasons concentration is also necessary with abnormal urines. Ordinary concentration methods of preparative chemistry cannot be applied to protein solutions as they easily denature. Several methods have been described

such as dialysis against the high molecular weight substances dextran or polyvidone. Both are expensive and impractical on a large scale. Ultrafiltration at high filtration pressures can be used but require special apparatus operating at high pressure and at these high pressures, proteins tend to be absorbed on to the filtering membrane. Low pressure ultra-filtration through dialysis tubing would appear to be the most suitable. No elaborate laboratory equipment is needed and neither protein denaturation nor loss of protein occurs, (Everall and Wright 1958).

Low Pressure Ultrafiltration: Method of Concentration:

After Everall and Wright 1958.

The dialysis material is visking tubing which is a seamless regenerated membrane with an average porosity of 24 angstrom units. The tubing is supplied by the Scientific Instrument Centre Limited, London W.C.1 and is permeable to water and will allow low molecular compounds

in aqueous solution to diffuse while refusing passage to molecules above 10,000 mol. wt. The tubing used in this work consisted of 500 cms. of 6 millimetre (8/32") diameter visking tubing previously immersed in distilled water for 30 minutes to soften. It is contained without external support in a 5 litre Buchner flask. One end of the tubing is tied and the other pulled over a glass tube which pierces the rubber bung of the flask. One litre of a twenty four urine collection or the total urinary output if the volume is less than 1000 ml. is run into the visking tubing and subjected to a negative pressure below 60 cms.of mercury using an Edwards high vacuum pump (Edwards Instruments Limited, Crawley, England). The dialysis process is conducted at 4^{o} Centigrade and a concentration between 100 and 500 times is obtained after 24 hours. A pressure guage is added to the pump system in order that any changes in pressure can be noted and corrected.

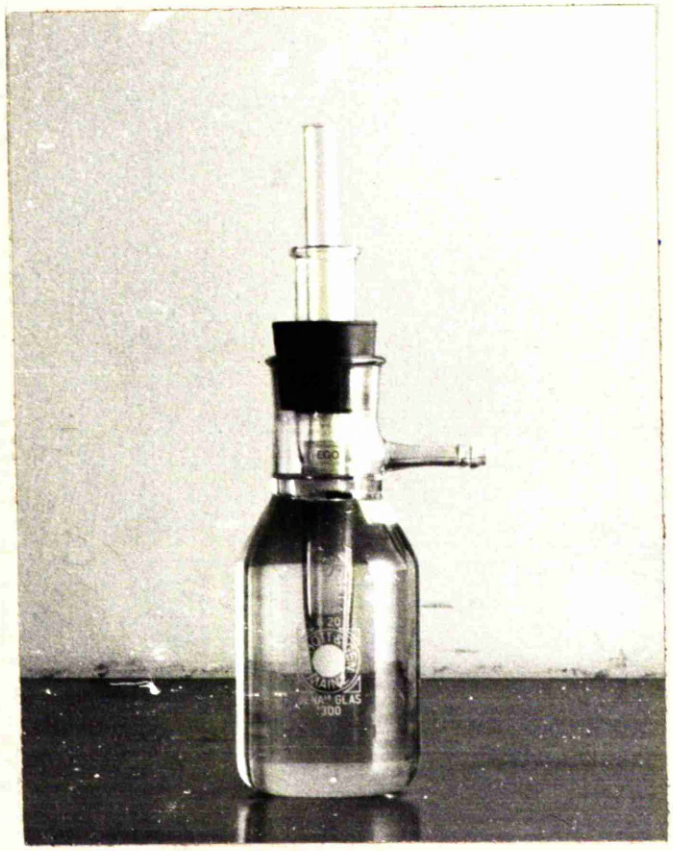
The residual volume of urine is measured and its protein content estimated by the biuret method. Although Berggard (1961), in his study on plasma proteins in the urine showed no alteration in precipitin pattern of urinary gamma globulin after the urine had been stored for 10 days at room temperature, there is no doubt that urine is a medium for bacterial growth, (Asscher et al. 1966) and that bacterial proliferation occurs during a twenty four hour collection. A quicker method to avoid any bacterial action on the proteins has been devised.

RAPID CONCENTRATION METHOD (After Goddard & Hobbs 1968).

A fresh 10 ml. mid-stream urine sample is placed in 15 ml. autoclaved Mackie McCartney containers using 17 mm. oxoid membrane filters to fit modified "Hemmings' Filters" and centrifuged at 3000 revolutions per minute for 10 minutes. These oxoid filters which will remove bacteria, are used in preference to the

FIG. I

APPARATUS FOR RAPID CONCENTRATION :
COLLODION MEMBRANE IN GLASS HOLDER
(SARTORIUS)



millipore filters used by Goddard and Hobbs which rupture easily. The urine is now dialysed at room temperature in a collodion bag contained in a Sartorius glass holder supplied by V.A.Howe London (FIG. I) against a 50 per cent. solution of 0.9 per cent. Sodium chloride (w/v) in barbital buffer pH 8.6 (Ionic strength 0.075) at a negative pressure of 60 cmsHg. Concentration to x 100 - 200 is obtained in 2 to 3 hours. The initial sample volume and volume of concentrate is recorded in order to calculate the degree of concentration. The removal of bacteria by the oxoid filters is confirmed by bacteriological culture of urine specimen before and after centrifugation. No change in protein content occurred by centrifuging.

Both the visking tubing and collodion membrane are easily traumatised with loss of sample. Reserve of urine to repeat the process is usually available for the collodion membrane method but not for dialysis with visking tubing. The number of proteins detectable in normal

urine has increased from 8 to 10 components (Grant 1957), (Patte et al. 1958), (Keutel et al. 1959) to over 20. (Bergarrd 1964). This is due not only to better identification by immuno-electrophoresis but is related to different methods and degrees of urine concentration. To identify proteins not previously found in normal urine Bergarrd (1964) concentrated urine 1000 to 2000 times.

The object of the present work is to try and make a comparative study of various states of proteinuria in pregnancy and if possible to express the findings quantitatively. While Bergarrd's method will demonstrate proteins present in very minute amounts, it is felt that with this degree of concentration the opportunity of comparing those proteins forming the main proportion of the urinary total is sacrificed. The main purpose is to isolate proteins covering a wide variety of molecular size, thus providing some index of renal permeability. A concentration of 100 to 300 times has been found sufficient

for this purpose and is in agreement with the protein content of 1.5g. per cent. suggested by Wolvius and Verskere (1955) as being suitable for filter paper electrophoresis. The concentration levels obtained by low pressure ultrafiltration with visking tubing and collodion membrane as described are within this range and allows identification of the more prominent proteins rather than a wide range of proteins present in minute fractions if the urine is concentrated to a greater degree.

Quantitative Test for Urinary Protein :

Colorimetric Method

Alkaline copper solution reacts with the peptide bonds in the protein molecule, producing a violet colour which is directly proportional to the amount of protein present (biuret reaction).

Reagents

20 per cent. trichloracetic acid.

1N. sodium hydroxide.

Standard protein solution of 500 mg. per 100 ml.

Store distributed into small volumes below 18°C.

Biuret reagent

Dissolve 9g. of potassium sodium tartrate in 500 ml. of 0.2 N. NaOH. Add 3g. of $CuSO_4$ $5H_2O$ and dissolve by stirring.

Now add 5g. of KI and make up to 1,000 ml. with 0.2 N. NaOH. Store in a polythene bottle.

METHOD:

1. To 1 or 2 ml. of urine add an equal quantity of trichloracetic acid.
2. Mix well and allow to stand for a few minutes.
3. Centrifuge.
4. Decant the supernatant fluid without disturbing the deposit.
5. Dissolve the precipitated protein in 1ml. of N. N aOH.
6. Add 2ml. of distilled water.
7. Add 5ml. of biuret reagent.
8. To 3ml. of distilled water and 3ml. of standard protein in each of two tubes add a further 5ml. of biuret reagent.

9. Mix all three tubes thoroughly and place them in the 37°C water bath for ten minutes for the colour to develop.
10. Allow the tubes to cool and compare the colours in a photo-electric absorpitometer, using a green filter or transmission at 540 m.

Use distilled water to zero the instrument.

Calculation:

T = Test reading. B = Blank reading S = Standard reading. V = Volume of urine used. 3 ml. of protein solution contain 15 mg. of protein.

$$\frac{T - B}{S - B} \times 15 \times \frac{100}{V} = \text{mg/100ml. of protein}$$

Therefore :

$$\frac{T - B}{S - B} \times \frac{1.5}{V} = \text{g. / 100 ml.}$$

Estimation of the daily protein excretion is calculated from the amount of protein in grams per cent. of a sample of urine and the twenty four hour urine volume. Twenty four hour protein excretion = $\frac{\text{Amount in grams}}{100} \times$ 24 hour urine volume (ml.)

Protein Separation:

The first electrophoretic study on protein in normal urine was published in 1951 by Rigas and Heller. The urine was concentrated by ultrafiltration and the moving boundary technique of electrophoretic separation employed. Fractions with mobilities corresponding to those of the main serum proteins were identified. The albumin/globulin ratio appeared reversed and there was poor separation in the globulin range. Slater and Kunkel (1953), using paper electrophoresis in urine for the first time showed that pathological urines contained all the main electrophoretic fractions present in the serum.

McGarry et al. (1955), obtained similar separation both with free and paper electrophoresis.

The latter has several disadvantages, (Jencks et al. 1955). There is irreversible absorption of albumin on paper during its migration i.e. albumin tailing. Variation in

the dye binding capacity occurs with the amount and area of application of the protein, with fading and incomplete elution of the commonly used dyes bromophenol blue and amidoschwarz 10B and lack of linear relationship of the dye in paper.

As a medium for electrophoresis a gel offers several advantages over the former method using filter paper.

The outline of protein bands is better and there is less retention of the protein molecules (Peetoom 1963).

MEDIA

The following gel forming substances were considered: pectin, starch, cellulose acetate, agar. Good separation is obtained with pectin which has also the property of being dissolved by enzymes allowing isolation of already electrophoretically separated protein fractions. The enzymes however may be inconsistent in their action and thus require analysis before use. Starch gel provides good protein separation but as it is opaque, it is useless for immuno-

electrophoresis. It has also been found to give electrophoretic mobilities different from those observed in liquid media. Membrane constructed of cellulose acetate is another alternative but is more difficult to use than agar and may exert a certain "filtering effect" and hence macromolecular proteins may be retarded in their movement. Agar was selected as the gel former. Although technical skill is needed in its use, with practise this can be overcome and on account of its high liquid content it does not impede electrophoretic transport. Its great advantages is in its transparency allowing identification of specific precipitation reactions which can be recorded photographically.

Agar - Electrophoresis

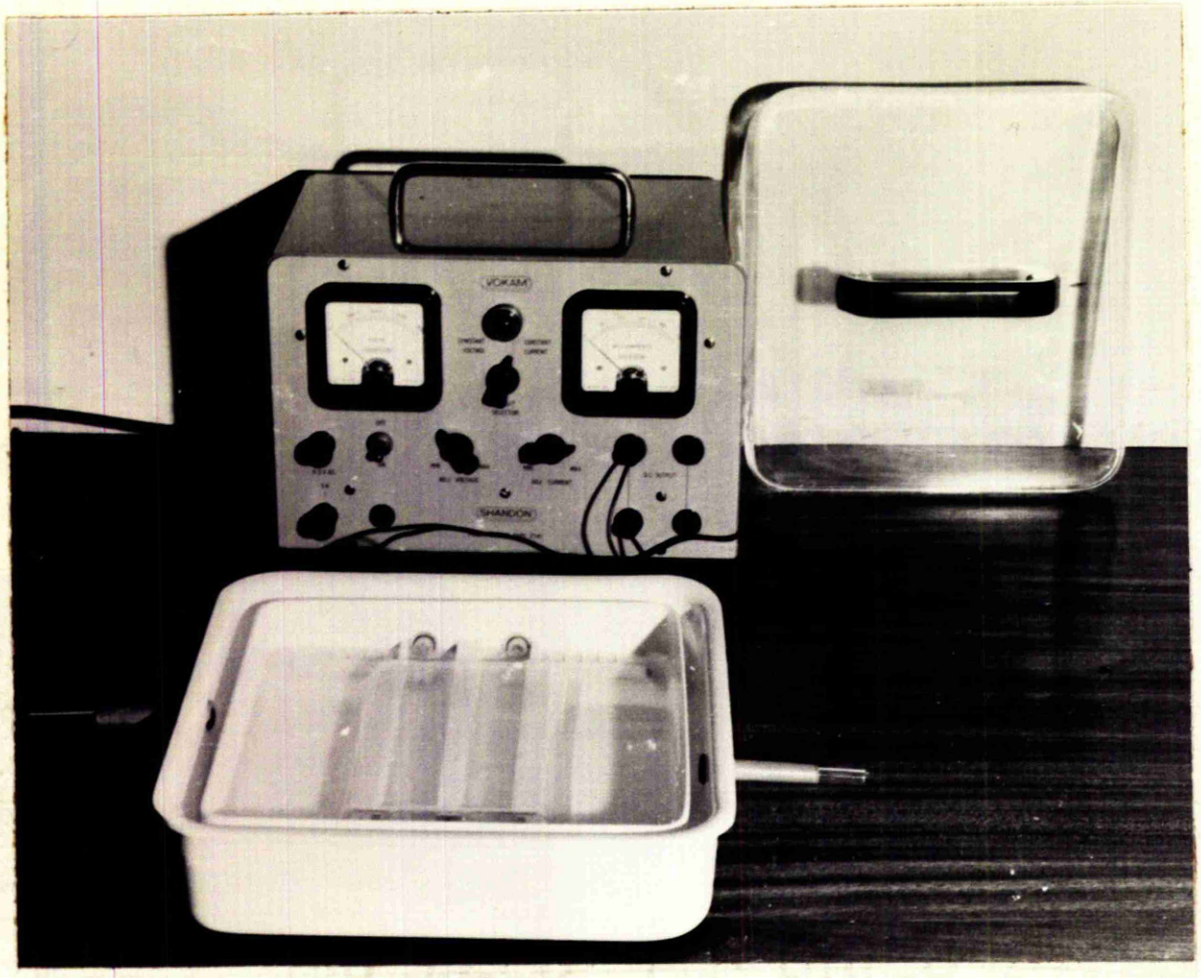
By preliminary trials 1 per cent. Agar gel was found to produce an elastic but firm gel. Powder agar is dissolved in heated buffer solution, sodium barbitone hydrochloride buffer pH. 8.6 ionic strength 0.075 avoiding prolonged

heating which denatures the agar. The resultant liquid if not absolutely clear is quickly filtered by suction through a double layer of Whatman's filter paper in a buchner flask. Latterly immuno-diffusion agar tablets (Oxoid Limited, London S.E.) were used, replacing the powder agar as the quality of agar gel obtained with the tablets did not necessitate filtration.

A uniform layer, 0.2 cm. thick, of buffered agar is applied to a photographic plate 16 x 9.5 cms., free of defects and cleaned with care. A good horizontal base for this is obtained by pouring a 5 per cent. molten agar layer into a dish fixed firmly on a level bench, placing the photographic plate on the agar base when set and then applying the agar buffer solution.

Filter paper wicks 4 cms. wide are placed at each end of the glass plate with 1 cm. of the filter paper resting on the glass plate. The strips are pressed to the underlying agar base after the molten agar has been poured and

FIG. II Immuno-electrophoresis Apparatus :
Shandon Power Pack and Bath



thus fixed in this position.

After one hour at room temperature the agar is set and the plate with filter wicks is detached from the agar base by incising the periphery. In addition, the agar is cut slightly at the edge of the glass so that the protruding strips of filter paper can hang perpendicular. This incision is refilled with molten agar to restore continuity after the plate has been placed on a block in the electrophoretic bath with the filter paper wicks immersed in the buffer of the electrode compartments. (FIG II)

The buffer bath consists of two oblong plastic tanks each containing a platinum electrode and sodium barbitone hydrochloride buffer pH 8.6 ionic strength 0.075 which is discarded after each electrophoresis. The source of electricity is a Shandon Power Pack (Shandon Lab., London N.W.10) at constant voltage. A direct current supplying 80 volts at 50 milliamps produces separation of protein

over a distance of 2.5 cms. per hour. Application zones 1 cm. apart are cut in the agar plate, 4 cms. from one edge, with a circular metal tube 0.2 cm.. in diameter, the agar being removed by suction. The urine concentrate is then pipetted into the application holes and the current switched on. The lid of the electrophoretic bath is then placed in position to prevent drying of the agar when exposed to room temperature. The electrophoresis is completed between $2\frac{1}{2}$ to 3 hours and subjected to specific antibodies, the process of immuno-electrophoresis, a technique developed by Grabar and Williams (1953). The principle of the method is electrophoretic separation of mixtures in a gel medium followed by diffusion of precipitating antibodies into the same gel at right angles to the direction of the electrophoresis. This is a macro-method and a modification of Scheidegger's micro-method (1955) is used. The advantage over Grabar and

William's method is that the amount of anti-serum and urine required is less. Grooves are cut, 0.2 cm. in width, at right angles to the application zones and equidistant from them. This is performed with the aid of cm. divided into mm. graph paper placed below the glass plate. The agar strips are removed and a thin film of molten agar is pipetted along each groove, in order to prevent any escape of fluid between the agar and the glass plate. The specific antisera is then pipetted into each groove. The following antisera are used :-

1 Goat antisera to whole human serum (Hyland Laboratories, Los Angeles California).

2 Specific plasma protein antisera (Behringwerke A.G. Marburg Lahn).

Rabbit anti-albumin

Rabbit anti-transferrin

Rabbit anti-gamma G globulin

Rabbit anti-alpha$_2$macroglobulin

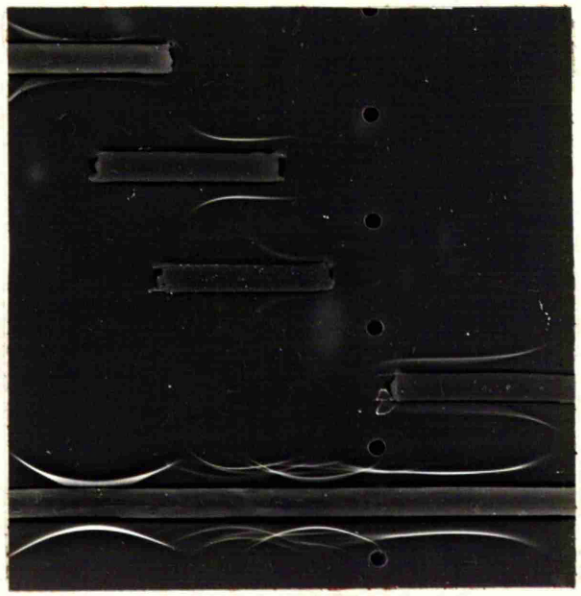
Within 24 hours at room temperature the precipitin areas are fully developed. These are most clearly outlined around 18 hours which is the optimal time for obtaining a permanent record. The arcs of the larger molecular weight proteins may be rather faint at this time and improvement in their intensity is obtained by washing the plates in physiological saline for several days although clarity of outline decreases.

Identification of Proteins:

Initially to identify all possible protein fractions which could occur in the urine, pregnant female serum is electrophoresed and subjected to goat antisera to whole human serum (Hyland). (FIG. III). Each protein band is identified and this reference chart kept for identification purposes of the immuno-electrophoretic patterns found in the urine. Pregnant female serum is also subjected after electrophoresis to specific protein antisera anti-albumin, anti-transferrin, anti-gamma G globulin and anti-alpha$_2$macroglobulin

FIG. III.

Immuno-electrophoresis Serum: Proteins



Albumin

Alpha$_2$macroglobulin

Transferrin

gammaGglobulin

Widespectrum

Human pregnant serum has been electrophoresed. In the lowest trough, wide range goat anti-serum to whole human serum has been applied and precipitin arcs to proteins present in human serum obtained. In the other troughs rabbit-antisera to specific proteins has been applied for accurate identification of these proteins on the electrophoresis.

(Behringwerke) so that the exact position of these proteins in the electrophoretic run is known. The same procedure is carried out in urine. Following electrophoresis it is subjected to goat antisera to whole human serum (Hyland) and then to the specific antisera (Behringwerke) Rabbit anti-albumin, rabbit anti-transferrin, rabbit anti-gamma G globulin and rabbit anti-alpha$_2$macroglobulin. These antisera are applied at the position on the electrophoretic run where the proteins have been found to separate from the serum chart.

Photographic Technique

Contact printing is employed. As the agar gel is clearly transparent, the precipitin lines are visible and can be recorded by direct exposure. Kodak 4 S.G./ 3.S. Bromide photographic paper is used. The agar plate, covered with a layer of distilled water, is laid on the

printing paper. A six second exposure is given and within two minutes of being immersed in developer fluid, precipitin lines appear. The photographs are then fixed, washed for 30 minutes and dried.

Protein Selectivity measurement by immuno-diffusion:

To determine quantitative variation in the pattern of protein excretion.

The principle of the method, demonstrated by Mancini (1963), involves radial diffusion of antigen into buffered agar, in which has been mixed antiserum raised against the appropriate protein. A disc of antigen antibody precipitate is formed.

Two methods have been employed :-

1. Commercially prepared : Immunodiffusion plates : Behringwerke
2. Plates prepared under standard conditions.

FIG. IV Immunodiffusion Plates

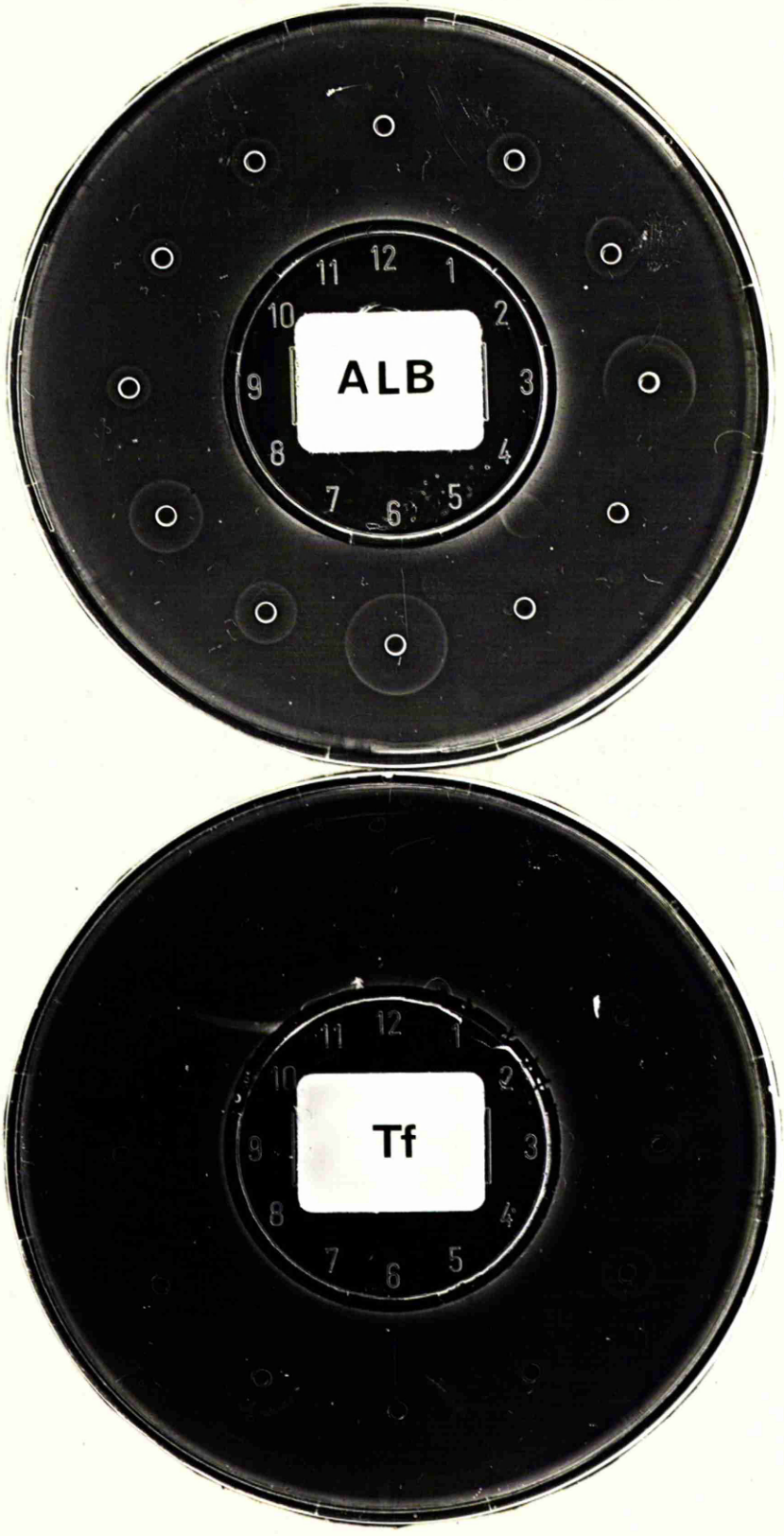
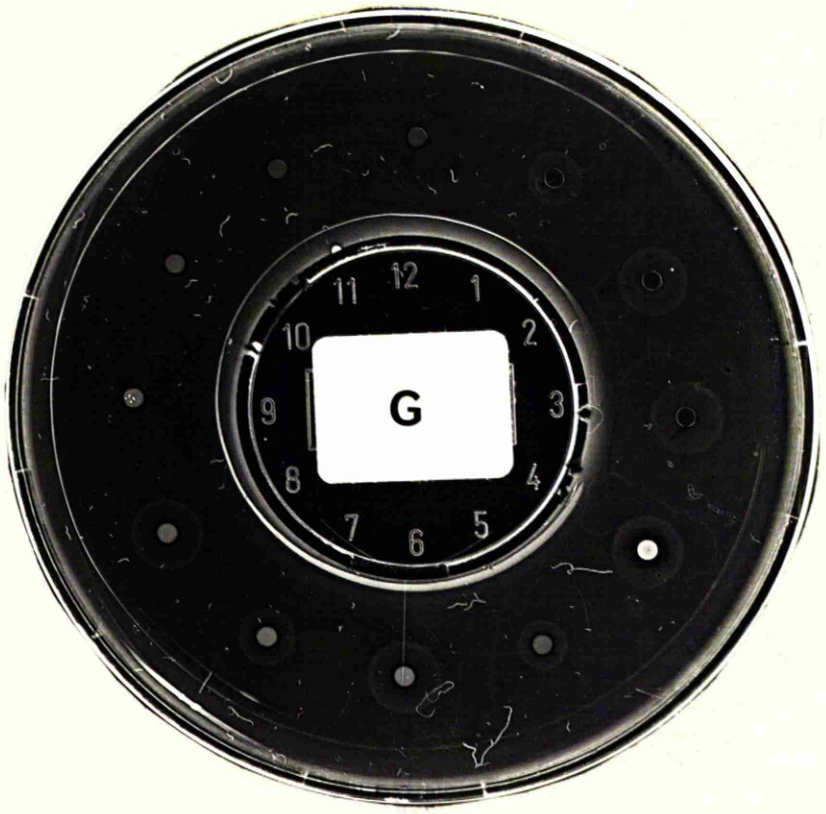


FIG. IV Immunodiffusion Plate



METHOD I

Immunodiffusion plates containing anti-albumin (alb), anti-transferrin (Tf.) anti-gamma G globulin (G) are used. (FIG. IV) (Behringwerke A.G. Marburg Lahn)

Each plate consists of a prepared agar gel bed incorporating a specific antiserum with 12 cylindrical wells for the application of the antigen solution: in this work urine and heparinised plasma. Numbers for recording purposes are moulded at the centre of the disc, and plates are enclosed in a waterproof-cover which holds a small amount of water in order to keep the agar gel moist. Each plate has a tight lid as it is important to prevent the gel from drying.

The wells are filled with exactly 2 microlitres by means of a 10 microlitre Hamilton graduated microsyringe (Scientific Glass Engineering Pty. Limited, Melbourne, Australia) The technique is as follows :- 2 cms. of P.T.F.E. Sleeving (T.W.T/26 VACTITE) supplied by Vactite Wire Company Limited, Bootle, Lancs.

is attached to the needle of the syringe. The plunger of the syringe is first raised to the 1 microlitre mark. The sleeving is then inserted into the sample and the plunger is raised to the 3 microlitre mark allowing 2 microlitres of sample to enter the sleeving. The plunger is now pushed down ejecting the sample in the form of a bleb at the end of the sleeving. The plate, placed on a moveable platform is raised into position with the application well maneouvered directly underneath the bleb which on touching the foot of the well spreads evenly with no loss of sample due to scatter of the fluid. The necessity of cleaning the syringe after each injection is avoided by use of the sleeving which is just discarded after each ejection.

METHOD 2

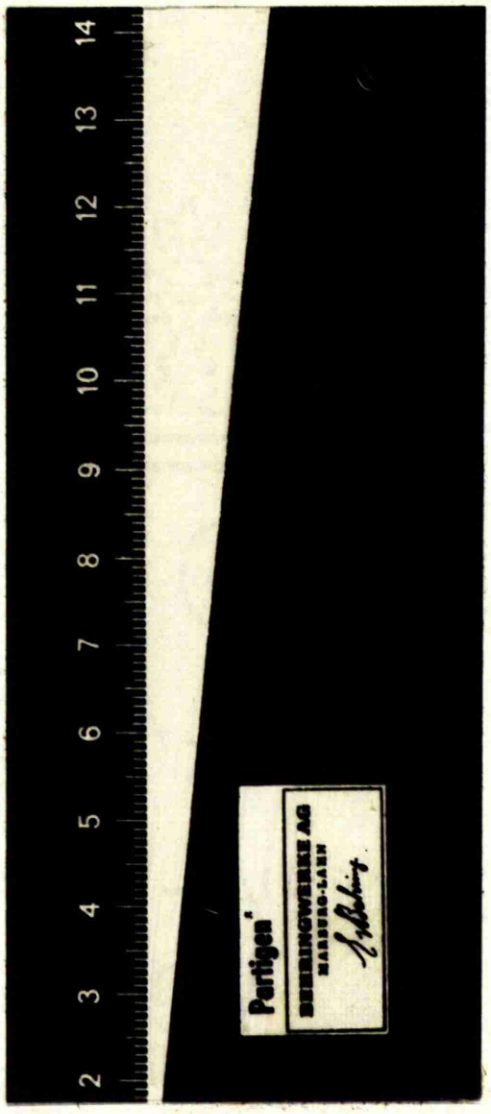
Prepared Plates

The agar gel bed is prepared in a manner similar to immuno-electrophoresis.

Three photographic plates 6 x 8.5 cms. are placed on an agar base in a plastic tray 19 x 9 x 3 cms. One oxoid agar tablet is dissolved in 50 ml. sodium barbitone hydrochloride buffer and heated until transparent. It is then cooled to 55°C when the specific antiserum is added and mixed. The resultant mixture is quickly poured over the photographic plates to give an agar gel bed of 0.2 cm. thickness. When set, one hour later, the plates can be cut out of the agar base and circular holes, 0.2 cm. in diameter, punched out by means of suction with 0.2cm. circular metal tube. After filling the wells, with a Hamilton syringe as described the plates are placed in a humidity chamber to prevent drying. In general, diffusion of the antigen is complete at 48 hours. If precipitates are too weak to

FIG. V

Measuring Ruler Calibrated in mm.



furnish clear results, the plates are washed for 3 days with physiological saline, the solution being changed several times per day. The diameter of the precipitin rings is measured in two directions with a ruler calibrated in mm. (FIG. V).

Thirty six estimations can be performed with the one modified plate (Method 2) compared with 12 using the commercially prepared plate and thus the former is less expensive. The disadvantage of the second method is that the potency of antiserum is unknown and may vary and that the volume required to produce satisfactory ring formation is determined by trial and error which is time consuming and wasteful in material.

Urinary clearances of albumin (molecular weight 69,000) transferrin (molecular weight 88,000), gamma G globulin (molecular weight 160,000) are assessed by estimations on samples of heparinised plasma and mid-stream specimens of urine taken simultaneously. A 10 ml. urine

sample is concentrated by the rapid concentration method. Both urine and plasma samples are stored at -20°C and thawed prior to use. The following dilutions of plasma are used 1/50, 1/100 for anti-albumin plates, 1/2, 1/4 for anti-transferrin and anti-gamma G globulin plates. The dilutions of urine required are neat, 1/10, and urine concentrate depending upon the total urine protein measured by the biuret method.

Urine Protein	Urine Dilutions for Immunodiffusion Plates		
g per cent.	Anti Albumin	Anti Transferrin	Anti IG
0.5 - 1 gm.	NEAT 1/10	NEAT	NEAT
0.1 - 0.5 gm.	NEAT	concentrate	concentrate

These dilutions are such as to yield precipitin rings between 4 mm. and 10 mm. and have been found by trial and error. The wells are then filled as described using the Hamilton microlitre syringe. At 48 hours the precipitin rings are fully developed and the diameters are measured in mm.

The clearance of IgG (i.) and Albumin (Alb.) are then expressed as a proportion of the transferrin (t.) clearance.
This enables the volume of urine to be ignored.
The calculation is made directly from the U/P ratios.

$$\text{Albumin clearance} \quad \left(\frac{UV}{P}\right)_{Alb.} \times \left(\frac{P}{UV}\right)_{t.}$$

$$\text{Gamma G globulin clearance} \quad \left(\frac{UV}{P}\right)_{i.} \times \left(\frac{P}{UV}\right)_{t.}$$

Material:

Section I

Five categories of patients have been studied.

1. Normal patients.
2. Patients with pre-eclampsia.
3. Patients with urinary tract infection.
4. Patients with nephrosis.
5. Patients with hypertension.

1. Normal Patients:

These were women who had been admitted to hospital in the last month of pregnancy to await delivery for obstetrical reasons. Blood pressure was normal in all cases and the Albustix test for the presence of protein in the urine was negative. Bacteriological count of mid-stream specimens of urine showed less than 10,000 bacteria per ml.

2. Pre-eclampsia:

Thirty five patients were investigated. Proteinuria and hypertension were present in all and in ten cases there was oedema

requiring diuretic therapy. The group was divided into moderate and severe pre-eclampsia by the degree of hypertension, the condition being moderate if the diastolic blood pressure was below 100 mm. Hg and severe if above 100 mm. Hg.

Moderate Pre-eclampsia

This consisted of fifteen cases aged between 20 and 38 years with an average age of 26. There were 9 primigravidae and 6 parous patients. Commencement of study was from 33 weeks onwards and on average from the thirty eighth week of pregnancy.

Severe Pre-eclampsia:

Ten primigravidae and 10 multiparous patients aged between 18 and 40 with an average age of 29 were investigated. The average stage of pregnancy at which study was commenced was thirty weeks.

3. Urinary Tract Infection:

Five cases were studied. The diagnosis of urinary tract infection was made on a clinical history and confirmed by examination of a mid-stream specimen of urine. A positive diagnosis was based on the presence in a direct film of more than 20 white blood cells cmm. and greater than 10,000 bacteria per ml. cultured. The patients' ages ranged from 19 to 30 years with an average age of 22. There were two primigravidae and three parous patients. The earliest study was at 18 weeks and the latest at 31 weeks.

4. Nephrosis

Four patients between 22 and 28 years with an average age of 26 were investigated; two were parous patients and had been noted to have proteinuria in their previous pregnancy. Proteinuria was detected in three cases in the first half of pregnancy and in the fourth when first seen at 36 weeks.

The diagnosis was a clinical one based on the presence of persistent proteinuria of more than 2g. daily detected prior to pregnancy. Oedema was not present and blood urea was raised to 50 mg. per cent. in one of the primigravidae. Initial study was begun in three cases in the first half of pregnancy and in the fourth case at 36 weeks.

5. Hypertensives:

The patients in this group had been hypertensive since first seen and gave a history of hypertension in a previous pregnancy. Seven women, one primigravida, the rest multiparae were studied, aged between 23 and 47 with an average age of 36. There was no clinical evidence of oedema. Protein was not detected in the urine by the Albustix test. Initial investigation ranged from 20 to 31 weeks, slightly earlier than the pre-eclamptic group.

Scope of Investigation:

Subsequent to their initial investigation each case was studied at montly intervals during the first half of pregnancy and thereafter every two weeks till 36 weeks, when weekly studies were performed and sometimes daily prior to commencement of labour and induction. A specimen was collected immediately after delivery. Contamination with lochial discharge invalidates this result and a further specimen was obtained at about the seventh day. If resolution had occurred a final specimen was obtained at six weeks but if proteinuria persisted, weekly collections were started. Investigations in all patients in this section consists of subjecting a concentrated urine specimen to immuno-electrophoresis with goat antisera to whole human serum (Hyland Lab.)

Section II

In this section a more detailed study was conducted in a group of patients with proteinuria. Thirteen women were studied, ten primigravidae and three multiparae. Two

of the latter had proteinuria in a previous pregnancy and three primigravidae had nephrosis. The remaining eight women had pre-eclamptic toxaemia of varying degree, two having eclampsia. The patients were selected from among those whose urines gave a positive test with Albustix on routine examination at ante-natal clinic, although the date of onset of proteinuria was unknown in most cases since many of the patients did not come under observation until the latter part of pregnancy. Each case was studied weekly and in most cases daily before and after delivery. Investigation in this section consists of immuno-electrophoretic analysis as carried out in section I but with four separate electrophoretic runs to which were applied anti-albumin, anti-transferrin, anti-gamma G globulin and anti-alpha$_2$macro-globulin subsequent to the wide range anti-serum to whole human serum run. It was thus possible to detect the presence of individual proteins with molecular weight

ranging from 70,000 to 900,000.

Section III

In this group of patients, the urinary protein pattern determined by immuno-electrophoresis as in Section II, is correlated with protein selectivity. Protein clearances are performed on albumin, transferrin and gamma G globulin in 14 patients. Two with proteinuria alone, one primigravid and one parous patient aged 23 and 29 years, the other 12 women had pre-eclampsia in varying degrees, 4 moderate in severity, 8 severe in type three of whom were eclamptic. The four cases with moderate pre-eclampsia were 3 primigravidae and 1 parous patient ranging from 19 to 30 years with an average age of 23. Of the eight women with severe pre-eclampsia two were multiparous patients and six were primigravidae aged between 19 and 39 with an average age of 22.

A minimum of three clearances, two before delivery and one post-natally was performed in all cases.

RESULTS

Section I

Normal Patients:

In this group of twenty patients, blood pressure ranged between 90/50 mm. Hg. and 100/80 mm. Hg. The minimum twenty four hour urine protein excretion was 0.08g. and the maximum 0.4g. with an average of 0.15g. Immuno-electrophoresis of the urine after concentration revealed trace amounts of albumin. These patients were studied over periods of five days at various stages of gestation from 33 to 41 weeks. Precipitin arcs were detected to albumin only which were too faint for photographic illustration.

Pre-eclampsia:

Moderate Pre-eclampsia.

In the fifteen cases studied, blood pressure level was between 130/90 mm. Hg. and 140/100 mm. Hg. Oedema was demonstrated clinically in seven cases which were given diuretic therapy. Blood urea level ranged between 12 and 25 mg.

per 100 ml. with an average of 14 mg. per 100 ml. The twenty four hour urinary protein excretion varied between 0.01 and 1.8g. with an average of 0.45g. Six proteins were identified in the urine concentrates by immuno-electrophoresis Albumin, Alpha$_1$ glycoprotein, transferrin, ceruloplasmin, haemopexin and gamma G globulin. The quantity of urinary protein in the cases of moderate pre-eclampsia remained relatively constant. Similarly there were no qualitative changes in the number of precipitin bands, being unvaried until the total protein diminished. In all cases by the seventh post partum day only a trace of albumin with occasional traces of transferrin and gamma G globulin were detected on immuno-electrophoresis of the concentrated urine sample.

The following are illustrative cases of moderate pre-eclampsia.

Mrs. K. Para 0+0 Aged 17 Diagnosis : Moderate Pre-eclampsia

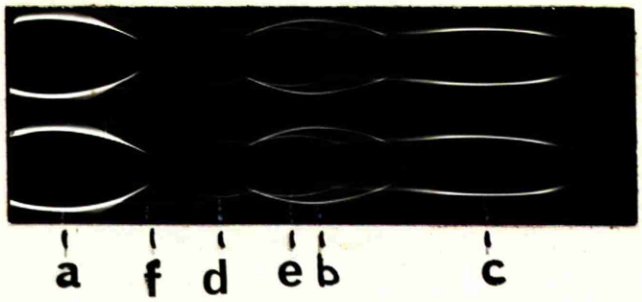
No serious illness.

First seen at 38 weeks. Blood pressure 130/85 mm. Hg. Albustix positive. Urinary protein 0.05 g. per cent. Immuno-electrophoresis showed albumin, transferrin and gamma G globulin, ceruloplasmin, haemopexin and alpha$_1$ glycoprotein (FIG. I). This pattern (FIG.II) remained until after delivery at term of a live female child weighing 4 kilos. Immuno-electrophoresis six days after delivery revealed only traces of albumin and transferrin (FIG.III). Blood pressure was normal. 0.001 g. per cent. protein was present and albustix was negative. The patient did not return to the post-natal clinic at six weeks.

Comment: The immuno-electrophoresis (FIG.I) demonstrates the urine protein pattern in moderate pre-eclampsia with an increase in number of proteins ante-natally and a rapid decrease after delivery. (FIG.III).

MRS. K. (O+O) Aged 17 Moderate Pre-eclampsia

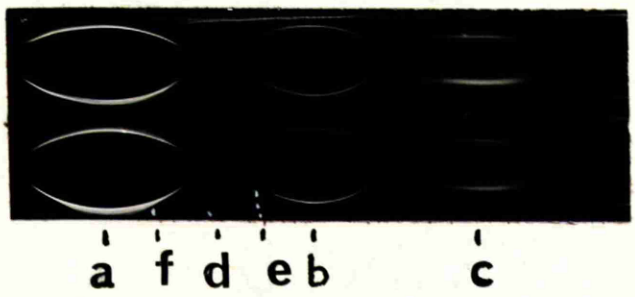
Immuno-electrophoresis Fig. I



a = albumin, b = transferrin, c = gamma G globulin

d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein

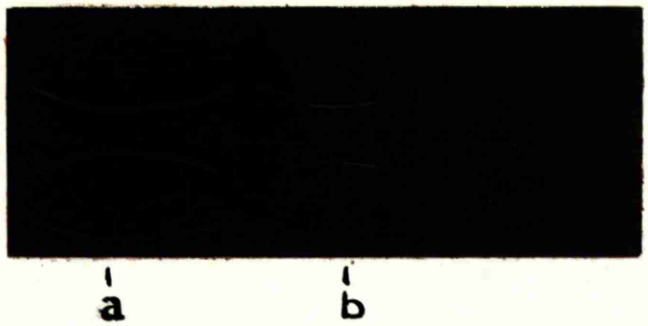
Immuno-electrophoresis Fig. II



a = albumin, b = transferrin, c = gamma G globulin

d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein

Immuno-electrophoresis Fig. III



a = albumin, b = transferrin

Mrs. G. Para 0+0 Aged 18 Diagnosis : Moderate Pre-eclampsia

No serious illness.

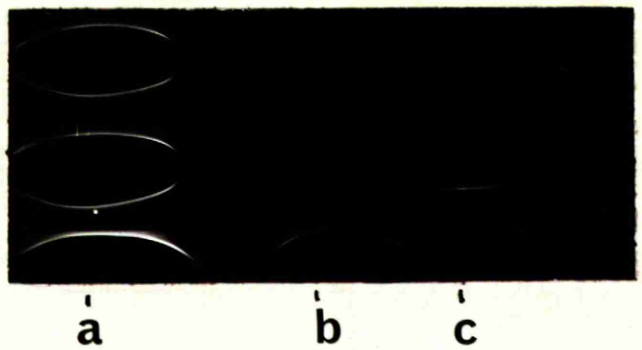
First seen at 16 weeks, blood pressure 120/80 mm. Hg. Poor attender at clinic and defaulted three times, however when seen at 32, 34 and 36 weeks blood pressure was normal and albustix negative. At 38 weeks blood pressure rose to 130/90 mm. Hg. and there was a trace of protein by albustix. Urine protein was 0.02 g. per cent. Immuno-electrophoresis revealed albumin, transferrin and gamma G globulin (FIG. I). Diastolic pressure remained between 85 and 95 mm. Hg. Urinary protein level rose to 0.032 g. per cent. Immuno-electrophoresis revealed an increase in the number of precipitin bands with the presence of ceruloplasmin, haemopexin and alpha$_1$ glycoprotein (FIG. II). Spontaneous labour occurred one day after expected date of delivery of a live male child weighing 3,2 kilos. Immuno-electrophoresis two days later, showed a very much increased number of proteins. This was due to lochial contamination of the

urine specimen and serum proteins were demonstrated (FIG. III). By the seventh day, only albumin and transferrin could be detected by immuno-electrophoresis (FIG. IV). Blood pressure was normal. The patient failed to attend the post natal clinic.

Comment:- This case illustrates the increased number of protein bands found in pre-eclampsia as this condition becomes manifest. Post delivery immuno-electrophoresis (FIG. III) demonstrates the caution that must be exercised to ensure that the urine is free of blood, producing a false urine protein pattern. FIG. (IV) gives a true picture of the rapid return to normal in moderate pre-eclampsia after delivery.

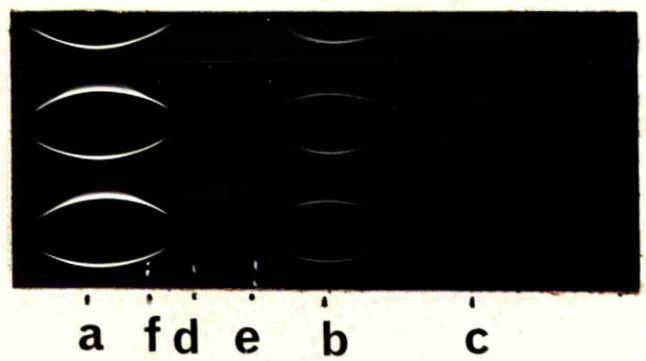
MRS. G Para (0+0) Aged 18 Moderate Pre-eclampsia

Immuno-electrophoresis Fig. I



a = albumin, b = transferrin, c = gamma G globulin

Immuno-electrophoresis Fig. II

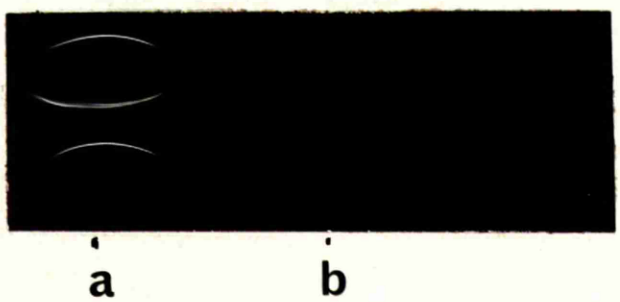


a = albumin, b = transferrin, c = gamma G globulin
d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein

Immuno-electrophoresis Fig. III

Serum Proteins

Immuno-electrophoresis Fig. IV



a = albumin, b = transferrin

Severe Pre-eclampsia:

In these twenty cases, blood pressure readings ranged between 145/95 and 180/100 mm. Hg. Oedema was present in four patients who were given diuretic therapy. Blood urea ranged between 16 mg. per 100 ml. and 48 mg. per 100 ml. with an average of 32 mg. per 100 ml. Twenty four hour urinary protein excretion ranged between 0.34 g. to 20.9 g. with an average of 4.8 g. Immuno-electrophoresis of the urine concentrate revealed the same six bands as in the moderate pre-eclampsia group but in addition gamma A globulin and traces of $alpha_2$ macroglobulin could be recognised. In seven cases, this picture represented a progressive increase in the number of bands from an initial moderate pre-eclamptic pattern. The maximum number of precipitin bands in all cases was observed just before termination of the pregnancy or occurrence of intra-uterine death, the main feature being the appearance of $alpha_2$ macroglobulin. Within twenty four hours of delivery in all cases $alpha_2$macroglobulin had

disappeared and in the majority of cases only albumin, transferrin and gamma G globulin remained. By six weeks, only traces of albumin were observed with occasional faint arcs to gamma G globulin and transferrin in some cases. An example of severe pre-eclampsia is now described.

Mrs. W. Aged 33 Para 0+0 Diagnosis : Severe Pre-eclampsia

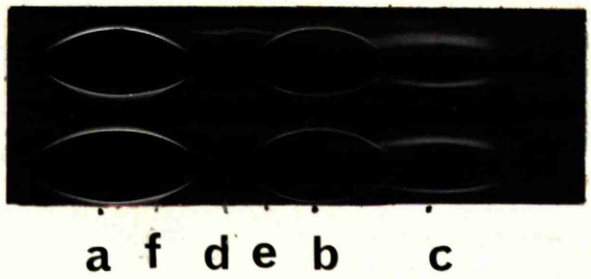
Apart from appendicectomy no serious illness. First seen when fourteen weeks pregnant, blood pressure normal, no proteinuria. Patient referred to her own doctor for ante-natal care for the next sixteen weeks. On her return visit at 30 weeks, blood pressure was normal, albustix negative. Two weeks later, blood pressure had risen to 140/100 mm.Hg. Intermediate reaction with albustix was present at a urine protein level of 0.5 g. per cent. Patient was admitted to hospital. The size of the uterus was also thought to be small for dates. Immuno-electrophoresis (FIG. I) pattern showed 6 precipitin bands, albumin, transferrin, gamma G globulin, ceruloplasmin, haemopexin and alpha$_1$ glycoprotein. Blood pressure remained elevated during the next five weeks despite sedation, the highest blood pressure being 170/120 mm.Hg. Heavy protein by albustix was present at a level of 0.5 g. per cent. Immuno-

electrophoresis picture (FIG. II) was similar to FIG. I, but no alpha$_2$macroglobulin could be seen. At 37 weeks labour commenced spontaneously, after five hours foetal distress occurred and Lower Segment Caesarean Section was performed resulting in delivery of a live female child weighing 2 kilos. Five days after delivery, blood pressure was normal. Albustix was negative at a protein level of 0.01 g. per cent. and the immuno-electrophoresis pattern showed traces of albumin, transferrin and gamma G globulin (FIG. III).

Comment:- The urinary protein pattern again illustrates the increased number of bands found in pre-eclampsia. The large molecular weight proteins were not seen and the foetal outcome was good. Despite the duration of the pre-eclampsia for over 7 weeks, within 5 days, there was reversal to the normal urine protein pattern.

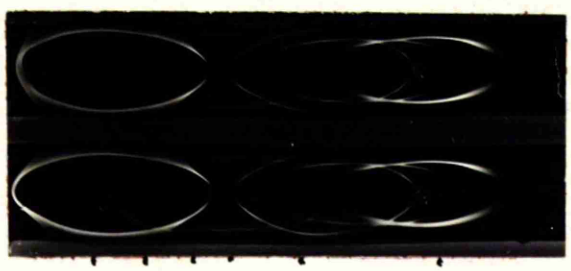
MRS. W. Para (0+0) Aged 33 Severe Pre-eclampsia

Immuno-electrophoresis Fig. I



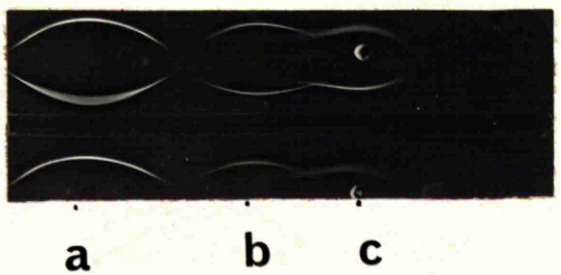
a = albumin, b = transferrin, c = gamma$_G$globulin
d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein

Immuno-electrophoresis Fig. II



a = albumin, b = transferrin, c = gamma$_G$globulin
d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein

Immuno-electrophoresis Fig. III



a = albumin, b = transferrin, c = gamma G globulin

Proteinuria associated with urinary tract infection.

There were five cases in this group. Significant bacteriuria in a mid-stream specimen of urine was present in all cases in association with a twenty four hour urinary protein excretion between 0.03 and 0.32g. with an average of 0.19g. Blood pressure and blood urea was normal in all cases. Immuno-electrophoresis of urine concentrate revealed trace amounts of albumin, transferrin and gamma G globulin with the density of the precipitin arc to gamma G globulin equal to that of the albumin arc. Following treatment of the urinary tract infection, the amount of protein decreased in all cases and the precipitin bands, albumin, transferrin, gamma G globulin became very faint. In one case hypertension developed with an increase in the protein excretion. Bacteriological culture of the urine was negative and thus pre-eclampsia had occurred with appearance of albumin,

transferrin, gamma G globulin, haemopexin, alpha$_1$glycoprotein and ceruloplasmin on immuno-electrophoresis of the urine concentrate. Three cases of urinary tract infection are described with illustrative immuno-electrophoretic tracings.

Mrs. J. Para 0+1 Aged 19 Diagnosis : Urinary Tract Infection

No previous history of urinary infection. Aborted in first pregnancy at 14 weeks. Admitted in present pregnancy at 24 weeks with symptoms of pyelonephritis. Mid-stream specimen of urine grew more than 100 white cells, 75,000 bacteria per ml. cultured. No protein was detected by albustix, 0.02 gm. per cent. protein present. Immuno-electrophoresis of urine concentrate revealed three precipitin bands: albumin and trace amounts of transferrin and gamma G globulin (FIG. I). A 5 day course of ampicillin 500 mg. six hourly followed by long term nitrofurantoin 100 mg. thrice daily was given. After three weeks in hospital the patient was discharged only to be re-admitted one week later in premature labour. An intermediate protein reaction was present on albustix, 0.05 g. per cent. protein was present. Bacterial count of mid-stream specimen of urine was 1000 white cells 400,000 bacteria per ml. were cultured. Immuno-electrophoresis

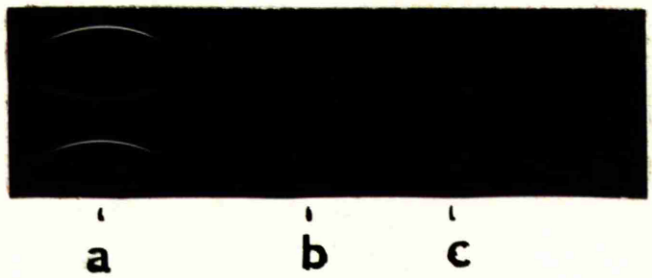
of the urine concentrate showed precipitin areas to albumin, transferrin and gamma G globulin (FIG. II) with an increase in density of the gamma G globulin band from the previous admission. Chemotherapy was changed from nitrofurantoin to nalidixic acid although sensitivity tests of the cultured urine showed it to be still sensitive to nitrofurantoin but resistant to ampicillin. Labour did not become established and the patient was discharged home at 30 weeks to attend the ante natal clinic. She remained symptom free till 38 weeks when re-admitted for the third time again in false labour. Albustix test for protein was negative. .02 gm. per cent protein was present. Bacterial count of a mid-stream specimen of urine was less than 20 cells. Immuno-electrophoresis revealed arcs to albumin, transferrin and gamma G globulin with a decreased density of gamma G than when last admitted (FIG. III). Nalidixic acid therapy was continued and the patient went

home after five days to be re-admitted twelve days later at term in labour and after six hours had a spontaneous delivery of live child weighing 3.6 kilos. Albustix was negative at a urine protein level of 0.03 gm. per cent. The immuno-electrophoresis showed albumin and trace arcs of gamma G globulin and transferrin. (FIG. IV). Bacterial count of mid-stream specimen of urine was less than 20 white cells.

Comment:- The immuno-electrophoretic pattern is characteristic of that found in association with urinary infection. A definite gamma globulin band is present in severe infection (FIG. II) decreasing with treatment (FIG. III. and IV).

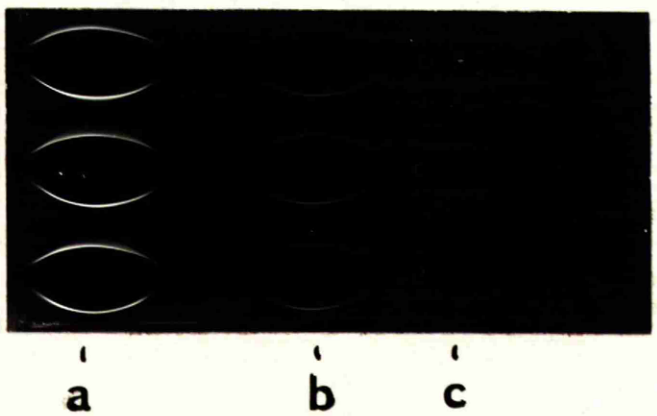
MRS. J. (O+1) Aged 19 Urinary Tract Infection

Immuno-electrophoresis Fig. I



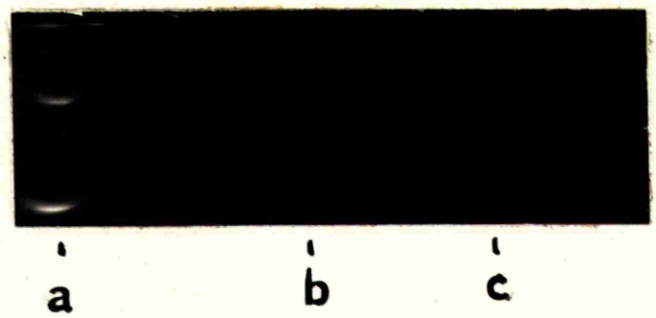
a = albumin, b = transferrin, c = gamma G globulin

Immuno-electrophoresis Fig. II



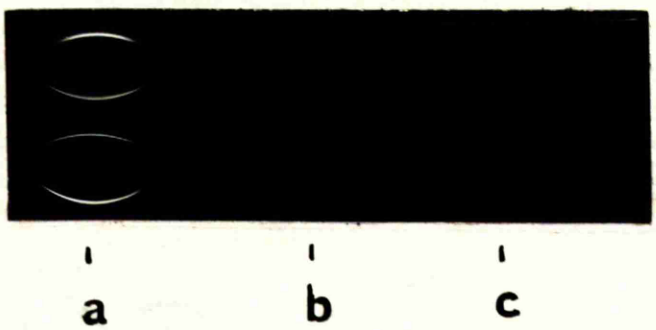
a = albumin, b = transferrin, c = gamma G globulin

Immuno-electrophoresis Fig. III



a = albumin, b = transferrin, c = gammaGglobulin

Immuno-electrophoresis Fig. IV



a = albumin, b = transferrin, c = gammaGglobulin (ABSENT)

Mrs. B. Para 0+0 Aged 21 Diagnosis : Urinary Tract Infection

No serious illness, no history of urinary infection.

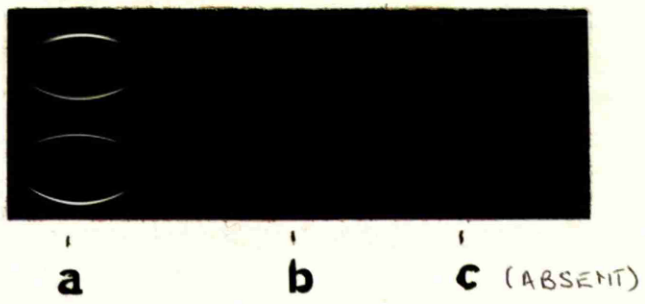
Admitted at 16 weeks with hyperemesis. Albustix negative. Urinary protein0.01 g.per cent. Mid-stream specimen of urine less than 20 cells on bacterial count. Immuno-electrophoresis:- trace amounts of albumin and transferrin (FIG. I) Patient responded to intravenous fluid and vitamin B treatment and was discharged 7 days later. Re-admitted at 34 weeks with recurrence of vomiting and symptoms of urinary tract infection. Mid-stream urine less than 20 cells on bacteriological count. Urinary protein0.05g. per cent. Immuno-electrophoresis:- albumin, transferrin and in addition increased gamma G globulin (FIG.II). Symptoms settled without treatment and patient dismissed home after 9 days in hospital. Re-admitted in labour ten days past expected date of delivery. Spontaneous delivery of live male child weighing 2.7 kilos

occurred after a 6 hour labour. Mid-stream urine prior to delivery : 40 cells on bacteriological count with immuno-electrophoresis still showing albumin, transferrin and gamma G globulin (FIG. III). Albustix negative for protein. Urine protein 0.01 g. per cent.

Comment:- In this case the immuno-electrophoresis (FIG. II) was typical of urinary infection and thus agreed with the clinical diagnosis. However bacteriological count was negative. The persistant finding of albumin, transferrin and gamma G globulin suggests the continued presence of latent urinary infection.

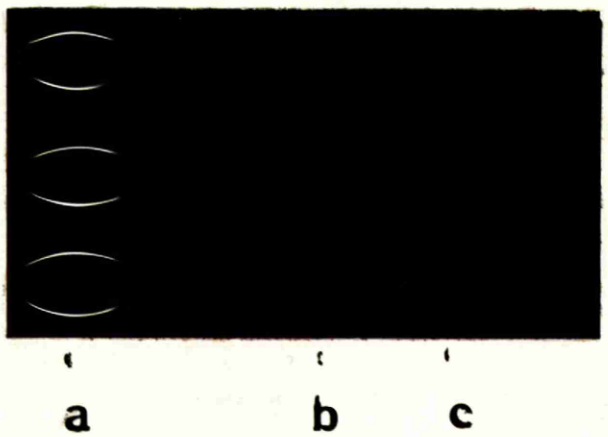
MRS. B. (0+0) Aged 21 Urinary Tract Infection

Immuno-electrophoresis Fig. I



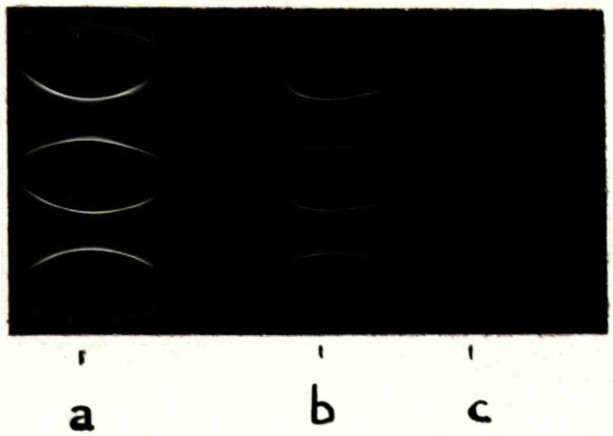
a = albumin, b = transferrin, c = gammaGglobulin

Immuno-electrophoresis Fig. II



a = albumin, b = transferrin, c = gammaGglobulin

Immuno-electrophoresis Fig. III



a = albumin, b = transferrin, c = gamma$G$globulin

Mrs. H. Para 0+0 Aged 22 Diagnosis : Urinary Tract Infection

No history of note.

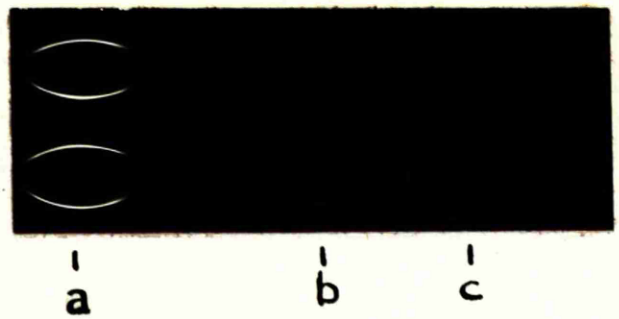
Admitted at 28 weeks with clinical urinary infection and combined iron and folic acid deficiency anaemia haemoglobin 7.9 g. per cent. Trace of protein by albustix. Urinary protein 0.04 g. per cent. Intravenous fluids administered and ampicillin course started. Bacterial count on mid-stream urine: 740 white cells and over 100,000 bacteria. Immuno-electrophoresis prior to treatment showed albumin, transferrin and gamma G globulin (FIG. I). Following completion of ampicillin course, patient started on long term sulphadimidine treatment 0.5g. twice daily. Mid-stream urine became negative to bacteriological culture. Immuno-electrophoresis showed albumin and transferrin but decreased gamma G globulin (FIG. II), at a urinary protein level of 0.02 g. per cent. Patient dismissed home 4 weeks after admission, symptom free with haemoglobin having

risen to 10.8 g. per cent. Re-admitted for induction at 42 weeks. Lower uterine segment caesarean section was performed for cephalo-pelvic disproportion with delivery of live male child weighing 5.1 kilos. Bacterial count of mid-stream urine prior to delivery was less than 20. Albustix negative. Urinary protein level 0.06 g. per cent. Immuno-electrophoresis revealed trace of albumin, transferrin and faint gamma G globulin (FIG. III).

Comment:- A further example of the immuno-electrophoretic pattern found in urinary infection (FIG. I). The change in immuno-electrophoresis is shown in (FIG. II and III) with decreased gamma G globulin reflecting the response to treatment of the infection.

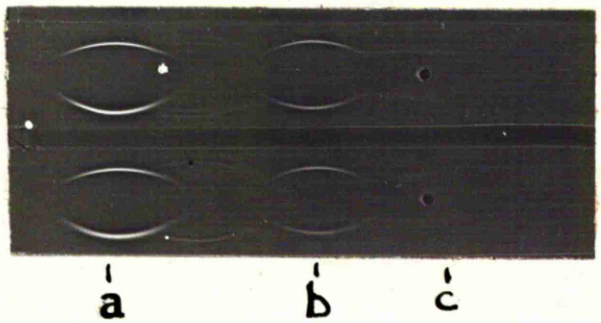
MRS. H. (0+0) Aged 22 Urinary Tract Infection

Immuno-electrophoresis Fig. I



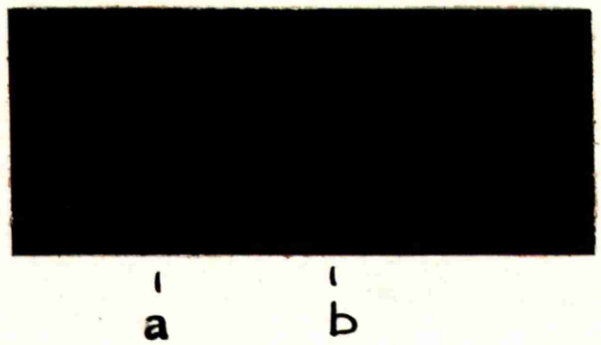
a = albumin, b = transferrin, c = gamma G globulin

Immuno-electrophoresis Fig. II



a = albumin, b = transferrin, c = gamma G globulin

Immuno-electrophoresis Fig. III



a = albumin, b = transferrin, c = gamma G globulin
(ABSENT)

Proteinuria without hypertension or urinary tract infection.

The total protein excretion over 24 hours in this group of four patients ranged from 1.5 g. to 15.3 g. with an average of 8.2 g. Blood pressure was normal. Blood urea levels ranged from 13 to 24 mg. with an average of 19 mg. per 100 ml. A greater number of protein arcs were present on immuno-electrophoresis of the urine concentrate than in the other groups. The main feature was clustering of proteins in the region of alpha and beta globulins. $Alpha_2$ macroglobulin was detected.

The onset of proteinuria occurred early in pregnancy, the earliest record being at 14 weeks. From the beginning numerous protein arcs were present and as pregnancy progressed the number increased. In most cases after delivery there was a rapid diminution in the number of proteins, the macroglobulins disappearing within the first weeks. Two cases were exceptional in that although these large protein molecules had disappeared by the end of the second week,

proteinuria persisted for 5 and 6 months respectively following delivery with the immuno-electrophoresis revealing many precipitin arcs. A typical case Mrs. H. is described in detail in association with immuno-electrophoretic tracings.

Mrs. H. Aged 25 Para 1+0 Diagnosis : Nephrosis

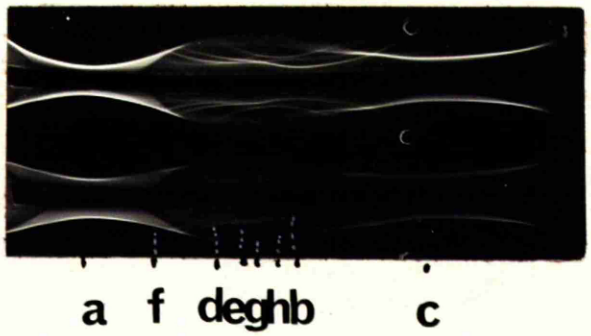
Previous history of proteinuria in first pregnancy in which induction was performed at 36 weeks with delivery of a live male child with difficulty which subsequently died at 48 hours from cerebral trauma. At post natal visit 8 weeks later, patient still had proteinuria of 8 g. per litre and was thought to have underlying renal pathology. In this present pregnancy, attended at 20 weeks: intermediate reaction with albustix present at a urine protein level of 0.6 g. per cent. Blood pressure and blood urea were normal. Immuno-electrophoresis (FIG. I) revealed numerous protein bands including alpha$_2$macroglobulin. This protein (FIG. II and III) pattern continued throughout pregnancy. Blood pressure was normal and urine protein level ranged between 0.6 g. and 1 g. per cent. Surgical induction was performed at 38 weeks. The patient however failed to go into labour and lower uterine caesarean section was performed with delivery of a live male child weighing 2.7 kilos.

Proteinuria continued to be present after delivery although by six months had decreased to 0.1 g. per cent. The immuno-electrophoretic pattern (FIG. IV and V) continued to show a wide range of urinary proteins.

Comment:- The immuno-electrophoretic pattern revealed a wide range of protein including alpha$_2$macroglobulin which was present throughout pregnancy and in the puerperium. This was present even at low protein concentration, although the protein excretion did tend to increase during pregnancy.

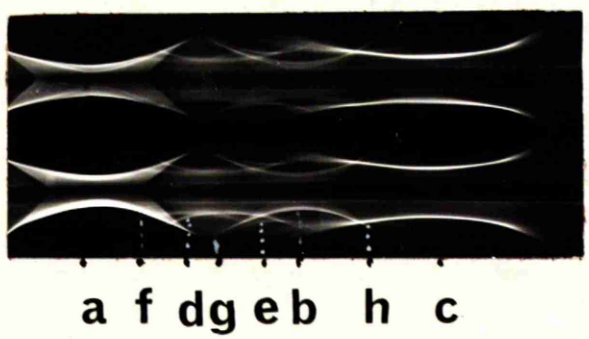
MRS. H Para (1+0) Aged 25 Nephrosis

Immuno-electrophoresis Fig. I



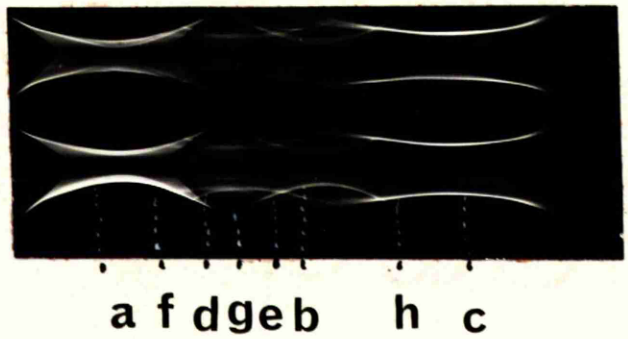
a = albumin, b = transferrin, c = gammaGglobulin
d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein, g = alpha$_2$macroglobulin
h = gamma A globulin

Immuno-electrophoresis Fig. II



a = albumin, b =transferrin, c = gammaGglobulin
d = eeruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein, g = alpha$_2$ macroglobulin
h = gamma A globulin

Immuno-electrophoresis Fig. III

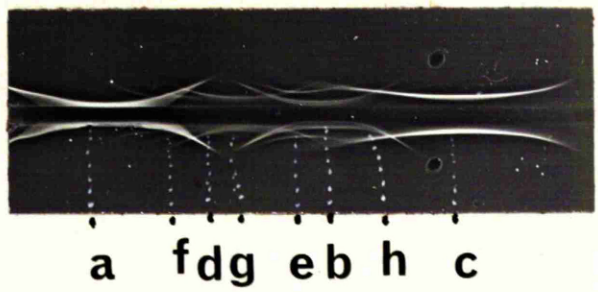


a = albumin, b = transferrin, c = gamma G globulin

d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein, g = alpha$_2$ macroglobulin

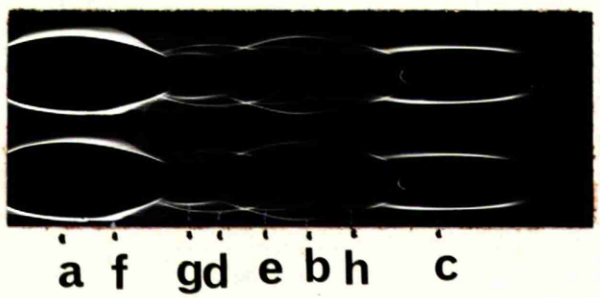
h = gamma A globulin

Immuno-electrophoresis Fig. IV



a = albumin, b = transferrin, c = gammaGglobulin
d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein, g = alpha$_2$macroglobulin
h = gamma A globulin

Immuno-electrophoresis Fig. V



a = albumin, b = transferrin, c = gammaGglobulin
d = ceruloplasmin, e = haemopexin, f = alpha$_1$ glycoprotein, g = alpha$_2$macroglobulin
h = gamma A globulin

Hypertensives

Seven patients were examined. Blood pressure ranged between 140/90 to 200/130 mm. Hg with an average of 160/105 mm. Hg. Twenty four hour protein excretion level was between 0.1 g. to 0.4 g. with an average of 0.16 g. Blood urea levels were between 11 and 37 mg. per 100 ml. with an average of 25 mg. per 100 ml. Immuno-electrophoresis of the urine concentrate revealed trace arcs to albumin, transferrin and gamma G globulin. Little change occurred in the immuno-electrophoresis of these cases prior to delivery or postpartum. In the majority a faint trace of albumin similar to the normal pattern was observed.

The following case is a typical example.

Mrs. G. Aged 47 Para 10+4 Diagnosis :

Essential Hypertension

Rheumatic fever as a child. Meningitis when 41 years of age. Ten full term uneventful pregnancies, all delivered at home. Last pregnancy seven years ago.

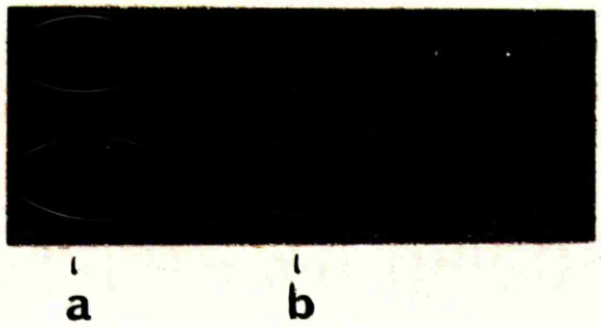
Present Pregnancy: First seen at twenty weeks. Blood pressure recorded at 160/90 mm. Hg. Albustix negative. Urine protein 0.01 g. per cent. and blood urea 26 mg. per 100 ml. Immuno-electrophoretic pattern showed traces of albumin and transferrin (FIG. I). Patient attended the ante-natal clinic every two weeks for next ten weeks. Diastolic blood pressure ranged between 90 and 100 mm. Hg. Albustix negative. Admitted at 32 weeks when found to have trace of protein on albustix at a urinary protein level of 0.02 g. per cent. Blood pressure was 150/100 mm. Hg. and immuno-electrophoretic pattern unchanged (FIG. II), Sod Amytal 200 mg. was given thrice daily with some lowering of blood pressure, the diastolic being recorded between

80 and 95 mm. Hg and the patient was allowed home at 34 weeks but re-admitted at 36 weeks, blood pressure having risen to 170/100 mm. Hg. Urinary protein level remained at 0.02 g. per cent. Albustix was negative and immuno-electrophoresis unaltered (FIG. III). It was thought necessary to effect delivery and lower uterine caesarean section with sterilisation was performed at the 37th. week as the lie was transverse. A live female child was delivered weighing 2.8 kilos. Immuno-electrophoresis on the tenth day showed albumin and a trace of transferrin (FIG. IV). Urinary protein level was 0.01 g. per cent and Albustix negative. Blood pressure had fallen to 125/85 mm. Hg. when dismissed on the twelfth day after delivery. The patient did not report for her post natal visit at six weeks.

Comment:- The urinary protein pattern was typical of that found in association with essential hypertension at little variance from the normal normotensive patient. Little change occurred in the immuno-electrophoresis despite alteration in blood pressure.

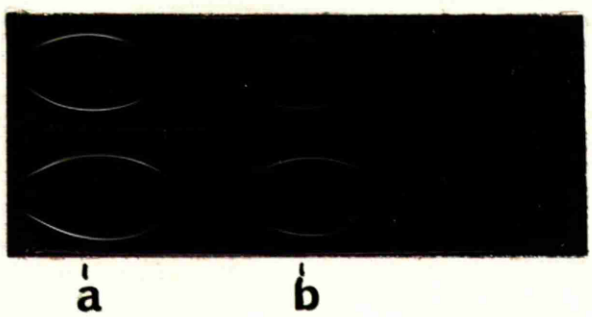
MRS. G. (10+4) Aged 47 Essential Hypertension

Immuno-electrophoresis Fig. I



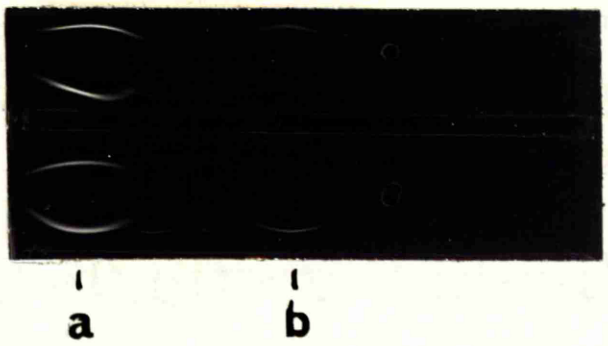
a = albumin, b = transferrin

Immuno-electrophoresis Fig. II



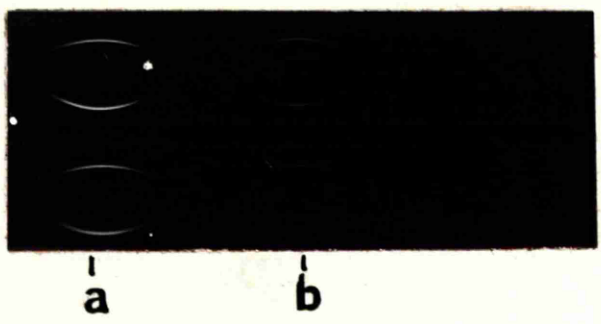
a = albumin, b = transferrin

Immuno-electrophoresis Fig. III



a = albumin, b = transferrin

Immuno-electrophoresis Fig. IV



a = albumin, b = transferrin

Foetal Outcome

No stillbirths or neonatal deaths occurred in the normal group, the patients with urinary tract infection, moderate pre-eclampsia or proteinuria alone cases. There were four stillbirths, one neonatal death and one abortion in the patients with severe pre-eclampsia. Two patients with hypertension had neonatal deaths, due to prematurity, the pregnancies being terminated in the maternal interest at 28 and 34 weeks with delivery of infants weighing 0.8 kilos and 1.2 kilos respectively.

The average birth weight in the different groups was as follows :-

	Infant Weight (Kilos)
Normotensives	3.1
Pre-eclampsia (moderate)	3.2
(severe)	2.05
Urinary Tract Infection	3.3
Hypertensives	2.9 (excluding the two neonatal deaths)
Proteinuria	3.07

Conclusions

Section I

1. The patients with proteinuria alone had the highest average protein excretion at 8.2 g/24 hours. The group with pre-eclampsia came next with an average 24 hour excretion of 4.8 g. in the severe group and 0.45 g. in those cases with moderate pre-eclampsia. The urinary tract infection group, the hypertensive cases and the normal patients had average 24 hours protein excretions of 0.19g, 0.16g. and 0.15g. respectively.
2. The number of precipitin bands detected by immuno-electrophoresis increased with the amount of protein excreted, the greatest number being observed in the proteinuria cases and severe pre-eclampsia.
3. The increase in precipitin bands in the pre-eclamptic group appears to be related to the blood pressure level and amount of proteinuria; the more severe the pre-eclampsia, the greater the number of proteins detected.

4. The large molecular weight protein, alpha$_2$ macroglobulin, is only found in cases of nephritis and severe pre-eclampsia.
5. The immuno-electrophoretic pattern and amount of protein in the three groups; normal, urinary tract infection and hypertensive patients did not differ greatly.
6. Residual immuno-electrophoresis changes were most marked in the proteinuria alone cases and in a few of the severe pre-eclamptics.
7. Birth weight is lowest and perinatal loss highest in the severe pre-eclamptic group.

Section II

Subsequent to the preliminary results recorded in section I it was decided to investigate more fully the protein pattern found in conditions associated with proteinuria in pregnancy. Particularly interesting was the discovery of large molecular proteins in both pre-eclamptic patients and in those with gross proteinuria and a history of previous renal disease. Nine patients suffering from pre-eclampsia, two of whom developed eclampsia, three patients in whom a diagnosis of nephritis had been made and one case of proteinuria of undetermined origin were studied.

Results in general:

The immuno-electrophoretic patterns obtained with the wide range antisera revealed a greater number of precipitin bands and therefore a wider range of protein fractions in the nephritic cases than in those with pre-eclampsia. By subjecting each specimen to tests with individual protein antisera: albumin, transferrin, gamma G globulin

and alpha$_2$macroglobulin, more precise information was obtained. All patients whatever the level of proteinuria from 0.15 to 23 g. per 24 hours, excreted albumin, transferrin and gamma G globulin. In contrast the alpha$_2$macroglobulin fraction occurred in only six of the thirteen women in this group: in all three nephritics, in the woman with recurrent proteinuria in pregnancy of unknown origin, and in two pre-eclamptic women with the greatest levels of proteinuria 7 and 23 g. per 24 hours, one of whom became eclamptic.

Pre-eclampsia:-

There were seven primigravida and two multiparous patients.

Primigravida with Pre-eclampsia:-

The following case of severe pre-eclampsia illustrated by immuno-electrophoretic tracings is described.

Mrs. M. Aged 24 Para 0+0 Diagnosis : Severe Pre-eclampsia.

No previous history of note.

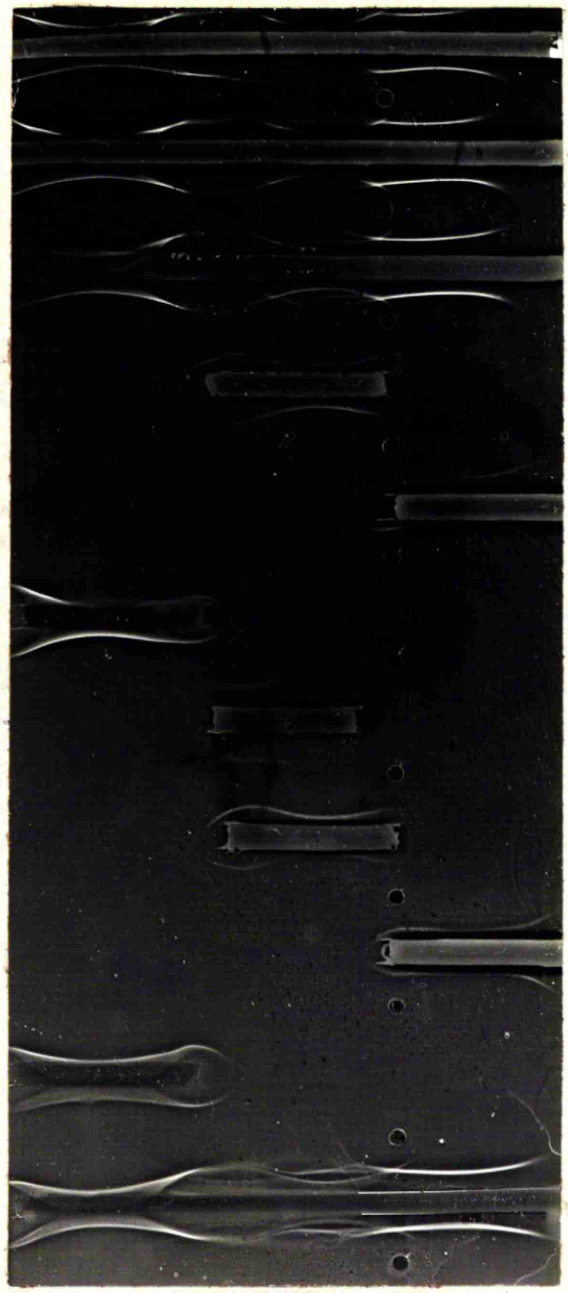
Admitted via the flying squad on account of severe pre-eclampsia at 36 weeks. Symptoms of headaches and vomiting present. Blood pressure 200/130 mm. Hg. Heavy reaction to protein by albustix, 0.7 g. per cent. protein in urine. Three eclamptic seizures shortly after admission. Immuno-electrophoresis (FIG. I) revealed a wide range of urine proteins including $alpha_2$ macroglobulin clearly demonstrated by the use of individual antiserum to specific proteins. Protein excretion increased to 23g/24 hours, the diastolic blood pressure ranged between 100 and 120 mm. Hg., and the blood urea rose to 70 mg/100 ml. with the immuno-electrophoresis continuing to show albumin, transferrin, gamma G globulin and $alpha_2$macroglobulin (FIG. II). Intra-uterine death occurred twelve hours after the eclamptic seizure with spontaneous delivery of a stillborn male child weighing

2.01 kilos 7 days later. The immuno-electrophoretic pattern remained unchanged till 48 hours after delivery when the blood pressure had fallen to 140/90 mm. Hg., the blood urea to 20 mg. per 100 ml. and urinary protein to 10 g. per 24 hours. Six weeks after delivery alpha$_2$ macroglobulin was no longer present on immuno-electrophoresis of the urine concentrate although albumin, transferrin, gamma G globulin were still seen (FIG. III). Blood pressure had returned to normal at 90/60 mm. Hg., and blood urea to 14 mg. per 100 ml. The urinary protein level was 0.9 g. per 24 hours.

Comment:- The presence of the large molecular weight protein in association with gross proteinuria is indicative of severe pre-eclampsia occurring in this case after eclamptic seizures. FIG. I and FIG. II both show definite alpha$_2$macroglobulin precipitin arcs which are no longer present in FIG. III; the postpartum picture at 7 and 42 days.

MRS. M. Para (0+0) Aged 24 Severe Pre-eclampsia

Fig.I



Widespectrum

Transferrin

Gamma G globulin

Albumin

$Alpha_2$macroglobulin

Fig. II

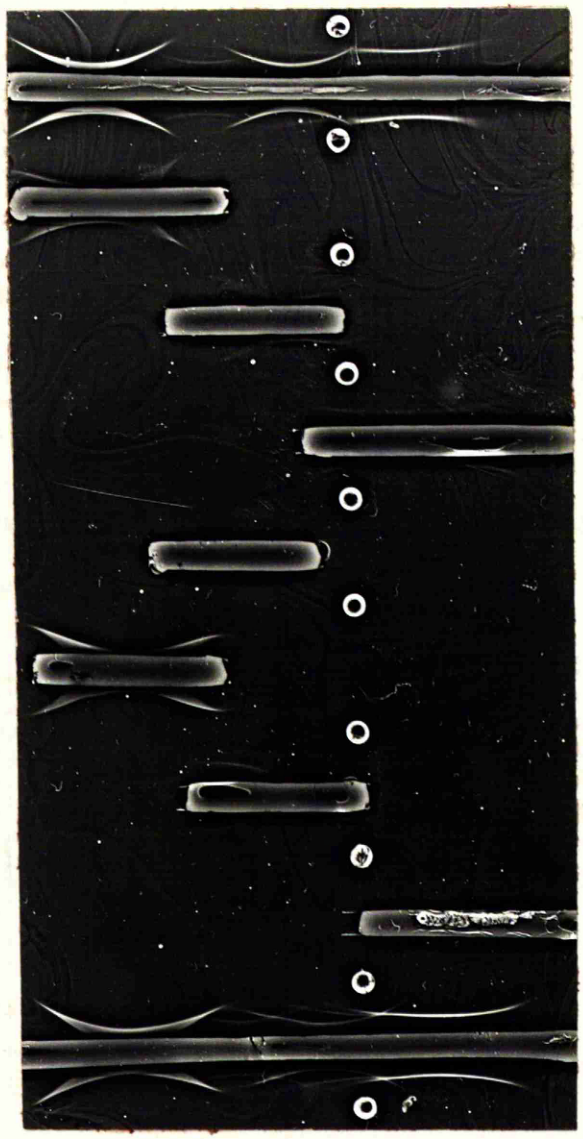
Transferrin

Gamma G globulin

Albumin

Widespectrum

Fig. III Post Partum



Widespectrum

Albumin

Transferrin

Gamma G globulin

Alpha$_2$macroglobulin
(ABSENT)

Albumin

Transferrin

Gamma G globulin

Widespectrum

The ensuing case, Miss C. is an example of moderate pre-eclampsia and comparison may be made with the foregoing patient.

Miss C. Aged 21 Para 0+0 Diagnosis : Moderate Pre-eclampsia

No previous illness.

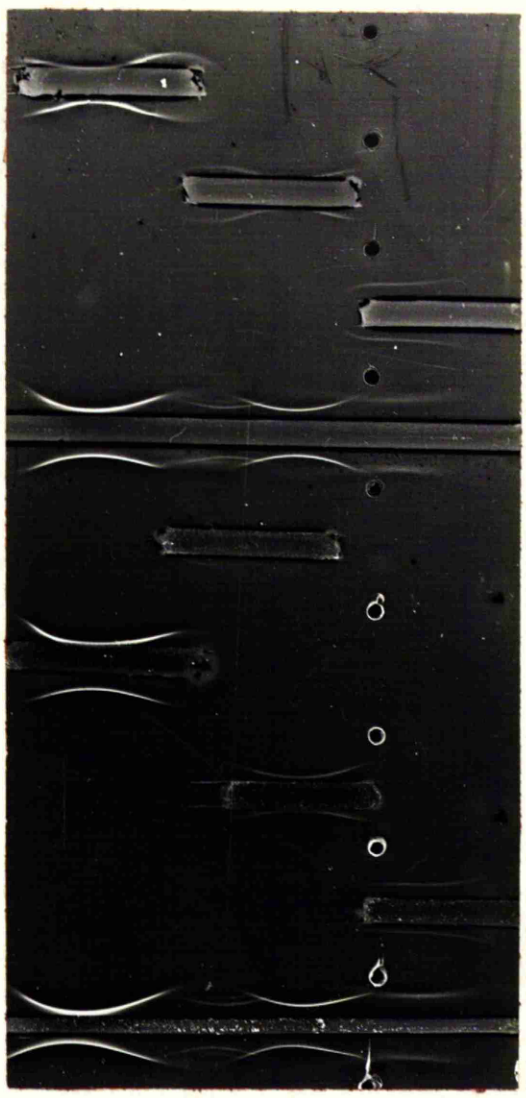
First attended antenatal clinic at 30 weeks. Blood pressure noted to be 150/85 mm.Hg. Albustix negative. One week later blood pressure was found to be 140/105 mm.Hg. Albustix positiveaat trace reaction, urine protein level was 0.2g. per cent. Immuno-electrophoresis (FIG. I) revealed definite reaction to albumin, transferrin and gamma G globulin but none to alpha$_2$macroglobulin. Patient admitted. Sedation and diuretic treatment given. Blood pressure remained between 140/100 mm.Hg. and 160/115 mm.Hg. Immuno-electrophoresis picture (FIG. II) remained unchanged. At 34 weeks pregnancy terminated. Surgical induction was performed with delivery of a live female child weighing 2 kilos. By the fourth post natal day urinary protein level was 0.05 g. per cent. Blood pressure was normal and immuno-electrophoresis (FIG. III) revealed traces of

albumin and transferrin to the individual antisera.

Comment:- The protein pattern is characteristic of moderate pre-eclampsia. Antenatally there is a consistent pattern present without the occurrence of alpha$_2$macroglobulin and a rapid change back to the normal protein pattern after delivery.

MISS C Para (0+0) Aged 21 Moderate Pre-eclampsia

Fig. I



Albumin

Transferrin

Gamma G globulin

Wide Spectrum

- - -

$Alpha_2$macroglobulin (ABSENT)

Albumin

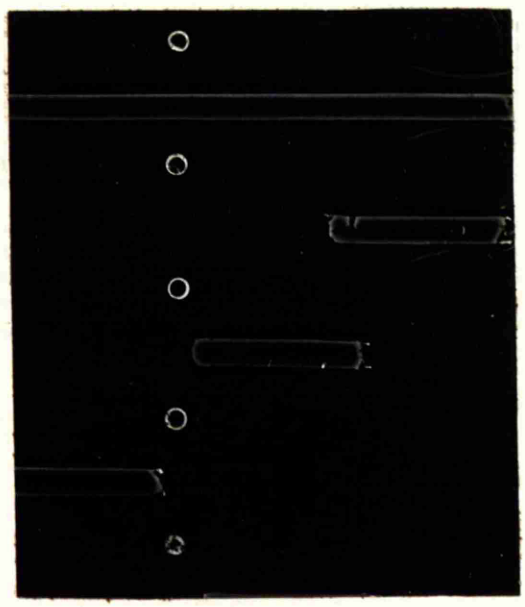
Fig.II

Transferrin

Gamma G globulin

Wide Spectrum

Fig. III



Wide Spectrum

Albumin

Transferrin

Gamma G globulin

Alpha$_2$macroglobulin
(ABSENT)

The second case of eclampsia developed in a primigravida aged 26 at 37 weeks with blood pressure of 150/90 mm. Hg. and protein excretion of 5.9 g. per 24 hours. Surgical induction was performed but during labour, an eclamptic seizure occurred. Immediate Caesarean Section was carried out with delivery of a live male child weighing 2.94 kilos. The diastolic blood pressure remained elevated for 24 hours and blood urea at 40 mg. per 100 ml. but urinary protein excretion fell to 1 g. per 24 hours. The immuno-electrophoresis of urine concentrate showed precipitin arcs to albumin, transferrin gamma G globulin prior to induction and following eclamptic seizure. On no occasion was $alpha_2$macroglobulin detected and by the fourteenth day after delivery, traces of albumin, transferrin and gamma G globulin only were present, the patient was normotensive, the blood urea 14 mg. per 100ml. and urinary protein level 0.1 g. per 24 hours.

In the four remaining primigravida with pre-eclampsia the onset of study was between 31 and 33 weeks. The highest blood pressure in each case was 130/90, 180/130, 160/110 mm.Hg. Albumin, transferrin and gamma G globulin precipitin arcs were present in all cases on immuno-electrophoresis of urine concentrates prior to delivery. In one case, a primigravida aged 17, there was a trace of $alpha_2$ macroglobulin at a urine protein level of 7 g. per 24 hours and blood urea of 28 mg. per 100 ml. Within 12 hours spontaneous delivery at 32 weeks occurred of a stillborn female child weighing 1.95 kilos. By the third postpartum day, blood pressure was normal, blood urea 22 mg. per 100 ml. and urine protein level 0.2 g. per 24 hours. Albumin, transferrin and gamma G globulin were still present, but $alpha_2$ macroglobulin had disappeared. In one other case, that of pre-eclampsia at 31 weeks in a primigravida aged 22 associated with a twin pregnancy with an initial blood pressure of 180/130 mm.Hg, 24 hour protein level of 3.2 g.

and blood urea 16 mg. per 100ml. Alpha$_2$ macroglobulin was suspected on immuno-electrophoresis just prior to the onset of premature labour, the protein excretion having risen to 5.1 g. per 24 hours and blood urea to 22 mg. per 100 ml. Spontaneous delivery occurred of live uniovular male children weighing 1.81 and 1.86 kilos. By the fourth postpartum day only traces of albumin, transferrin and gamma G globulin were present with a decrease in the protein excretion to 0.1 g. per 24 hours at a blood pressure level of 150/90 mm. Hg.

The two other primigravida were first investigated at 31 and 33 weeks. The patient aged 29 at 31 weeks had a protein excretion in 24 hours of 0.6 g, blood urea was 22 mg. per 100 ml. and on immuno-electrophoresis of urine concentrate, albumin, transferrin and gamma G globulin were seen. By 34 weeks the pre-eclampsia had become more severe, blood pressure rose to 160/110 mm. Hg., blood urea 38 mg. per 100 ml. and 24 hour urinary protein level to 1.5g. A trace of alpha$_2$

macroglobulin appeared on immuno-electrophoresis of urine concentrate together with albumin, transferrin and gamma G globulin. Elective Caesarean was performed with delivery of a live child weighing 2.17 kilos. By the tenth post partum day, the 24 hour urinary protein level had fallen to 0.8g, blood pressure to 135/90 mm. Hg. and blood urea returned to a normal level; The primigravida at 33 weeks, aged 36 had a blood pressure of 170/110 mm. Hg., with a blood urea of 23 mg. per 100 ml. and 24 hour urinary protein level of 0.6 g. The pre-eclampsia remained unchanged till 37 weeks when the twenty-four hour urinary protein output rose to 2g. Immuno-electrophoresis of urine concentrate revealed albumin, transferrin and gamma G globulin prior to delivery persisting at six weeks postpartum but in diminished amounts. Blood pressure on the seventh post natal day was 140/80 mm. Hg., urinary protein level 0.15 g. per 24 hours. Elective Caesarean Section was carried out with delivery of a live female child weighing 2.7 kilos.

Multipara with Pre-eclampsia:-

Two patients were studied. One aged 23 years had pre-eclampsia in her first pregnancy. Moderate pre-eclampsia occurred in this her second pregnancy at 37 weeks. Blood pressure was 135/85 mm.Hg., urinary protein excretion per 24 hours, 0.15g.and blood urea 23 mg.per 100 ml. The immuno-electrophoresis of urine concentrate revealed albumin, transferrin and gamma G globulin. Induction was performed with spontaneous delivery of a live male child weighing 3.7 kilos. By the fourth postpartum day trace amounts of the proteins present antenatally were detected. Blood pressure and urea were normal at a 24 hour protein excretion level of 0.05 g.

The second parous patient aged 36 who had two previous pregnancies, in none of which pre-eclampsia had occurred, developed pre-eclampsia in this pregnancy at 35 weeks. Blood pressure was 145/90 mm.Hg., protein 24 hour urine excretion 3.2g. and blood urea 28 mg. per 100 ml. The pre-eclampsia clinically became more severe at 36 weeks, blood pressure rising to 150/120

mm.Hg., and urine protein excretion to 6.2g. per 24 hours. The immuno-electrophoresis of urine concentrate remained unchanged. Albumin, transferrin and gamma G globulin were present but there was no alpha$_2$macroglobulin. Surgical induction was performed with delivery of a live male child weighing 2.43 kilos. The blood pressure level following delivery was slow to fall, the diastolic reading at 6 weeks postpartum still being elevated at 90 mm Hg. The protein level however, had fallen to 0.3 g. per 24 hours with trace amounts of albumin, transferrin and gamma G globulin present on immuno-electrophoresis of the urine concentrate.

Nephritis:-

Three primigravida with a history of nephritis were studied from 8, 32 and 36 weeks.

Case one is described in conjunction with the immuno-electrophoretic tracings.

Mrs. G. Para 0+0 Aged 24 Diagnosis : Nephritis

Known to have type II glomerulonephritis since age of 15. In remission stage for a year prior to pregnancy.

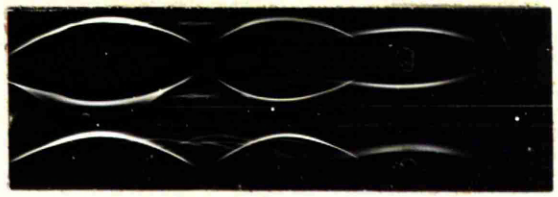
First seen at 12 weeks, heavy reaction with albustix. Urine protein level 0.4g. per 24 hours. Immuno-electrophoresis of urine concentrate with wide spectrum antiserum demonstrated many protein bands (FIG. I). Blood pressure was normal at 120/80 mm. Hg. and blood urea 16 mg. per 100 ml. Throughout antenatal period immuno-electrophoresis (FIG. II and III) revealed arcs to albumin, transferrin, gamma G globulin and alpha$_2$macroglobulin with the individual antiserum. Blood pressure was normal and urine protein level ranged between 0.1 to 0.4 g. per 24 hours. At 38 weeks the patient was delivered by Caesarean Section of a live male child weighing 1.87 kilos. Post-partum immuno-electrophoresis (FIG. V) continued to show a wide range of protein bands although alpha$_2$macroglobulin could not be

demonstrated. Urine protein excretion after delivery lay between 0.1 to 0.3g. per cent. Blood pressure was normal.

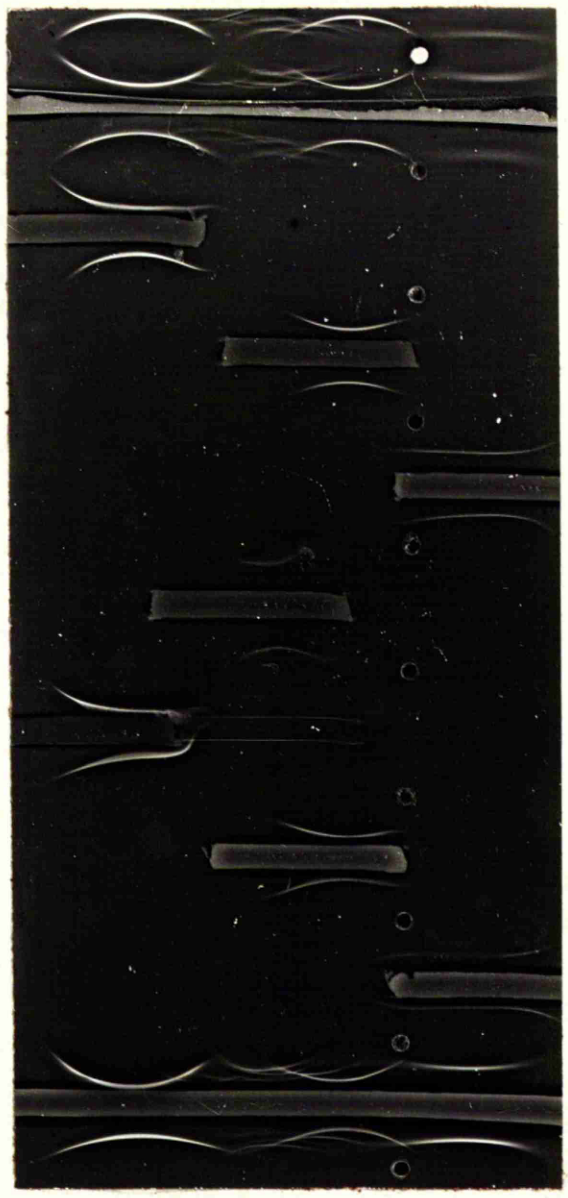
Comment:- FIG. I to FIG. III illustrate the urine protein picture in nephrosis. A wide range of protein is present including alpha$_2$ macroglobulin from the early weeks of pregnancy. This continued after delivery although alpha$_2$macroglobulin was not detected in this case (FIG. IV).

MRS. G Para (0+0) Aged 24 Nephrosis

Fig. I



Wide Spectrum



Wide Spectrum

Albumin

Transferrin

Fig.II

Gamma G globulin

$Alpha_2$macroglobulin

- - -

Fig.III

Albumin

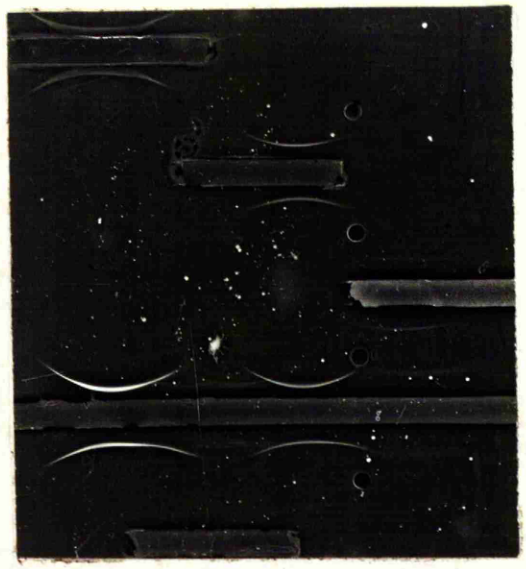
Transferrin

Gamma G globulin

Wide Spectrum

POST PARTUM

Fig. IV



Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Alpha$_2$macroglobulin
(ABSENT)

Case Two:-

A nephritic of 6 months standing aged 25 was first investigated at 32 weeks. Blood pressure was normal but 24 hour urinary level was 5.4. g and blood urea 100 mg. per 100ml. On immuno-electrophoresis of the urine concentrate albumin, transferrin, gamma G globulin and alpha$_2$macroglobulin were present. The condition remained unchanged and at 38 weeks when the blood pressure was 130/90 mm.Hg., blood urea 58 mg. per 100 ml. and protein output of 1.8 g. in 24 hours induction was performed with spontaneous delivery of a live male child weighing 2.83 kilos. At six weeks postpartum blood urea was still elevated at 62 mg. per 100 ml., protein excretion in 24 hours was 2 g. with a normal blood pressure. The immuno-electrophoresis of urine concentrate continued to show alpha$_2$macroglobulin was well as albumin, transferrin and gamma G globulin.

Case Three:-

The third case who had nephritis for 2 years and was now pregnant for the first time at the age of 21 was first investigated at 26 weeks. Blood pressure was elevated at 140/90 mm.Hg., blood urea 60 mg. per 100 ml. and protein excretion 0.4g. per 24 hours. During the subsequent weeks the blood urea and blood pressure fell to normal but rose again at the 37th week when the blood pressure was found to be 180/100 mm.Hg. and the blood urea 50 mg. per 100 ml. The protein excretion had also risen to 2g. per 24 hours. The initial immuno-electrophoresis of urine concentrate demonstrated albumin, transferrin and gamma G globulin but the second assessment two weeks later at 28 week revealed the presence of alpha$_2$ macroglobulin which remained throughout the pregnancy and was still demonstrated at six weeks.

The pregnancy was terminated at 37 weeks by surgical induction with spontaneous delivery of a live male child weighing 2.26 kilos.

Probable Nephrosis with superimposed Pre-eclampsia:

The final case presented is that of multiparous patient aged 32 who had proteinuria in each of her previous pregnancies.

Mrs. K. Aged 32 Para 4+0 Diagnosis : Nephrosis Superimposed Pre-eclampsia

History of Pulmonary Tuberculosis.

Four previous pregnancies with only two surviving children, stillbirth having occurred in the first pregnancy at 32 weeks and neonatal death after induction at 38 weeks in the fourth pregnancy. Proteinuria known to be present in the last three pregnancies and persisting after delivery.

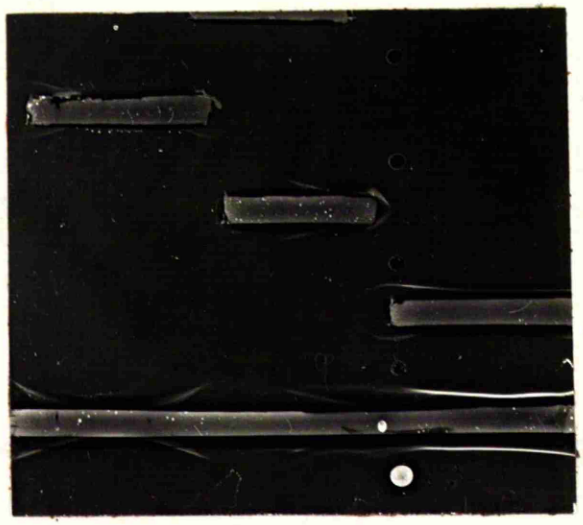
Attended local authority antenatal clinic in this pregnancy from twenty weeks, noted to have a positive albustix with a normal blood pressure. Referred to hospital at 32 weeks and admitted. Albustix positive at a protein level of 0.08 g. per cent. Blood pressure 130/85 mm.Hg. Immuno-electrophoresis (FIG.I) with wide range antiserum

revealed bands to six proteins and with individual antiserum albumin, transferrin and gamma G globulin but not with alpha$_2$macroglobulin. For the next five weeks urine protein level ranged between 0.05 and 0.08 g. per cent. Albustix was negative or occasionally a trace. Diastolic blood pressure was between 95 and 100 mm. Hg. At 38 weeks protein excretion increased to 0.2g. per cent. and a change in the immuno-electrophoretic pattern (FIG. II) occurred with the appearance of alpha$_2$macroglobulin. Two days later, intra-uterine death occurred with delivery of a stillborn male child weighing 2.8 kilos, forty-eight hours after disappearance of the foetal heart. By the third postpartum day urine protein level had fallen to 0.1g. in twenty four hours. Blood pressure remained at 135/90 mm. Hg. Alpha$_2$macroglobulin was not present on immuno-electrophoresis (FIG. III) with only traces of albumin, transferrin and gamma G globulin present.

Comment: This case demonstrates the difficulties which may arise when pre-eclampsia developes in a case of renal disease. FIG. I is not typical of nephrosis as $alpha_2$ macroglobulin is not present although there is a wide range of proteins at a very low protein level. The clinical outcome with foetal death following the appearance of $alpha_2$macroglobulin and the rapid reversal to an almost normal pattern postpartum suggests that pre-eclampsia is the main element with perhaps some mild degree of renal pathology.

MRS. K Para (4+0) Aged 32 Nephrosis
Superimposed
Pre-eclampsia

Fig. I



Alpha$_2$macroglobulin

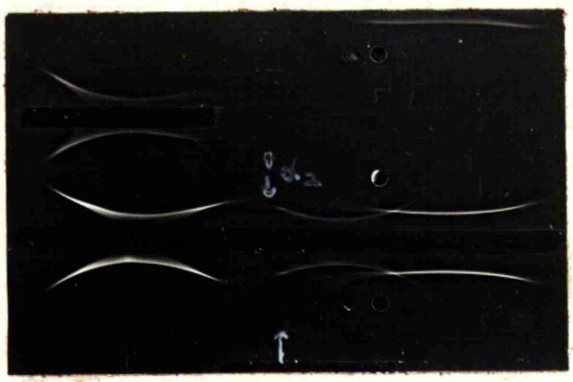
Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Fig. II



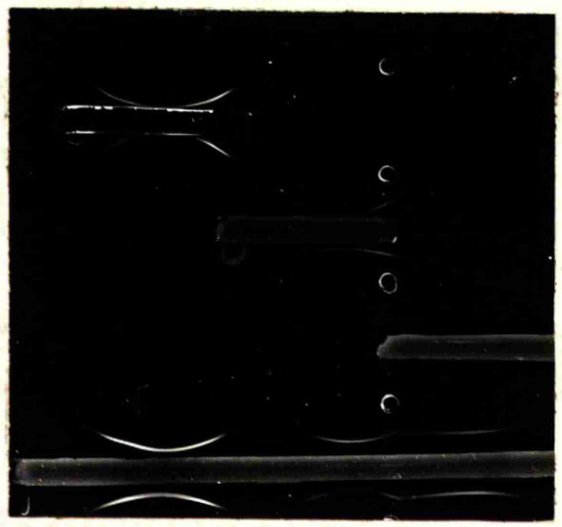
Gamma G globulin

Albumin

Wide Spectrum

Alpha$_2$macroglobulin

Fig. III



Alpha$_2$macroglobulin (ABSENT)

Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Foetal Outcome:-

No infant deaths occurred in the patients whose immuno-electrophoretic pattern shows albumin, transferrin and gamma G globulin but there were three stilbirths in three of the patients showing alpha$_2$macroglobulin on immuno-electrophoresis. Two of these had severe pre-eclampsia and the other, recurrent proteinuria in pregnancy of unknown origin. Alpha$_2$macroglobulin was present in the urine of the pre-eclamptic patients on initial examination. In one case this followed eclamptic seizure and remained until after delivery two weeks later. In the other it was observed when first seen at 32 weeks with intra-uterine death occurring forty-eight hours later. The third case in which proteinuria was present in a previous pregnancy, was found to have albumin, transferrin and gamma G globulin from 20 weeks of pregnancy. At 37 weeks, alpha$_2$macroglobulin was detected and within two days, intra-uterine death occurred.

All three patients with nephritis and showing alpha$_2$macroglobulin in the urine had live babies. The average birth weight in this section was 2.5 kilos. One case with pre-eclampsia and one of the nephritic cases had babies weighing less than the tenth percentile weight for gestational age.

Conclusions:-

1. The most striking feature is the contrast between the cases of nephritis and those of pre-eclampsia.
2. Alpha$_2$macroglobulin was present in the nephritic cases even when the proteinuria was of slight degree, whereas, in pre-eclampsia this large molecular protein was present only in the two patients with gross proteinuria.
3. Alpha$_2$macroglobulin in the puerperium disappeared rapidly in the pre-eclamptic cases whereas it persisted in the nephritic cases.
4. The appearance of alpha$_2$macroglobulin in the urine during pre-eclampsia is associated with severe pre-eclampsia and with a poor foetal prognosis.
5. In the nephritic cases, no infant deaths occurred.

6. Not all cases are clear cut, the difficulty arising when pre-eclampsia occurs in a patient with a history of previous kidney damage. In this case, $alpha_2$ macroglobulin appeared in the urine when proteinuria was of mild degree and was associated with intra-uterine death.

Section III

Having studied urinary proteins qualitatively it was decided to try and examine the quantitative aspect of renal protein excretion. Excretion of protein must be related to the permeability of the glomerulus and the greater the permeability the largerthe molecules which will be passed. If this were the only factor controlling the appearance of proteins in the urine then the pattern of protein excretion in any particular disease would be fairly constant. This does not appear to be the case. As well as permeability it would be reasonable to think that structural changes in the glomerulus would affect the protein pattern. In addition there is the unknown factor of the tubular re-absorption of protein.

It was decided to examine the rate of excretion of protein in some of the clinical conditions already mentioned. The urinary clearance of protein was calculated by the formula commonly used for renal clearance of other substances. UV/P where U = concentration of protein in mg. per 100 ml. :

V = the urine volume per ml. per minute and P = plasma concentration of protein in mg. per 100 ml. This was carried out for three proteins: albumin, transferrin and gamma G globulin in the following cases. Three cases of eclampsia, five cases of severe pre-eclampsia, four cases of moderate pre-eclampsia, and two cases of proteinuria, un-associated with hypertension. It was decided to express also the clearances in terms of a standard protein molecule. Transferrin was chosen as the standard molecule. It is of small molecular weight, is homogeneous and clearly defined. Following measurement of the ring diameters from the immunodiffusion plates as described in the methods and illustrated in FIG.V and FIG.VI, the values were corrected for concentration and dilution factors. The U/P ratios of albumin and gamma G globulin were then expressed in terms of the U/P ratios of transferrin and the log albumin/transferrin clearance and log gamma G globulin/transferrin

clearance plotted in relation to the molecular weight of albumin and gamma G globulin for each particular set of clearances. The line between these two log clearances forms a regression line decreasing with increasing molecular weight. The angle of this line is taken as the index of selectivity for that particular set of clearances.

In the five cases of moderate pre-eclampsia the average clearance ratio gamma G globulin/transferrin was low. (Antenatally 0.2, postnatally 0.1) and the index of selectivity high (antenatally: average 58, postnatally : average 59) indicating little change in renal permeability either antepartum or postpartum. The severe pre-eclamptic patients antenatally had a high gamma G globulin/transferrin clearance (average 0.6) and low selectivity index (average 40). In the postpartum period however gamma G globulin/transferrin clearance fell to (average 0.09) and the index of selectivity rose to (average 60), thus returning to a normal selectivity.

In the three patients with eclampsia two had antepartum and the third postpartum seizures. In one of the antepartum cases and in the postpartum case, the gamma G globulin/transferrin clearance ratio was initially high 0.9 and 0.6 respectively with low selectivity indices of 28 and 30. These however improved in the puerperium the index of selectivity of the antepartum eclamptic case rising to 56 and the clearance gamma G globulin/transferrin ratio falling to 0.04; in the postpartum case the gamma G globulin/transferrin ratio fell to 0.2g. and the selectivity index rose to 50. The third case of eclampsia behaved abnormally the antepartum ratio gamma G globulin/transferrin was high at 0.45 with a selectivity index of 60, but in the puerperium the gamma G globulin/transferrin clearance ratio increased to 1.2 and the selectivity index fell to 17. This case is still being followed, permanent renal damage being suspected.

In the proteinuria cases, one had little renal defect, the ratio of gamma G globulin/transferrin clearance was high antenatally at 0.7 but fell to 0.03 postnatally, with an antepartum and postpartum selectivity index of 50 and 60 respectively. The changes observed being due to the increased permeability of pregnancy. The other case had gross renal damage, antenatally the gamma G globulin/transferrin clearance ratio was 0.5 and the index of selectivity 20. Postnatally the gamma G globulin/transferrin ratio still remained relatively high at 0.24 in comparison to the other cases and the selectivity index low at 22, renal biopsy four months after delivery showed membranous glomerulo-nephritis.

Foetal Outcome

In the proteinuria alone cases, no babies died. In the severe pre-eclamptic group, there was three neonatal deaths and one still-birth. No death occurred in the cases of moderate pre-eclampsia. The average birth weight in the nephritic group was 3.25 kilos

in the moderate pre-eclamptic group 2.9 kilos and in the severe pre-eclamptic group 1.9 kilos.

Conclusion:

1. In 13 out of the 15 cases there was a rise in the index of selectivity and a fall in renal clearance post delivery. The two cases in which this did not occur both continued to show alpha$_2$macroglobulin on immuno-electrophoresis in the puerperium. In one of these cases, the diagnosis of membranous glomerulo-nephritis was made on renal biopsy, the renal clearance gamma G globulin/transferrin was 0.24 postnatally and the index of selectivity 22. In the other case the renal clearance gamma G globulin/transferrin was 1.2 and the index of selectivity 17, renal damage suspected.
2. Thus it would appear that renal function is upset if the clearance of gamma G globulin/tranferrin is above 0.24 and the index of selectivity is below 25 in the puerperium suggesting that structural change has taken place.

The following cases were chosen to illustrate the immuno-electrophoretic patterns and selectivity indices in relation to the clinical history of each patient.

Mrs. A. Aged 26 Para 2+0 Diagnosis : Nephrosis

Known case of nephrosis, persistent proteinuria throughout and between previous pregnancies both of which were uneventful. First seen in this pregnancy when twelve weeks pregnant. Heavy proteinuria by albustix. 0.7 g. per cent. urine protein. Normotensive. Immuno-electrophoresis (FIG.I) with wide spectrum antiserum and with antiserum to albumin, transferrin and gamma G globulin and alpha$_2$ macroglobulin, good precipitin arcs. This pattern continued throughout pregnancy and was still seen six months after delivery (FIG. II - VI). Labour was induced at term with delivery of a live female child weighing 3.6 kilos. Urine protein level ranged from 0.4 to .9 g. per cent. Renal biopsy was performed four months after delivery and showed membranous glomerulo-nephritis.

Comment: The urinary protein pattern is characteristic of that found in nephrosis with the constant presence of the large molecular

weight protein, $alpha_2$macroglobulin during and after pregnancy. The finding is in agreement with the elevated gamma G globulin clearance and low index of selectivity which is present.

Antenatal selectivity indices : 1 - 3

Postnatal selectivity indices : 4

MRS. A Para (2+0) Aged 26 Nephrosis

Fig. I

Alpha$_2$macroglobulin

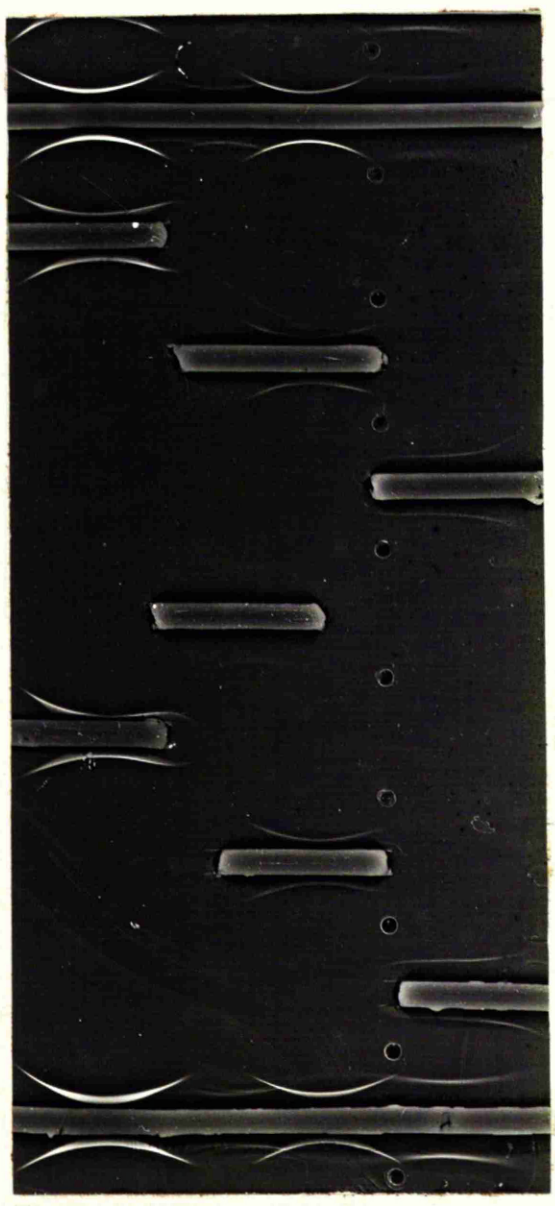
Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Fig. II



Wide Spectrum

Albumin

Transferrin

Gamma G globulin

Alpha$_2$macroglobulin

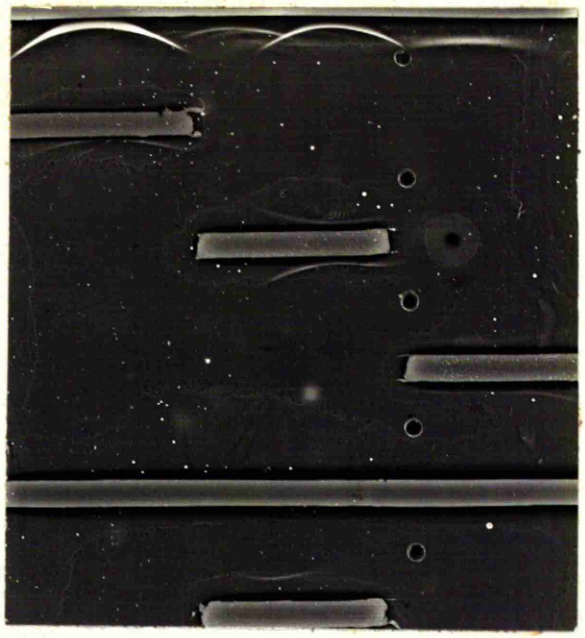
Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Fig.III



Wide Spectrum

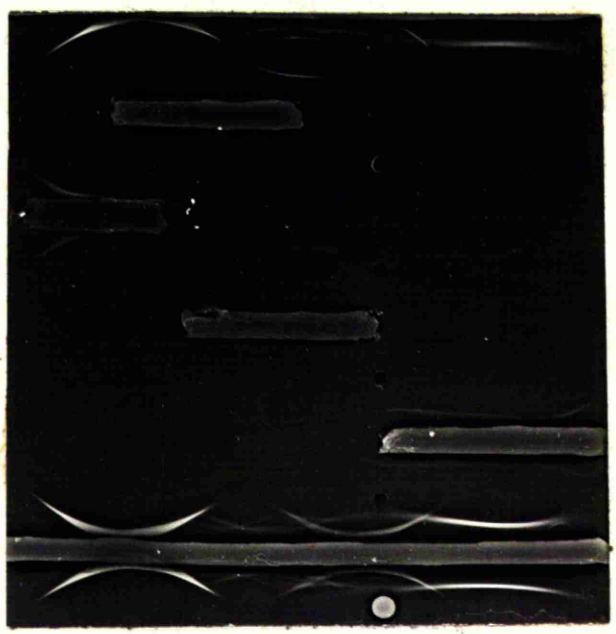
Albumin

Transferrin

Gamma G globulin

$Alpha_2$macroglobulin

Fig. IV



Wide Spectrum

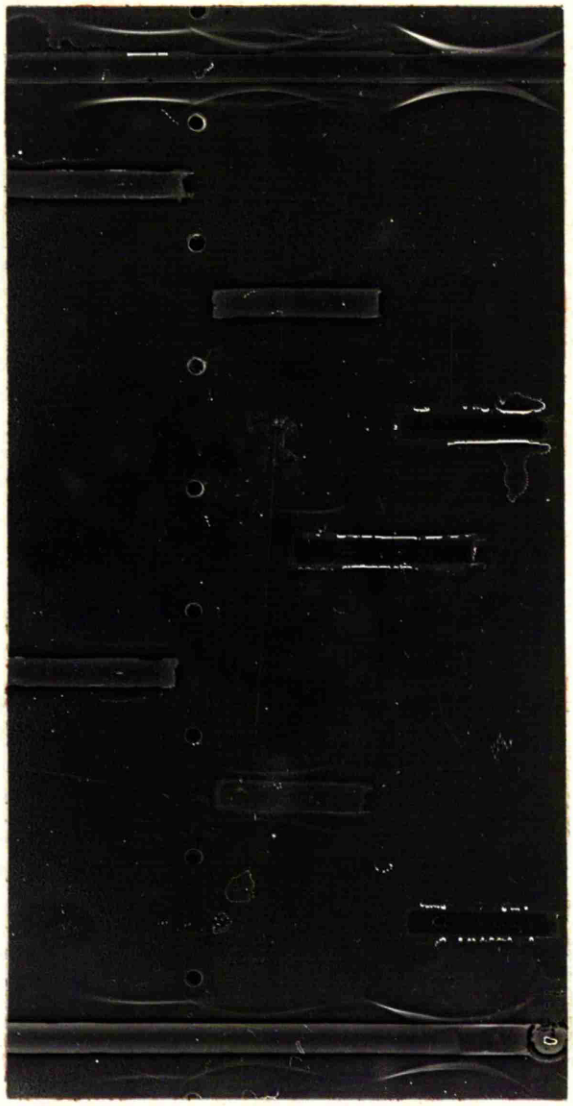
$Alpha_2$macroglobulin

Albumin

Transferrin

Gamma G globulin

Wide Spectrum



Wide Spectrum

Albumin

Transferrin

Gamma G globulin

Alpha$_2$macroglobulin

Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Fig. V

Wide Spectrum

Albumin

Transferrin

Gamma G globulin

Alpha$_2$macroglobulin

Fig. VI

Needle biopsy of Kidney in Mrs. A

Histological abnormalities were obvious in both glomeruli and tubules. The glomerular tufts (FIG.I) were much enlarged and filled their capsules. At several points there were adhesions between the tufts and the capsules. A patchy increase of mesangial substance was apparent. Capillaries varied in diameter and some showed a prominent sub-endothelial deposit. In some the basement membrane was obviously thickened.

The epithelium of the convoluted tubules was of low type and de-differentiated. In many the cytoplasm showed a frothy vacuolation. Basement membranes were prominent and many were thickened.

The appearances were those of a membranous glomerulo-nephritis showing considerable tubular degeneration.

FIG I

RENAL BIOPSY (PERIODIC ACID SCHIFF STAIN X 200)

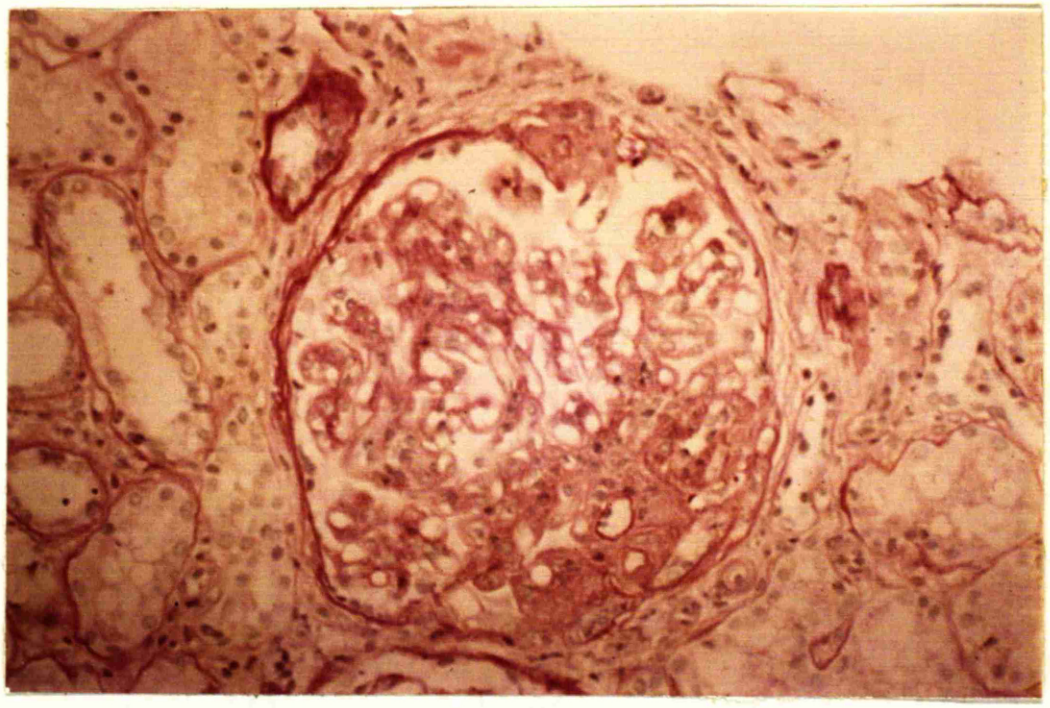


FIG II

RENAL BIOPSY (PERIODIC ACID SCHIFF STAIN X 800)

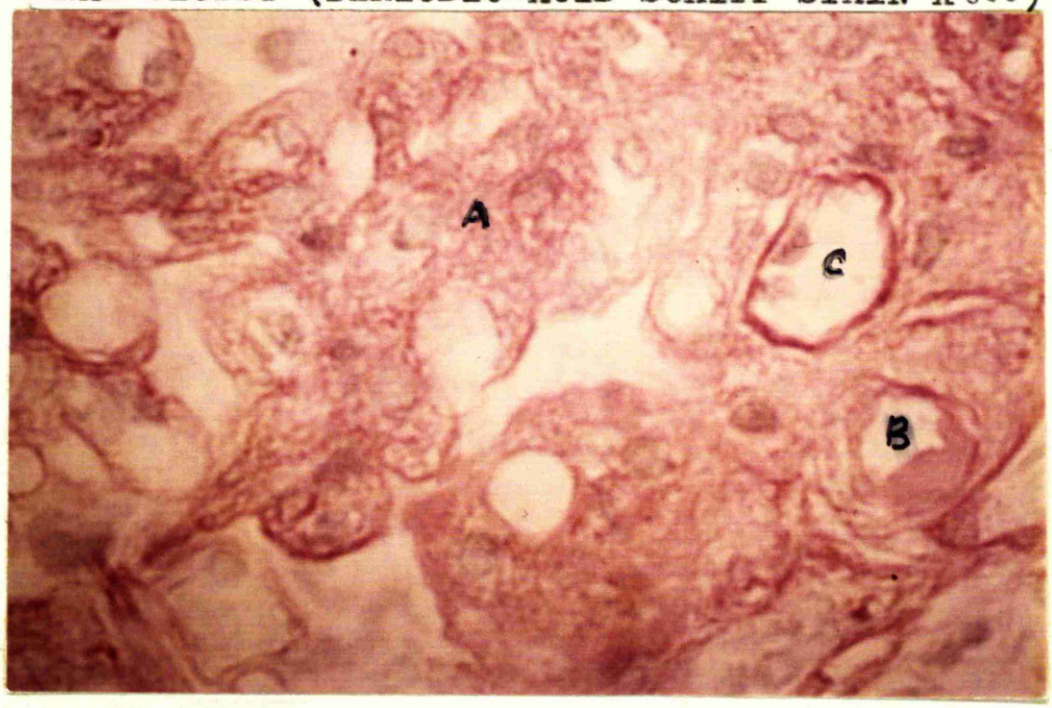
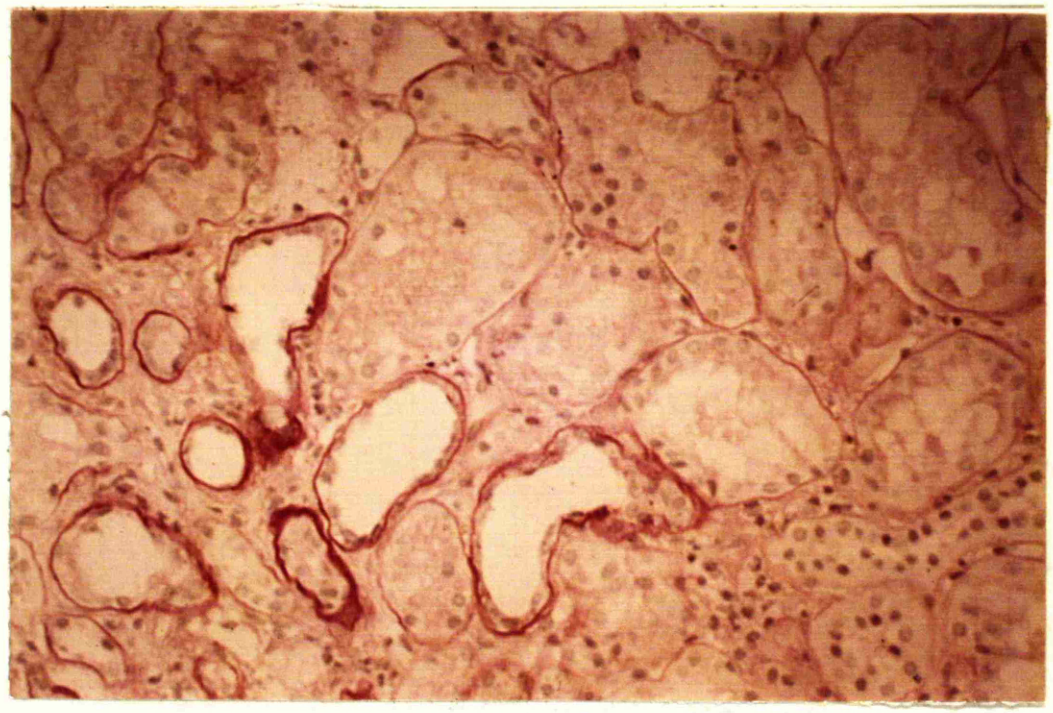


FIG III

RENAL BIOPSY (PERIODIC ACID SCHIFF STAIN X 200)

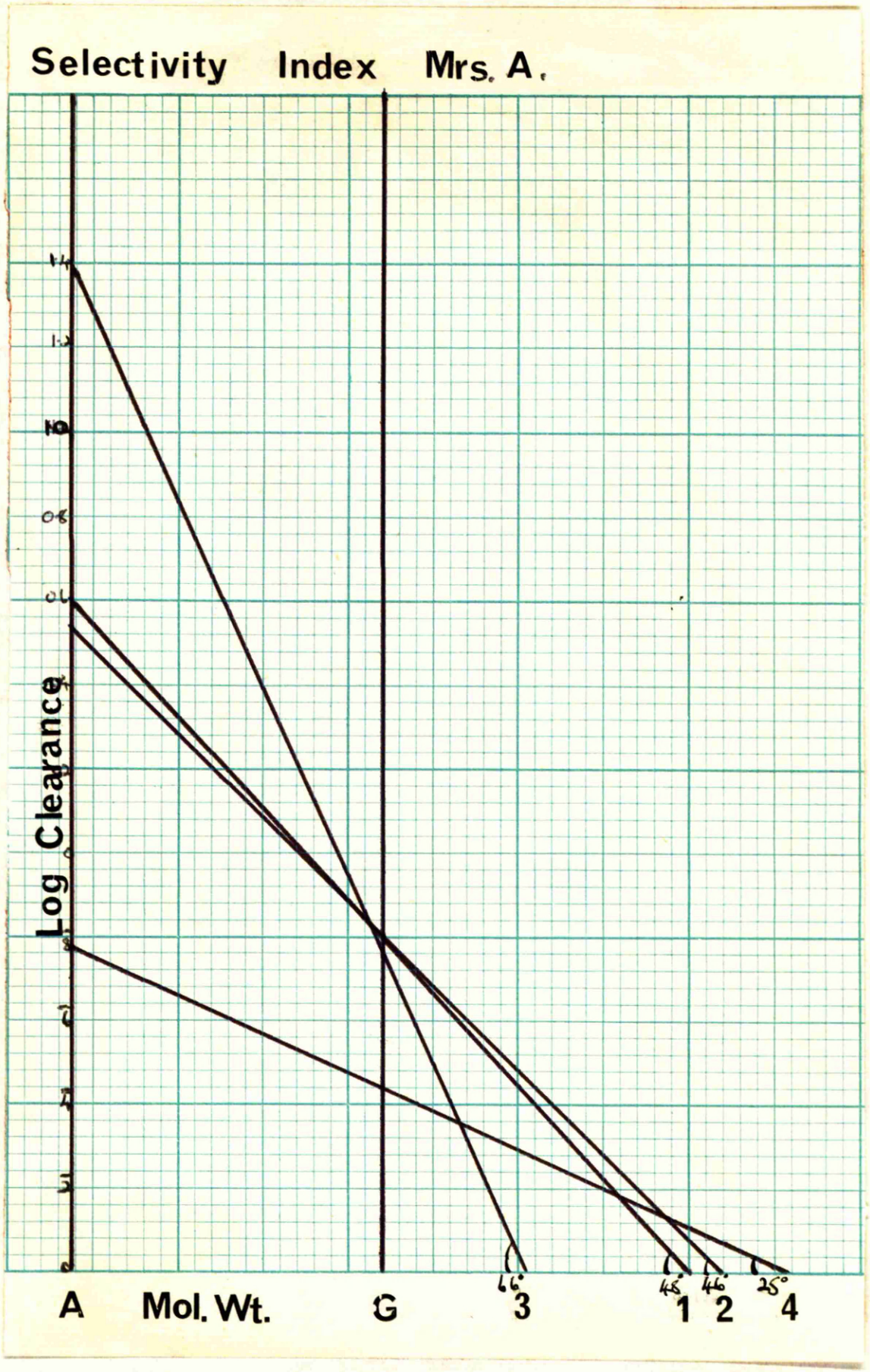


LEGENDS FOR FIGURES

FIG. I This is a glomerulus showing typical changes. There is an increase of mesangial substance, varying from point to point. Capillaries are widely patent but vary in diameter. The accompanying tubules are lined by low cuboidal cells and many nuclei are pyknotic.

FIG. II A high power view of the same glomerulus. At (A) the fibrillar mesangium is apparent. An eccentric subendothelial deposit is present in capillary(B,) and at (C)there is thickening of the basement membrane.

FIG. III The degenerate appearance of the tubules is obvious in this figure. Many nuclei have disappeared and others are pyknotic. A foamy vacuolation affects the cytoplasm and thickening of the basement membrane is present.


Selectivity Index Mrs. A.
Log Clearance
A
Mol. Wt.
G
3
1
2
4
46°
45°
46°
25°

Mrs. S. Aged 29 Para 0+0 Diagnosis : Severe Pre-eclampsia

No serious illness.

First attended antenatal clinic at twenty weeks albustix negative. Blood pressure 150/90 mm. Hg. Patient referred to own doctor for ante-natal care for next 10 weeks. Admitted at 30 weeks with eclamptic seizure. Heavy reaction with albustix, urine protein level 0.7g. per cent. Blood pressure 210/130 mm. Hg. Immuno-electrophoresis (FIG.I) with individual antiserum showed albumin, transferrin, gamma G globulin and $alpha_2$macroglobulin. This picture continued. (FIG.II). Conservative management undertaken for next two weeks. Blood pressure did not respond and blood urea rose from 48 to 70 mg. per cent. Pregnancy was terminated by elective Caesarean Section at 32 weeks with delivery of a live female child weighing 1.3 kilos. Immuno-electrophoretic pattern (FIG.III) continued to show $alpha_2$ macroglobulin one month after delivery at a urine protein level of 0.2 g. per cent. Blood

pressure had however returned to normal.

Comment: Although underlying renal disease cannot be ruled out as there is no pre-seizure immuno-electrophoresis, the clinical history is of fulminating eclampsia, with renal damage. The continued high gamma G globulin clearance and low index of selectivity agree with the persistence of $alpha_2$macroglobulin in the urine postpartum indicating more permanent renal impairment.

Antenatal selectivity indices : 1 - 3

Postnatal selectivity indices : 4

MRS. S Para (0+0) Aged 29 Severe Pre-eclampsia

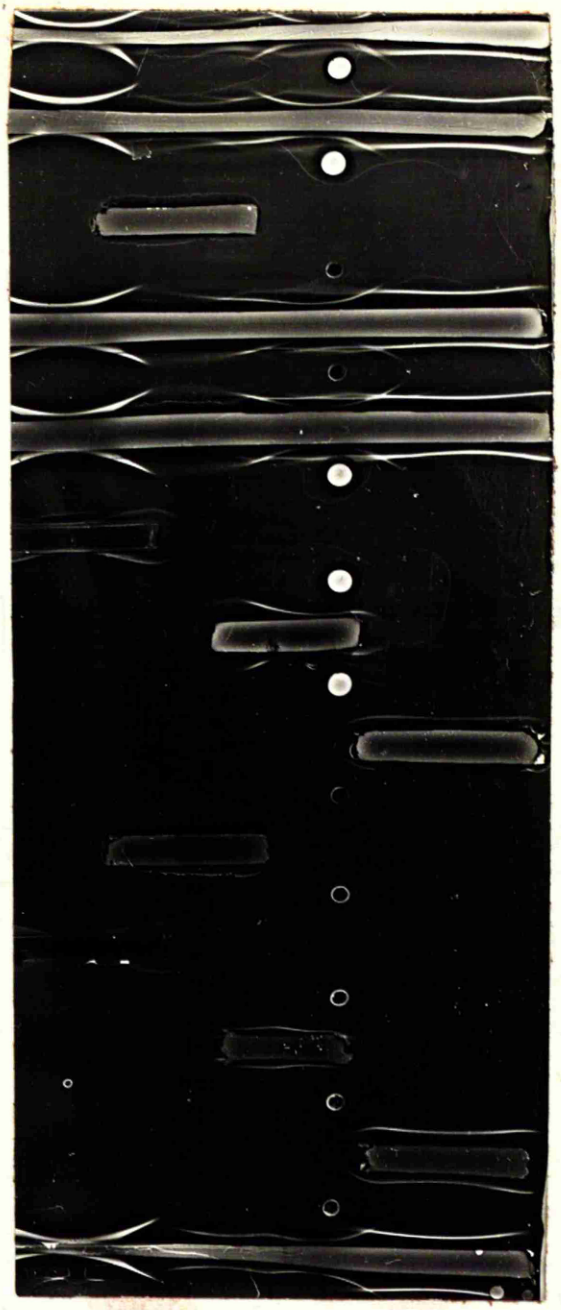


Fig.III — — — —

Fig. II

— — —

Fig. I

Wide Spectrum

Wide Spectrum

Alpha$_2$macroglobulin

Wide Spectrum

Wide Spectrum

Albumin

Transferrin

Gamma G globulin

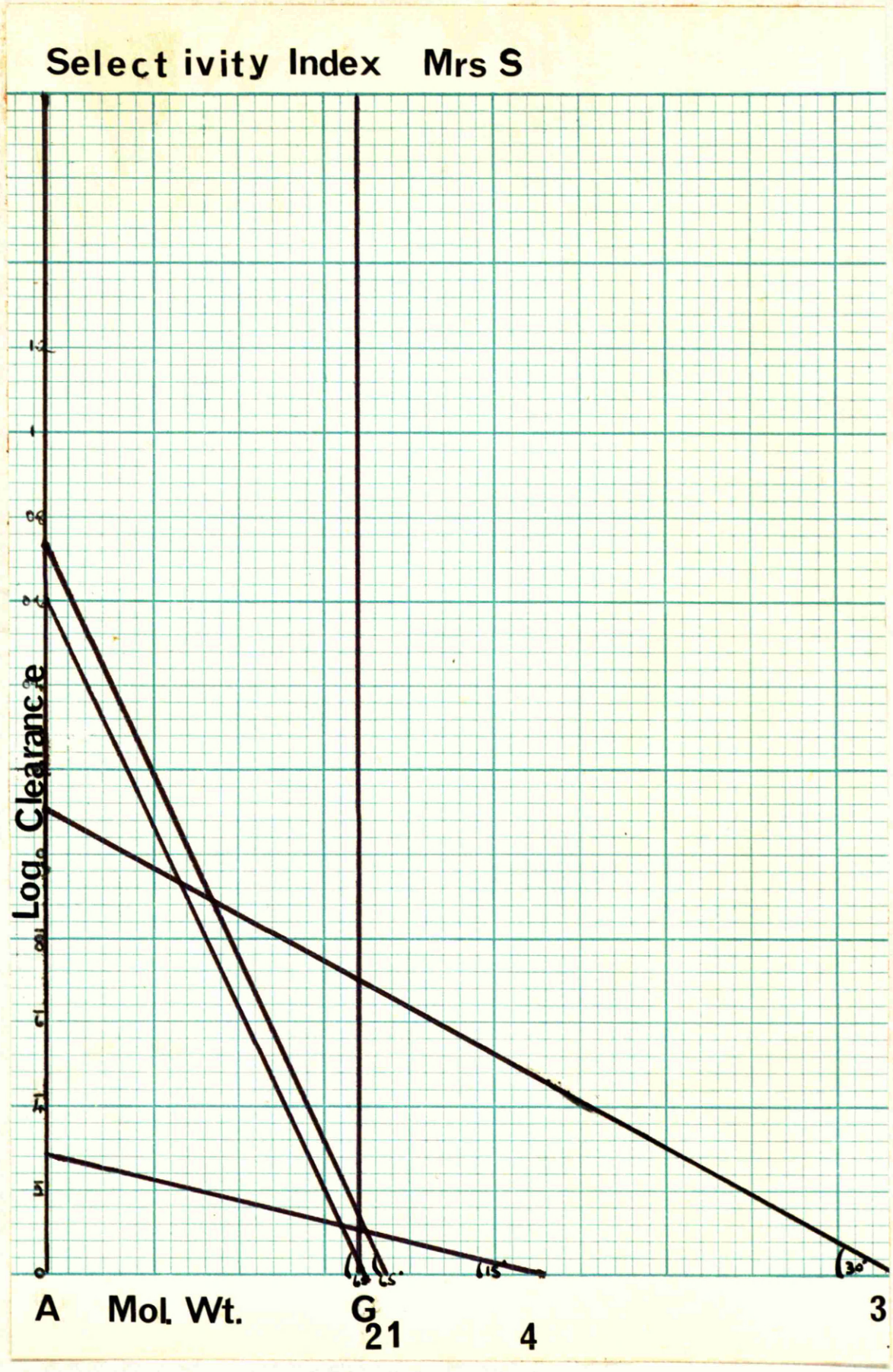
Alpha$_2$macroglobulin

Albumin

Transferrin

Gamma G globulin

Wide Spectrum


Selectivity Index Mrs S
Log Clearance
A Mol. Wt.
G
21
4
3

Mrs. McM. Aged 19 Para 0+0 Diagnosis : Severe Pre-eclampsia

No history of previous illness.

First attended antenatal clinic at 24 weeks. Blood pressure normal. No proteinuria. When seen at 28 weeks intermediate reaction with proteinuria present, urine protein level 0.4 g. per cent. Blood pressure had risen to 150/110 mm.Hg. Immuno-electrophoresis (FIG.I) revealed at least six different urine proteins with wide spectrum antiserum and arcs to albumin, transferrin and gamma G globulin with individual antisera. Following admission, with diuretics and sedation the condition remained unchanged the immuno-electrophoresis (FIG.II) continuing to show albumin, transferrin and gamma G globulin but no large molecular protein. At 31 weeks blood pressure rose to 175/115 mm. Hg., urine protein level to 0.6 g. per cent. and $alpha_2$ macroglobulin was demonstrated on immuno-electrophoresis (FIG.III). Caesarean Section was undertaken and a live

male child weighing 1.6 kilos was delivered which unfortunately died when three weeks old from infection and prematurity. Three days after delivery, the urine protein level had fallen to 0.15 g. per cent. and blood pressure to 130/85 mm. Hg. Alpha$_2$macroglobulin was no longer present (FIG.IV). Six weeks after delivery albustix was negative at a protein level of 0.1 g. per cent. and the immuno-electrophoresis (FIG.V) revealed traces of albumin. Blood pressure was normal at 120/70 mm.Hg.

Comment: This case illustrates the immuno-electrophoretic changes which occur in severe pre-eclampsia; the appearance of alpha$_2$ macroglobulin just prior to termination of the pregnancy and the rapid reversal after delivery. A close relationship of renal clearance and selectivity with the immuno-electrophoresis is again found. A raised gamma G globulin clearance of 0.8 fell to 0.2

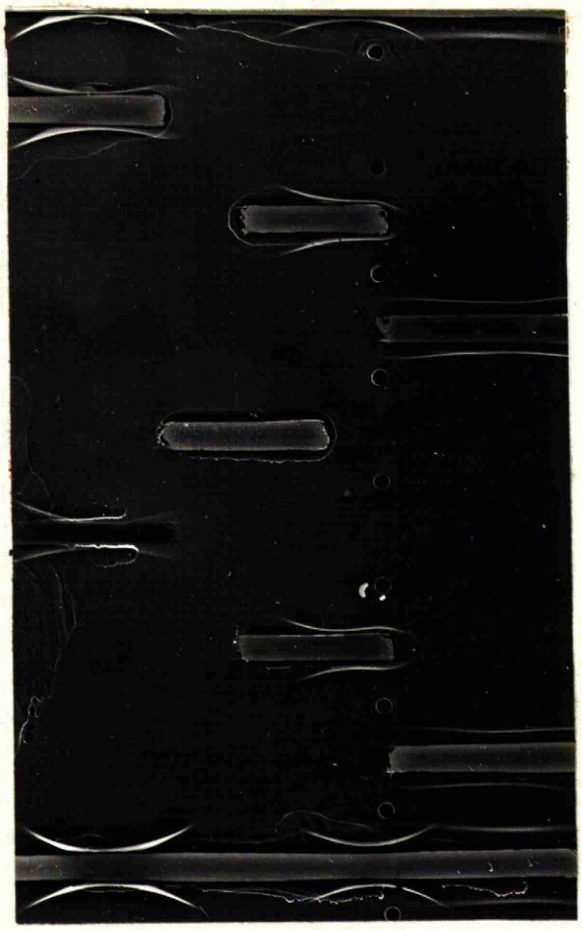
after delivery with a corresponding rise in index of selectivity from 22 to 66, when the immuno-electrophoretic pattern had returned to normal.

Antenatal selectivity indices : 1 - 2

Postnatal selectivity indices : 4

MRS.McM Para (0+0) Aged 19 Severe Pre-eclampsia

Fig.I



Wide Spectrum

Albumin

Transferrin

Gamma G globulin

Alpha$_2$macroglobulin

Fig.II

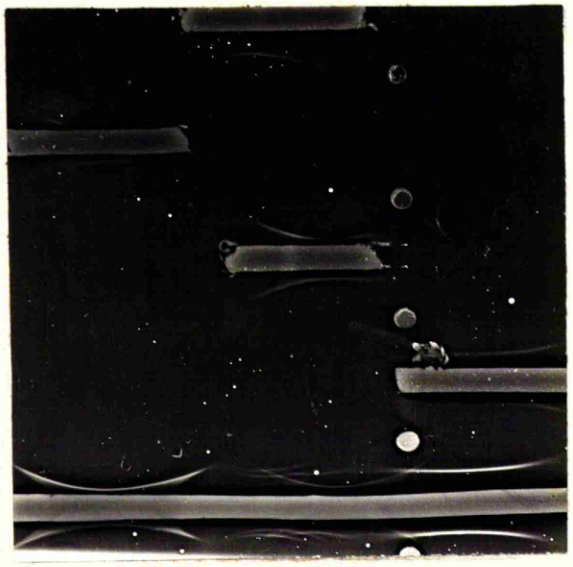
Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Fig.III



Alpha$_2$macroglobulin

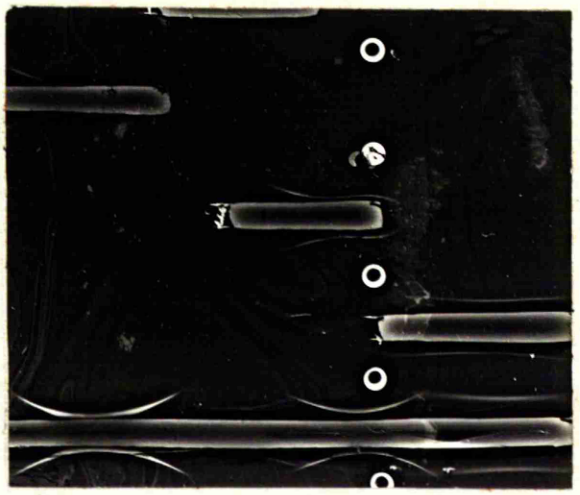
Albumin

Transferrin

Gamma G globulin

Wide Spectrum

Fig. IV



Alpha$_2$macroglobulin (ABSENT)

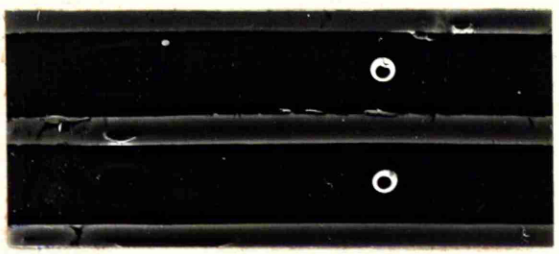
Albumin

Transferrin

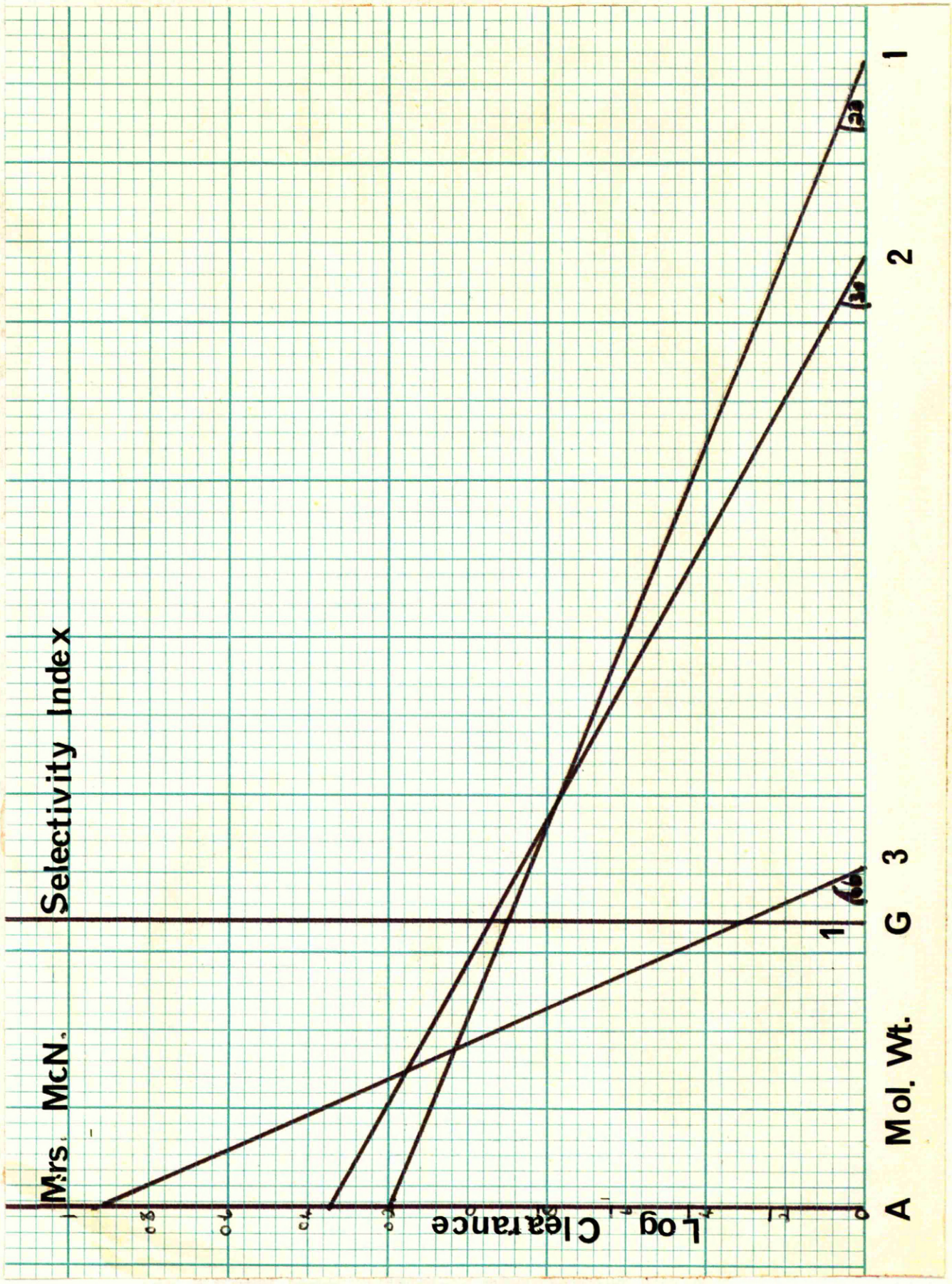
Gamma G globulin

Wide Spectrum

Fig. V



Albumin


Mrs. McN.
Selectivity Index
Log Clearance
A Mol. Wt. G
1
2
3

DISCUSSION

In this study an attempt has been made to investigate, by immuno-electrophoresis, proteinuria occurring in pregnancy: as a result it is hoped that a contribution will have been made towards a fuller understanding of this condition. As indicated in the introduction, it seemed to the author that no truly comparable investigation had ever before been attemped; consequently there is a meagre literature to which reference can be made.

A short historical account has been given on the diverse views held on the significance of proteinuria in pregnancy and on the development of methods as a means of investigation to enable a more precise definition of the proteins in the urine. From this it may be inferred, with modifications of methods and introduction of new techniques, changes do take place from time to time: on this basis it may be argued that some of the techniques employed may be open to doubt or question.

Nevertheless, on present day knowledge fundamentals of the method are generally accepted by all authorities. In particular this would seem to apply to the use of immuno-electrophoresis and immunodiffusion techniques which have been used so extensively in the present investigation.

On this assumption it is believed that the findings now presented are of some significance in the study of proteinuria in pregnancy and may perhaps be regarded as a basic point from which fuller investigation and study may emanate.

It is realised that the presentation of observations is one thing, whilst the interpretation of these observations is quite another. With this in mind, and accepting the significance of the techniques which have been employed as reliable, it is now proposed to discuss the findings here reported and attempt an interpretation.

In the first section of this study, various groups of patients were investigated in order to give a broad outline, covering the circumstances in which proteinuria may occur and be subject to change in pregnancy. A wide range of urine protein level and type of protein was found in the different groups. Despite the statement in Harrison's text book that normal urine contains either no albumin or, only an insignificant amount not great enough to be detected by regular tests, protein is present in normal urine in not inconsiderable quantity with a narrow dividing line between normal and abnormal urine. Dieckmann (1952) considered a twenty-four protein excretion which does not exceed 0.3gm. physiological. Addis (1948) however found that although normal individuals could excrete 10 to 30 mg. protein in 12 hours, the range of excretion in a group of patients with known renal disease was from 15 mg. to many grams and that

the spread of observation in normals and in those with renal disease overlapped so that thirty per cent. of patients with renal disease had levels of protein excretion within normal limits and therefore the absence of renal disease cannot be assumed even with a normal protein level. It is this wide range in both normal and abnormal subjects which makes it difficult to deduce much from total protein values with regard to the nature of any disease process. A more precise definition of the individual proteins which may reflect selectivity is necessary and immuno-electrophoresis is a technique which allows this to be done.

Since a metabolic interrelationship exists between the plasma proteins and other tissue proteins many workers have attempted to study the distribution of serum proteins in pregnant women. (Hoch et al 1948) (Paton et al 1964) (Brown 1954) (Von Studnitz 1955) (MacGillivray and Tovey 1957) (Paaby 1961) (De Alvarez 1961) (Bagga 1966). Although there is agreement that

serum protein concentration falls in pregnancy, there are extraordinary differences of opinion about the extent and pattern of fall. General opinion is of fall in albumin and rise in total globulin fraction in normal pregnancy. Tovey (1959) suggested without evidence that the fall in albumin is due to increased renal catabolism and Mack (1955) that the rise in the beta globulin is a manifestation of the high serum cholesterol found in pregnancy. It could be that the alterations and changes in protein pattern found in the urine in pregnancy to be discussed, are a reflection of the serum changes. This statement finds some justification on Wunderly Wuhrmann's (1953) evidence that protein content of several normal body fluids is similar to serum and that diffusion of proteins through a capillary membrane is determined to an important degree by molecular size. Although the question of increased or altered permeability in pregnancy is far from settled, reports by Pappenheimer et al (1951) on filtration, diffusion and molecular seiving through capillary membranes

lend support to the "Pore Theory of Capillary Permeability" and quite convincingly account for restricted diffusion as a function of molecular size. This concept suggests proteinuria results from increased permeability with "defects" or "pores" in the glomerular membrane of such dimension to permit the passage of proteins into the glomerular filtrate. Factors other than size and shape that may influence the rate of diffusion of molecules are considered negligible (Pappenheimer 1951). Although only indirectly applicable to proteins, clearance studies in animals utilising dextrans of varying molecular weight have demonstrated the distribution of "pore size" in the normal glomerulus within a range of molecular weight from 5,000 to 85,000. (Brewer 1951) (Wallenuis 1954). In addition these studies confirm the relationship between clearance of a molecule and its molecular weight.

As indicated in the introduction, paper electrophoresis can differentiate the conditions associated with proteinuria in pregnancy to a certain extent by comparison of the albumin/globulin ratios. (Lorincz et al 1961). (Parviainen et al 1951). This approach of assessing the relative increase and decrease in the main groups of proteins is too inaccurate to define differences present in the various conditions and it was on this account that the techniques of immuno-electrophoresis were explored. All protein fractions present in the blood may be found in the urine, although many of high molecular weight are present in extremely small amounts. The detection of these large molecules, it is suggested, is the important factor, not the quantity or relative amount as their presence would, on speculation, reflect changes in the glomerular membrane according to the "Pore Theory". Immuno-

electrophoresis provides a means of identifying various antigenic substances even in very complex mixtures as the precipitation reaction is specific. Serial studies with urine of reasonably uniform concentration subjected to immuno-electrophoresis give an indication of change in protein pattern and the employment of specific antisera to selected proteins reveals definite alteration in the character of the proteins present in the urine in different conditions. Thus it emerges that immuno-electrophoresis offers a more precise means of isolating proteins in the urine. Its value as a procedure however must lie in its significance. Several important aspects have to be considered.

The value of immuno-electrophoresis in the diagnosis of urinary infection must be assessed in relation to previous work in this field. Extensive studies of urinary tract infection were made by Dodds (1931-32) and Baird (1935-36) but with the introduction of antibiotics and the assumption that cure could be simply

achieved, a decrease in interest in this condition occurred. During the last decade however, it became evident that this assumption was false and there has been a revival in the study of bacteriuria, mainly resulting from the work of Kass (1956, 1960) who developed quantitative bacteriological methods. His work showed a strong link between asymptomatic bacteriuria found in early pregnancy and the subsequent development of pyelonephritis. The presence of symptomless bacteriuria or asymptomatic bacteriuria, a term implying multiplying bacteria within the urinary tract in the absence of symptoms of urinary infection is a frequent finding in pregnant women. Little (1962) found an incidence of 5.3 per cent. in a study of 5000 women antenatally and Savage et al (1967) reported a prevalence of 6 per cent. among women attending antenatal clinics. These workers and many others confirmed the relationship between asymptomatic bacteriuria and

subsequent development of acute pyelonephritis. On this account screening for bacteriuria has been developed, not only by bacterial counts but by chemical means. In the latter, two tests have been used, the triphenyl tetrazolium chloride (TTC) test and the Greiss test. Unfortunately both have proved unsatisfactory because they are insensitive to counts less than 1 million organisms per ml. Contrary to original reports screening for bacteriuria and its treatment are unlikely to eliminate acute pyelonephritis. (Kincaid-Smith 1965). One of the probable factors accounting for the higher incidence of bacteriuria in pregnancy is the increase growth rate of bacteria found in the urine of pregnant women (Roberts and Beard 1965). The problem facing the clinician is not so much the primary diagnosis of renal infection as how to determine when the condition can be considered cured.

Indirect methods employed have been the measurement of antibody levels against the O

antigen of gram negative bacteria. (Neter et al 1952) (Brumfitt et al 1963), urinary concentrating ability tests (Winberg et al 1958-59) (Katz 1961) (Elder and Kass 1965) (Seligman and Hewitt 1965) (Reeves and Brumfitt 1968) and urinary enzyme levels particularly beta glucuronidase (Bank and Bailine 1965) (Roberts et al 1967). Provocative tests have also been studied in an attempt to reveal latent urinary tract infection (Pears and Houghton 1959) (McFadyen and McCallum 1964) (Katz et al 1962) (Little and De Wardener 1962) (Kennedy et al 1964) (Briggs et al 1963) (Hutt et al 1961)

Recently several urinary protein studies have been performed. Tidstrom (1963) demonstrated a definite predominence in the beta-gamma region by paper electrophoresis of concentrated pyelonephritic urine and Goddard and Hobbs (1968) obtained similar results by electrophoresis of urine on cellulose acetate. In the present study we have confirmed this. There was a definite increase in the gamma G globulin compared to the albumin component. More

important were the findings in our cases following treatment. The protein content of the urine diminished and the significant finding was the virtual disappearance of gamma G globulin. At the same time the bacterial count of the urine diminished to acceptably normal limits. Although the number of cases is small and therefore would not probably stand up to a statistical analysis, it seems reasonable to postulate in view of the recent urinary protein studies, and since hypergammaglobulinaemia is known to occur in chronic infections and connective tissue disorders (Ishizaka 1963) (Cohen et al 1964) that the detection of urinary gamma G globulin may be valuable in assessing urinary tract infection.

In studying the question of proteinuria two main problems immediately faced us. The first of these is of immediate practical nature:- the possibility of using the

detection of proteinuria as a diagnostic and prognostic tool. Secondly, there is the more academic question of the nature and mechanism of proteinuria itself. In considering renal infection it was shown that the most characteristic feature was the appearance of gamma G globulin, a protein of large molecular size compared to albumin. If the theory of pore size of the glomerular capillaries is correct, then at first sight it would appear that bacterial toxins or at least the products of inflammation can alter this pore size. It would also appear from the reduction in gamma G globulin in treated cases that this alteration in pore size is of functional nature and is not due to permanent structural changes. Since, however, gamma G globulin can be found in normal urine it could be that the increase found in urinary infection is not so much a change in glomerular capillary pore size as an alteration in the ability of tubules to re-absorb protein. Whichever of these is

correct, it is still obvious that the passage of large molecular proteins in the urine can be the result of functional change.

One of the central problems considered in the present thesis is the difficulty in distinguishing between primary renal disease (nephrosis) and pre-eclampsia, since in both conditions one may find proteinuria and varying degrees of hypertension. In our cases of nephrosis, the degree of proteinuria was moderate and hypertension was not a feature. This might seem to differentiate them from cases of pre-eclampsia. It is well recognised, however, that hypertension is not always the presenting feature in pre-eclampsia and proteinuria may precede a rise in blood pressure. Again, increase in blood pressure may be present in nephrosis and the problem of differentiating between this state and established pre-eclampsia appears once more.

The most apparent difference between the cases of nephrosis and those of pre-eclampsia was the constancy of the degree of proteinuria in the former and the variation in the latter. This, however, by itself is not a reliable diagnostic test since many cases of moderate pre-eclampsia can have a low grade proteinuria for a considerable time. It is in these circumstances that immuno-electrophoresis becomes useful. Analysis of the pattern obtained showed that alpha$_2$macroglobulin was present at all stages of pregnancy and at all degrees of proteinuria in our cases of nephrosis, whereas it was absent in moderate cases of pre-eclampsia. It did appear in severe pre-eclampsia but only late in the condition when the degree of proteinuria became gross. In addition, this protein disappears rapidly in the puerperium in cases of pre-eclampsia whereas in nephrotic cases it persists. The mechanism of proteinuria in these cases of nephrosis is not entirely clear. The normal kidney conserves

protein to a remarkable degree and analysis of the fluid obtained by tubular puncture in animals has shown that the glomerulus is responsible for the major part of its retention (Wearn and Richards 1904). (Walker et al 1941). The rate of production of glomerular filtrate concentration in this fluid could produce a major proteinuria in the absence of tubular re-absorption. There is good, if circumstantial, evidence of tubular re-absorption of protein from studies on pathological proteinuria (Hardwicke and Squire 1955) and from the histological demonstration in animals of the accumulation of protein-bound dyes and radio-active labelled proteins within the cells of the proximal tubule (Oliver 1948) (Spector 1954). The presence of protein in the urine therefore might also give arise from failure of the tubules to re-absorb all the protein of the glomerular filtrate. Numerous observations on pathological proteinuria have

shown that the diseased kidney is selectively permeable to plasma proteins according to their molecular weight, smaller protein molecules appearing more readily in the urine than the larger ones (Hardwicke 1954) (Rowe 1957) (Blainey et al 1960) (Cameron et al. 1965).

In our cases of nephrosis the main feature was the appearance of large molecular proteins in the urine when proteinuria was of moderate degree. If the basic lesion had been a general increase in pore size of the glomerular capillaries one would have expected a gross degree of proteinuria with large amounts of albumin when macroglobulins appeared in the urine. It might be, of course, that the small amount of protein in the urine was the result of active tubular re-absorption. This is to suggest that the tubules were healthy if not over-active although the glomeruli were diseased; and to produce the pattern observed the tubules would require to exert a highly selective re-absorption. A more reasonable

explanation might be to suggest that in nephrosis the glomerular lesion is patchy, many glomeruli being normal. This would account for the low-grade proteinuria. Not only so, but there are structural changes and individual capillaries of diseased glomeruli are not all affected in the same way. In some, the pores will be larger than normal thus allowing large molecular proteins to escape as well as small molecular proteins. On the other hand many capillaries may well have thicker walls and smaller pores. The variability in the structure of the capillaries of the glomeruli is obvious from the illustrations in the text, (Pages 142-3). In the illustrations too it is obvious that the tubules are far from healthy. Such a concept would account for the peculiar protein pattern in the urine.

Another feature noted in these cases was the reduction of protein excretion in the puerperium, although the pattern remained the

same. This suggests that pregnancy did influence the renal function in these cases by increasing permeability.

Pre-eclampsia itself is an extremely puzzling disease. It appears to be a self-limiting disease in the sense that it disappears with the termination of pregnancy and leaves no trace of its existence although proteinuria may have been extremely gross. The degree of proteinuria appears to run roughly parallel with the severity of other signs such as hypertension and oedema. The hypertension in pre-eclampsia, however, in terms of general medicine is rarely severe. Even in very severe pre-eclampsia, the systolic pressure rarely exceeds 190 mm. Hg. and the diastolic 115 mm. Hg. In essential hypertension pressures greatly in excess of this are commonly found in medical wards, but it is uncommon to find proteinuria of any significant degree if at all. Even in pregnant subjects suffering from essential

hypertension studied by us, the findings were similar to those in the normal pregnant patient. From this it would seem that hypertension per se plays little part in the causation of proteinuria.

Study of the cases indicates that the protein increases steadily as the disease increases in severity. At the same time, it has been demonstrated the larger protein molecules appear in the urine suggesting that the main effect of this disease is to increase the permeability of the renal apparatus perhaps by an increase in the pore size of the glomerular capillaries. Unfortunately, the renal lesion of pre-eclampsia is complex and not easily analysed (Dennis et al 1963) (Dieckmamnet al 1958) (Hopper et al 1961). There is histological evidence however, that abnormal protein material of large molecular size is passed through the glomerulus in cases of pre-eclamptic toxaemia and eclampsia. (Govan 1954) (Pirani et al 1963) (Pollack et al 1960).

Clinically and biochemically the renal changes regress in the puerperium (Fadel et al 1969). Whether the histological change disappears and whether the ultimate prognosis is good gives rise to some dispute and controversy still extists regarding the late effects of eclampsia and severe pre-eclamptic toxaemia. In an attempt to quantitate the findings in our studies, clearance rates and indices of selectivity were calculated. These showed that in the antenatal period in toxaemia the clearance of protein increased as the disease progressed and the selectivity became poor as indicated by a low index. These findings were even more marked in eclampsia. Similar results were shown in the cases of nephrosis and therefore this method of study did not differentiate between these conditions. However, in the postnatal period it became obvious that in most of the toxaemic cases there was a rapid return to normal with a fall

in clearance rate and marked rise in the index of selectivity . In contrast, was the finding in the case of proved membranous glomerulonephritis of an increase in the gamma globulin/transferrin clearance and a fall in the selectivity index during the puerperium. It is to be noted that this case deteriorated even further, months later. One case of eclampsia showed a similar pattern in the postnatal period and from this we suspect that there is underlying structural damage to her kidneys. This case is being followed.

Foetal Loss and Proteinuria

The assessment of foetal loss in the various conditions studied is difficult. Infants lost both from intra-uterine death and from prematurity must be taken into consideration. Thus the perinatal mortality rate gives a more accurate picture combining the number of stillbirths and the number of babies dying in the first week of life. Prematurity is a major factor in the neonatal deaths, arising

not only from spontaneous labour but also following induction where termination of pregnancy has been undertaken in the maternal welfare or foetal interest.

Much as been written on the effect of pyelonephritis on the foetus. The risk of prematurity and increased foetal loss in pregnant women with persistant bacteriuria was first reported by Kass (1960). This issue has proved to be controversial Leblanc and McGanity (1964) Kincaid-Smith and Bullen (1965) Stuart et al (1965) and Layton (1964) agree with Kass. While Whalley (1965) Norden and Kilpatrick (1965) Sleigh et al (1964) Bryant et al (1964) failed to confirm his findings.

Although controversy continues unabated the important thing at present is to treat this condition adequately. As previously stated it is difficult to determine when cure has been achieved. By use of immuno-electrophoresis it is possible to monitor the effects of treatment and determine if infection has been cleared or is likely to recrudesce.

The reports on foetal loss in association with maternal hypertension are very conflicting. Walters (1966) found the perinatal mortality three times as high as in normotensive primigravida although sustained hypertension of mild to moderate degree did not influence the growth of the foetus. Harley (1966) stated that the foetal loss was higher in hypertensive multiparous patients over the age of 35 than in hypertensive primigravida below that age. Others however have stated the hypertension per se has little effect on foetal prognosis. This controversy illustrates the pitfalls and unreliability of retrospective studies. Many factors may influence the outcome in this type of case, particularly the incidence of premature delivery. Pregnancies are frequently terminated prematurely in the interests of the mother.

There have also been studies of apparent essential hypertension in pregnancy associated with proteinuria and several observers have

noted an adverse effect on the foetus (Chesley and Annito, 1947; Taylor et al 1954; Townsend 1959). On the other hand Harley (1966), matching cases for age, parity and initial diastolic pressure, found that the difference in foetal mortality between cases with proteinuria and those without proteinuria was not statistically significant, but that foetal loss occurred irrespective of proteinuria when the diastolic pressure was above 100 mm. mercury. The first criticism which might be offered is that it is not clear whether these authors were studying the same condition. Patients known to have hypertension prior to pregnancy do not always have proteinuria nor do they necessarily develop it during pregnancy. One is left wondering therefore if many of these subjects were suffering from a primary renal condition or if many had developed a superimposed pre-eclampsia. In the patients studied by us there was no clinical evidence

of proteinuria and the outcome of the pregnancies was satisfactory. It is true that in patients suffering from proteinuria plus hypertension the foetal prognosis is roughly related to the degree of hypertension but in the individual case this does not necessarily follow. This is apparent from one of our cases of antepartum eclampsia which was treated conservatively with the subsequent birth of a live baby. It is equally true that proteinuria per se can not be accepted as a guide to prognosis. Originally we analysed a large number of case histories on the basis of the degree of proteinuria, to find if the estimation of total urinary protein in hypertensive cases could be a reliable guide to prognosis but we abandoned this when it became obvious that such a crude method was useless. It was only by studying the question of proteinuria in some depth that some sense could be made of this problem.

Even when the urinary proteins could be individually identified it became apparent that the presence of particular proteins varied in significance according to the basic nature of the condition. Comparison of cases of nephrosis and pre-eclampsia provides an illustration of this. A wide range of foetal loss in women with renal disease has been reported. McKay (1963) in a ten year survey involving 150 patients found an overall foetal wastage of 34 per cent. Hamilton (1952) studied 19 cases of chronic nephritis and the foetal loss was 72 per cent. Wilson (1958) in a more detailed investigation of 60 pregnancies found an overall foetal loss of 16 per cent.; but if the only sign of renal disease was proteinuria this figure dropped to 6 per cent., whereas if other features of chronic nephritis were present the incidence rose to 39 per cent. Schweitz and Seftal (1957) reported on 20 cases of the nephrotic syndrome during 21 pregnancies resulting in 22 live births. Renal biopsy in 7 of these cases showed membranous (Type II Ellis)

glomerulonephritis. Good results have also been reported by Eden and Holland (1948), Tillman (1951), Kellar (1963) and Hopper et al (1961). In MacKay's (1963) series he noted that foetal prognosis was affected by superimposed pre-eclampsia and the occurrence of an acute phase in the renal disease. Tillman (1951) came to the same conclusion. Seven cases of the nephrotic syndrome were studied in the present thesis. None of these subjects had hypertension and there was no sign of pre-eclampsia. All gave birth to live babies. It is interesting to note this in view of our constant finding of large molecule proteins such as alpha$_2$macroglobulin in the urine of these patients. Apart from being an indication of damage to the renal tissues the presence of these proteins appeared to have no other significance so far as the pregnancy was concerned. In contrast to this were the cases of severe pre-eclampsia and eclampsia. In the severe group of pre-eclampsia in Section I of this thesis, the foetal loss was 26 per cent.

due mainly to intra-uterine death with the birth weight of 25 per cent. of the cases below the tenth percentile for gestational age. In the moderately pre-eclamptic group birth weight and outcome were unaffected, thus the foetal loss would appear to depend on the degree of hypertension and urinary protein level as babies were lost in the group of patients where the diastolic blood pressure was above 100 mm. Hg. and protein excretion level above 1.8g. per 24 hours. The appearance of large molecular proteins in the urine was always associated with a deterioration in the patient's condition and was always of particular significance in relation to foetal prognosis. The was especailly true of $alpha_2$macroglobulin. Whenever this protein made its appearance it heralded the approach of intra-uterine death. In addition it seemed to be a more reliable guide in some cases than the clinical condition of the patient. The picture can be complicated however, particularly where the patient is seen as an emergency with signs of pre-eclampsia but has in addition underlying renal disease.

This was apparent in one case of antepartum eclampsia which was treated conservatively. Although alpha$_2$macroglobulin was present in her urine she subsequently gave birth by Section to a live baby two weeks after the eclamptic episode. During her puerperium she continued to excrete alpha$_2$macroglobulin and proteinuria was still present some months later. Nevertheless, in retrospect one would suggest that such a case ought not to be treated conservatively and that the presence of alpha$_2$macroglobulin should be a signal for interference.

The appearance of protein in the urine in pregnancy causes concern with regard to foetal well being because of the frequent association with placental insufficiency and intra-uterine death. The optimum time for termination of pregnancy in such cases is usually chosen empirically, irrespective of the period of gestation. An index of placental function, thus rationalising the timing of termination is required. Oestriol excretion has been found to be low in pre-eclampsia (Smith and Smith 1933) (Zondek and Goldberg 1957) (Kellar et al 1959) (Coyle et al 1962) (Roy et al 1963) (Michie 1967)

However Muller et al (1967) were unable to demonstrate that oestriol assays were of any value in the management of patients with pre-eclampsia and Booth et al (1965) felt that definite changes were only seen in severe conditions. A suggestion has also been made that the renal threshold of oestriol may be raised in pre-eclampsia (Nachtigall et al 1968) and variation in oestriol level may be due to renal impairment rather than diminished production (Roy 1963). Although Reid et al (1968) found good correlation between oestriol excretion and the perinatal mortality rate in conditions associated with proteinuria in pregnancy, oestriol estimates cannot foretell the time of foetal death nor indicate the optimum time for termination of the pregnancy.

From our results it is obvious that the appearance of alpha$_2$macroglobulin in severe pre-eclampsia is of considerable significance. Its appearance is usually of sudden onset, and heralds imminent danger to the foetus. It may

be pertinent to observe that once intra-uterine death associated with pre-eclampsia has occurred and delivery taken place there is rapid disappearance of the alpha$_2$macroglobulin although not of the other proteins present in the urine which is similar to earlier reports on the variability in albuminuria following foetal death, (Powilewicz et al 1924) (Dexter and Weiss 1941) (Kobes 1930). Immuno-electrophoretic analysis of urine in these cases can be achieved in a reasonably short space of time and the method could be used to monitor cases of severe pre-eclampsia.

CONCLUSIONS:

From this study the following conclusions would appear to be justified.

1. The technique of immuno-electrophoresis and immunodiffusion provide satisfactory means of investigation of proteinuria in pregnancy.
2. Definite identifiable protein patterns exist in various types of proteinuria in pregnancy and by serial investigation throughout pregnancy changes in the progress of the disease can be monitored.
3. In cases of urinary tract infection, proteins such as gamma G globulin are constantly present but gradually disappear with treatment. The reduction in the amount of gamma G globulin parallels the diminution in the bacterial count and in some ways is a more sensitive index of treatment.

4. The range of proteins, according to molecular size, is related to the degree of protein excretion in cases of pre-eclampsia.
5. In severe pre-eclampsia and in nephritis, the large molecular weight protein, alpha$_2$ macroglobulin is present in the urine. Distinction between the two conditions can be made by the constancy of alpha$_2$ macroglobulin in nephritis where as in pre-eclampsia, it is only present in severe stages of the disease when proteinuria is marked. It disappears rapidly after delivery.
6. Distinction between severe pre-eclampsia and nephritis is important not only in the interests of the mother but also in regard to the well being of the foetus. The presence of alpha$_2$macroglobulin in the maternal urine is a bad prognostic sign to the foetus in pre-eclampsia, whereas in proteinuria of renal origin,

the foetal outcome is good. Thus assay of urinary alpha$_2$macroglobulin may be used as a monitor of the foetal state.

7. There is an altered permeability of the glomerulus in pregnancy as demonstrated by protein clearances and selectivity indices. This would appear to be of functional nature in pre-eclampsia, but the findings in nephrosis can only be explained on the basis of structural damage.
8. Protein clearance and the selectivity index are related to the passage of large molecule proteins and therefore are of limited value as prognostic tests in the antenatal period. They are however useful im the puerperium in detecting these cases where pre-eclampsia has been superimposed on an underlying organic renal lesion. In these cases the clearance of protein remains high and the selectivity index is low.

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ADDENDUM

Two papers "Investigation of Proteinuria in pregnancy by immuno-electrophoresis" and "Investigation of Proteinuria in patients with hypertension in pregnancy" have been published in the Journal of Obstetrics and Gynaecology. British Commonwealth 1968, 75:289-296 , 1969 76:809-812.

These papers are incorporated in this thesis.