P R E - E C L A M P S I A

Renal and Hepatic Factors in Relation to Proteins.

by

J.T.S. Brown,
M.B., Ch.B., M.R.C.O.G.

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ProQuest LLC.
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PREFACE

The work described in this thesis was carried out at the Research Department, Glasgow Royal Maternity and Women's Hospital under the direction of Dr. A.D.T. Govan.

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SECTION 1

INTRODUCTION.

The proteins of the blood alter during pregnancy. Many authors have contributed to the literature on this subject, notably Dieckmann 1941, Dieckmann and Wegner 1934, Bergmann 1924, Eastman 1930, Plass and Bogert 1924, Plass and Matthew 1926, Strauss 1935 and 1937, Coetzee and Marrack 1924, and Undaliar, Nayar and Nenon in 1940. These authors mentioned all agree that the plasma proteins decrease during pregnancy. As long ago as 1871, Hamilton drew attention to the "watery" appearance of the blood in the toxaemias of pregnancy. The blood similarly becomes "watery" to some extent in normal pregnancy and Dieckmann (1952) states that the average serum protein concentration in pregnancy is 6.5 gms. per cent. and 1 gm. per cent. lower than in the non-pregnant state. Plass and Matthew (1926) reported that the fall in blood proteins was a progressive phenomenon, which continued until the ninth month of pregnancy; thereafter it rose, but the value at term was still below normal.

A degree of physiological dilution of the blood

or hydraemia does occur in normal pregnancy and the apparent loss of protein is relative not absolute, as confirmed by Wegner and Dieckmann (1934). According to their report, Dieckmann and Wegner found an average drop in serum protein concentration of 7 per cent.

Various investigators have studied the total serum proteins in toxaemic patients and in normal pregnant patients. All agree that the total serum protein and the serum albumin decrease in normal pregnancy, and that the decrease is more marked in toxaemic cases. In 1924 Coetzee and Marrack observed that, in toxaemia of pregnancy, there was a uniform fall in plasma albumin. Investigations of Plass and Bogert (1924), Eufinger (1928), Eastman (1930), Strauss (1937), Reinhart (1945) and Moller-Christensen (1946) also confirm that, in toxaemia of pregnancy, the protein concentration of the plasma decreases and mostly with regard to the albumin fraction.

In recent years considerable attention has been drawn to the effects of high protein diet on the incidence of toxaemia of pregnancy. The investigations of the People's League of Health (1942) and the

Toronto Experiment conducted by Ebbs, Tisdall and Scott (1941) point out that the incidence of pre-eclamptic toxaemia could be reduced if pregnant women were provided with an adequate diet of optimum protein content. These findings are corroborated in the literature by the work of Harden (1937), Strauss (1937) and by Harden, McEllroy and Huggins (1935), whereas Williams (1945) stated that he was "unable to make any positive correlation between the intake of protein and the severity of toxaemia".

According to Strauss (1937) there is a relation between hypo-proteinaemia and the oedema which occurs in pregnancy toxaemia. In support of this it is known that starvation causes a fall in plasma protein values, (Schittenhelm and Schlecht (1919), Hansen (1920), Bruckman and Peters (1930), Kumpf (1931), Weech and Ling (1931), and Liu, Chu, Wang and Chung (1932), and that oedema frequently accompanies starvation. Dieckmann (1952) and Tillman (1939), however, do not agree and affirm that oedema has nothing to do with plasma protein concentration. There is therefore variance on the question of the relationship, if such exists, between oedema and the concentration of the protein in the blood. It is

claimed however that the symptoms of pre-eclamptic toxaemia are alleviated (Harden (1936), Strauss (1938), and the incidence diminished, ((Interim report of the People's League of Health, (1942), and Ebbs, Tisdall & Scott (1941)) by a high-protein diet. In this respect it is interesting to note that Smith, Belt and Whipple (1920), Barker and Kirk (1930), Leiter (1931), Shelbourne and Egloft (1931) and Weich, Snelling and Goettsch (1933) state that plasma proteins can be regenerated by correction of dietary deficiencies. This may prove helpful in the treatment, and prevention, of pre-eclamptic toxaemia.

In 1856 Tarnier suggested that there was a degree of hepatic insufficiency in normal pregnancy and in 1907 Hofbauer described histological changes occurring in the liver during pregnancy. Seitz (1927) stated that unobilinuria was present in 64 per cent. of his cases of normal pregnancy. Schmidt (1928), Mikeldase (1928) and Breda (1929) reported similar findings. The investigations of Heinrichdorff (1913), Schikele (1917) and

Rolleston and McNee (1929), on the other hand did not wholly support the theory that the liver's function was impaired nor it's anatomy deranged during normal pregnancy. Various hepatic function tests, notably those dealing with bilirubin excretion, were utilised by Hofbauer (1933), Soffer (1933), Kauffman (1932) and Sullivan, Tew and Watson (1934). These authors all found an increased bilirubin retention in normal pregnancy.

In the pregnancy toxaemias a great many investigators have found an increase in serum bilirubin (Herrmann (1929), Eufinger and Bader (1926), Cantarow, Stuckert and Gartman (1935) and bilirubin retention has been found to be abnormally high by Lyon (1933) and other workers. It is, then, not surprising to find that many authorities have subscribed to the view that a state of hepatic dysfunction exists in toxaemia of pregnancy (Hofbauer (1933), Botella-Llusia (1936), Herold (1928), Rowe, McManus and Plummer (1936). Subnormal findings in hepatic function tests performed on pre-eclamptic patients have been reported by Kaufmann (1932), Hofbauer (1933),

Sullivan, Tew and Watson (1934) and Hirscheimer (1939). It is now fairly well established that the liver is the source of a number of the plasma proteins (Best and Taylor, 1950) and it is known that the level of the plasma proteins is low in hepatic dysfunction. Foster and Whipple (1921) found that the fall in plasma proteins was related especially to the albumin fraction. Apart from primary liver failure, Elman and Heifetz (1941) produced evidence to suggest that prolonged hypoproteinaemia upsets liver function and this is supported by the work of Himsworth and Glynn (1944) who showed that protein deficiency could result in hepatic necrosis.

The occurrence of albuminuria has naturally resulted in a number of theories relating pre-eclamptic toxaemia with renal pathology. The renal lesions of toxaemia are sometimes compared with those occurring in nephritis and nephrosis. Diminution of the plasma proteins is a constant feature of cases of nephrosis (Squire, 1953). Similarly, attempts have been made to compare toxaemia with malignant hypertension. Lewis and Page (1947) have shown that the latter condition

is accompanied by characteristic changes in the plasma protein pattern. If hypertensive toxaemia has any fundamental relationship with either of these conditions one might expect comparable changes in the plasma proteins.

From the above it is apparent that a study of plasma proteins might yield considerable information regarding the nature of the pathological process which is operative in the toxaemia of pregnancy. Studies of total plasma proteins and of albumin:globulin ratio have already been carried out by a number of observers as indicated above. There has, however, been little investigation of the various protein fractions in these conditions, although distinctive changes have been shown to occur in pathological conditions affecting the non-pregnant individual. Lewis and Page (1947) have investigated the electrophoretic pattern of the plasma protein in patients suffering from hypertension, as previously noted, and Griffiths and Brews (1953) have reported on characteristic changes in the serum in Multiple Myelomatosis. A further investigation of serum, and urinary, proteins has been carried out by Jamieson and

Addis (1951) who studied cases of pre-renal (non-myelomatous) proteinuria and reported variations from the normal protein fraction pattern. There is practically nothing known of the various protein fractions of the serum in normal pregnancy and we have been unable to find more than a few references to the literature. Longsworth, Shedlovsky and MacInnes (1939) for example, report their findings in one pregnancy serum. In 1945 Longsworth, Curtis and Pembroke report on the protein fraction values of four post-partum sera. In each instance these authors have utilised the electrophoretic technique, which seems a well chosen method of investigation.

The present thesis is a study of the serum protein fractions in pregnancy with particular reference to pre-eclamptic toxaemia. It is apparent from the foregoing that a number of problems present themselves and the thesis has been arranged accordingly.

It is obvious that, before any assessment of results can be made in toxaemia standards for the normal pattern of serum protein fractions in pregnancy must be established. As noted above reports in the literature are almost non-existent.

This was the first problem tackled and an attempt has been made to provide average values for the various trimesters of pregnancy and the effect of labour and lactation on these levels.

Apart from determining the changes in pattern, if any, in pre-eclampsia, it is obviously necessary to try and relate the serum protein values to the severity of the disease, and this has been done.

If the serum protein pattern is related to the occurrence of oedema in pre-eclampsia one would expect that some degree of similarity would be found between the values in that condition and other conditions in which this sign exists. For this reason it is necessary to study pregnant subjects suffering from oedema in the absence of pre-eclampsia. Similarly in the case of high blood pressure, it is essential to try and correlate the findings in pre-eclampsia with those in patients known to have established hypertension, pregnant and non-pregnant.

Renal and hepatic dysfunction may be etiologically related to pre-eclampsia and if so, the serum protein pattern in pre-eclampsia ought

to resemble those found in known cases of nephritis and liver disease.

All of these problems have been tackled and although more intensive research will be required before all of them are finally resolved, a certain amount of information has been acquired which will allow us to eliminate many of them. The results show certain trends which suggest that future work may be, with profit, concentrated on a very few of these problems.

Throughout this study the terms "pre-eclampsia", "pregnancy toxaemia", "Pre-eclamptic toxaemia" and "late toxaemia of pregnancy" are synonymous. Their varied usage has been dictated by a desire to avoid monotonous repetition in writing this thesis.

While the word "albuminuria" has been used in many instances throughout these pages it is understood that "proteinuria" more correctly denotes the presence of proteins in the urine. The words albuminuria and proteinuria have been used synonymously.

2 - METHODS AND MATERIALS

Sera from normal and complicated cases of pregnancy together with sera from non-pregnant patients suffering from certain pathological conditions have been studied by electrophoretic methods. Tiselius has done much to bring these methods within the bounds of practicability for the average laboratory. His original apparatus however is too costly and the technical difficulties too great for ordinary clinical analysis. It was decided to make use of the now popular paper strip method, which provides easily reproducible results at a minimum cost. Although the absolute accuracy cannot be compared with that of the original Tiselius method, it gives good comparable results provided the conditions are standard and care is taken with the pH of the buffer and with the current force passing across the field.

The method of fractionating proteins on filter paper has been developed in various countries and various authors have described the technique, notably Cremer and Tiselius, 1950. The separation of the various protein fractions is dependent on the fact that each molecule of the protein complex possesses acidic and basic groups, which cause the molecule to

act as a positive ion in an acid medium and as a negative ion in an alkaline field.

When the electrophoresis cell used by Tiselius in his method of protein fractionation is replaced by a strip of filter paper one is enabled to carry out adequate and inexpensive separation of the proteins of human plasma or serum. If a small amount of serum, or similar protein-containing fluid is applied to the middle of a strip of suitably prepared filter paper and a current is then passed from end to end through the paper, the protein molecules will react to the passage of the current by moving to the positive or negative end of the paper strip. The electric current is usually applied to the ends of the paper from electrodes immersed in a suitable buffer solution. The buffer solution is allowed to soak the filter paper, by capillary action, either before or after the application of the protein containing fluid which one wishes to analyse. When an electric current is passed through a paper strip soaked in alkaline buffer solution the protein molecules act as negative ions and migrate towards the anode electrode. The rate of migration of each protein fraction varies according to its physical characteristics and we find that the

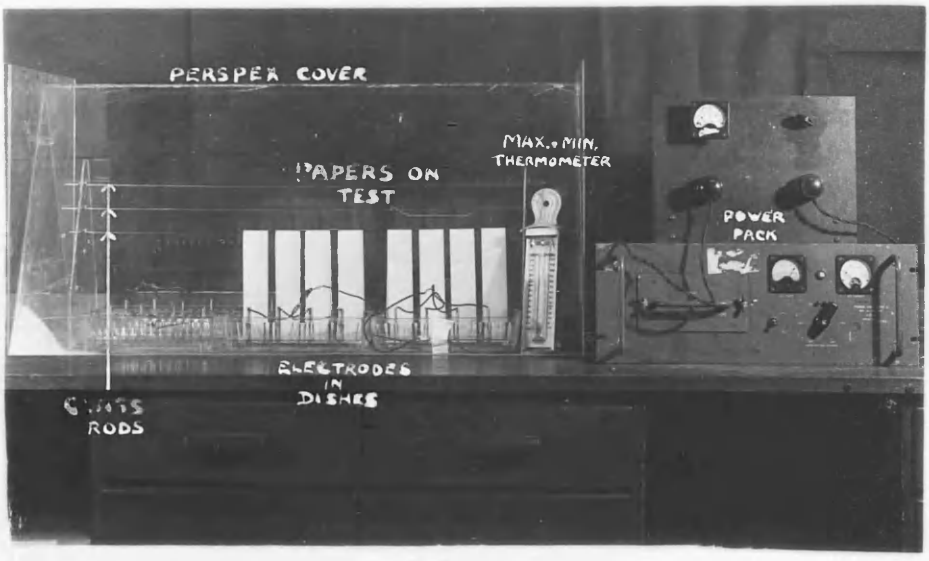


FIG. 1.

PHOTOGRAPH OF APPARATUS

albumin fraction moves furthest from the point of application of the protein to the paper whereas the gamma globulin stays close to that point. Plasma or serum may be applied to the paper in the form of a spot or as a line of fluid running transversely across the paper strip. The use of a spot of plasma or serum serves well enough as a qualitative method of analysis but quantitative studies are facilitated by using the "line application" technique. Very small quantities of protein-containing fluid may be analysed in this fashion and the method is therefore particularly suitable for the study of body fluids which are difficult to obtain in quantity. Use has been made of the latter technique in this study.

A considerable amount of experimental work required to be carried out before the electrophoretic unit, as now used, was fabricated in order to define and standardise the conditions under which good separation of the proteins might be obtained. A photograph of the completed unit appears opposite with the various parts named. The simplest method of description of the development of this electrophoretic apparatus is probably to describe it as follows, in its separate parts.

1. Paper

Experiments with various types of paper soon showed that Whatman's 3 MM was the most suitable. It is thin and smooth enough to allow accuracy in pipetting fluids, and at the same time strong enough to withstand the frequent immersions and washings during separation and staining of the protein moieties. Other papers such as Whatman's 2 MM paper, while suitable for the initial process of separation, proved too fragile to withstand the subsequent washing. Coarser types of paper did not permit the serum to be applied evenly.

Allowing 1.5 cms. at either end for immersion in the buffer solution varying lengths of paper were used in preliminary tests. It was found that a paper measuring 40 cms. in length allowed adequate separation of the various protein fractions and even after running for 18 to 20 hours with the apparatus later described, there was no danger of the proteins running completely off the paper into the buffer solution. The paper was, of course, of the standard 4 cm. width.

Source of Power

The normal alternating current of the mains supply

requires transformation to a direct form, and the voltage, which is somewhat variable, must be smoothed out. This can be done in various ways. Initially use was made of a simple metal rectifier delivering 1.5 amps. at 12 volts. It was found however that the heat generated quickly altered the buffer and interfered with the movement of proteins. Practically no separation of the fractions was obtained. Recourse was then had to a valve type rectifier as supplied to the Air Ministry, (tropical power unit No.3). This is capable of supplying 150 milliamps D.C. at voltages ranging from 200 to 250 volts with an input of 15 amps. at 250 volts A.C. This was modified by the addition of a variable potentiometer, 50,000 ohms maximum, and a milliameter reading from 0 to 40 milliamps. It was decided to fix the voltage at 250 and to vary the current strength. Four papers, of standard 4 cm. width and 40 cms. length were put in parallel and run for 18 hours in each instance. Runs were made at current strengths varying from 1 milliamp to 10 milliamps. This provided a range of strengths varying from 0.0625 mA. to 0.625 mA. per cm. width of paper.

At 1 mA total current there was no appreciable

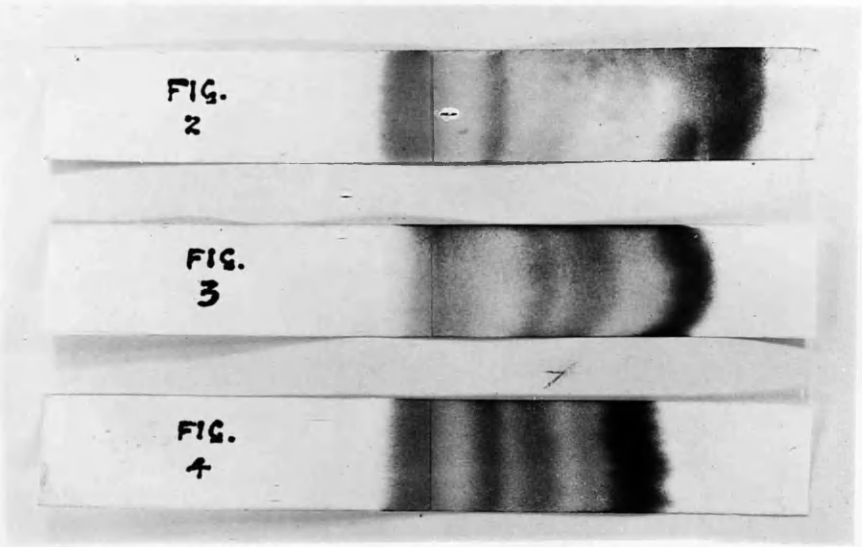


FIG.
2
FIG.
3
FIG.
4

separation of the proteins, while at the opposite end of the scale 10 mA caused the proteins to run too fast. Part of the protein in the latter instance was found to have run into the buffer fluid at the anode or all the protein bars assumed a curved outline (See Figs.2 & 3). With four papers it was decided that a total current of 4 mA was the optimum strength. This gave good separation without causing the proteins to run too fast. (Fig.4). In all further experiments the current was thus fixed to give 0.25 mA per cm. width of paper at 250 volts.

Period of Run

Numerous papers were run for varying lengths of time. Naturally separation of the proteins can be obtained fairly quickly if sufficient strength of current is applied to the paper but it was soon found that rapidity of movement did not provide the best result for analysis. As previously mentioned distortion of the protein bars occurred, making reading in the densitometer inaccurate. A steady, even separation is best obtained by applying a moderate current over a fairly long period of time, and from the experiments it was decided that an overnight run of 18 hours was the most convenient. This allowed

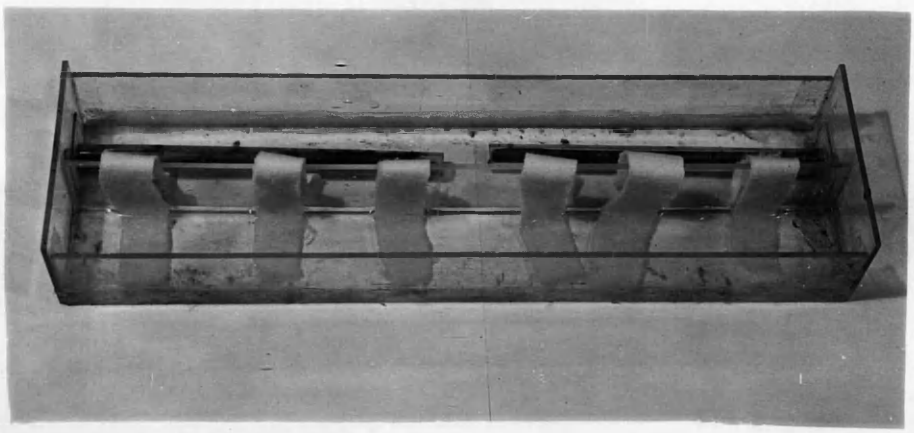


FIG. 5.

good separation to occur and sufficient time remained for drying, staining, washing, and analysis of the completed paper during daytime.

Buffer

The usual barbitone buffer at pH 8.6 was employed. This is made up by dissolving 10.3 g. of sodium di-ethyl barbitone and 1.84 g. of diethyl barbituric acid in one litre of distilled water. Although this mixture acts as a buffer we were surprised to find that, after running several papers under standard conditions, the separation of the proteins became less and less satisfactory. The run was shortened and it was impossible to distinguish the a_1 complex. A test of the buffer at this point showed that the pH had changed considerably on the cathode side. It was obvious that the resistance to the current had altered and that if comparable papers were to be reproduced the buffer would require to be made up fresh for each run. This was done and no further trouble was experienced.

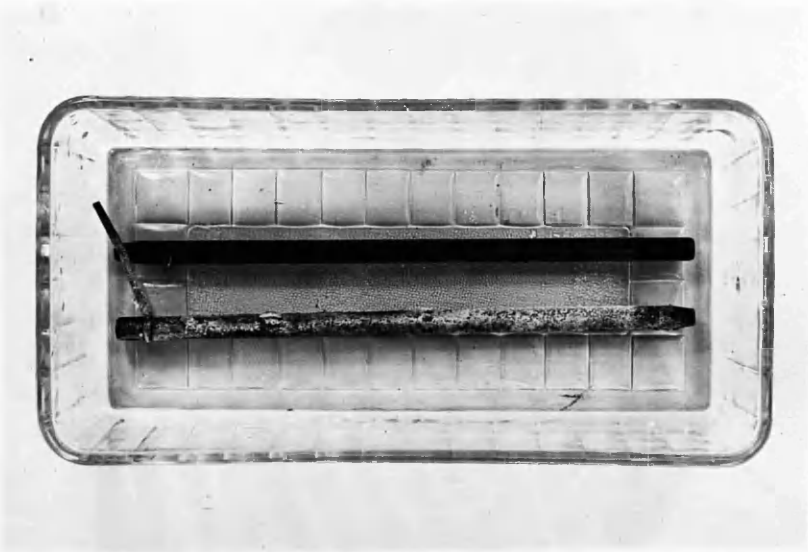


FIG. 6.

The Electrodes

Most workers have used carbon electrodes and our early experiments were conducted with a similar type. Soiling of the buffer solution, with subsequent colouring of the paper was troublesome. Protection of the papers and buffer solution was achieved, to a certain degree, by enclosing the carbon rods in a perforated Perspex tube and, later, by dividing the Perspex buffer dishes into two longitudinal sections (Fig.5) with wicks of white lint passing through holes in the division between electrode compartment and the compartment into which the papers dipped. After several papers had been analysed it was found that the carbon electrodes became incrusted with deposits of electrolyte (Fig.6) and that the separation of protein fractions was less and less complete in succeeding papers. This could be obviated to some extent by constant care in cleaning of the carbon rods but other faults arising in subsequent experiments eventually led us to discard this type of electrode. Up to this point the investigation of the electrode problem had been carried out employing a single paper strip. As soon as the number of papers was increased a serious fault was noted. Figure 7 shows the result

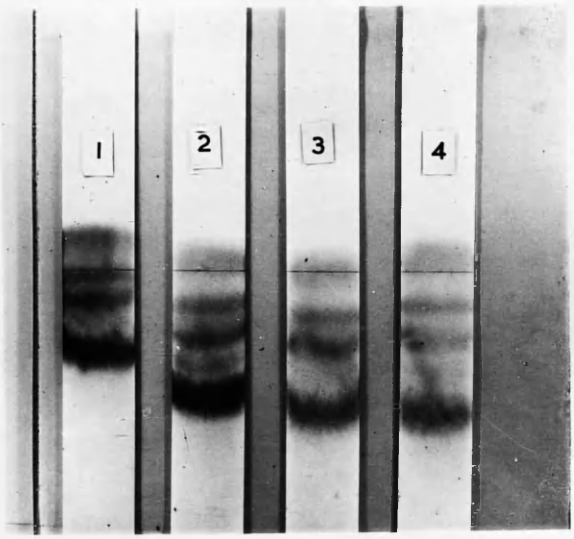


FIG. 7.

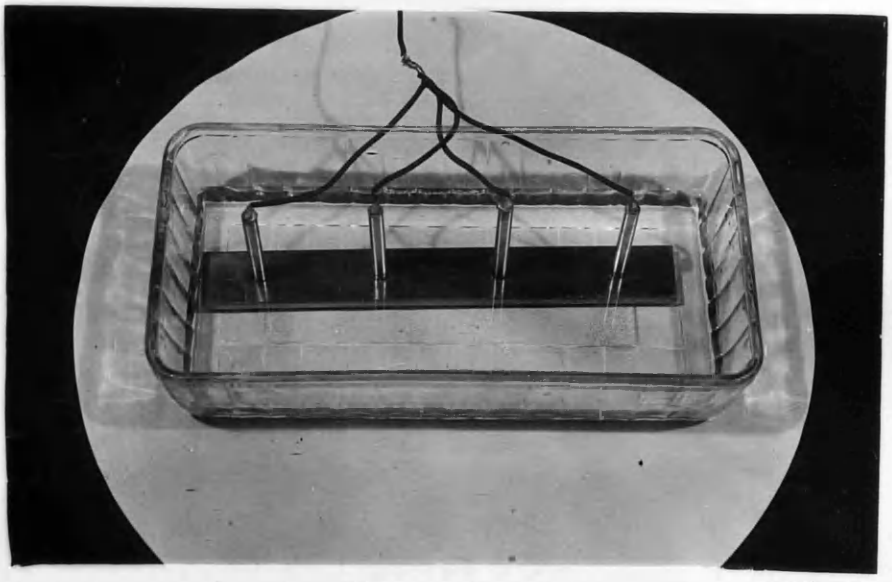


FIG. 8.

of running four papers simultaneously, equal quantities of the same serum being applied to all four. It is apparent from the figure that the current failed to run equally in every paper, the best separation being obtained in the paper nearest the point of input. The position was somewhat improved by attaching leads to either end of each electrode but it was still impossible to obtain identical results with several papers. Platinum wire was then tried but did not appear to be quite satisfactory. Ultimately stainless steel bar electrodes (Fig.8) with multiple tappings for leads were designed. These were of a size to fit the glass tanks. The tappings were made by drilling the rectangular bars at equi-distant points and fitting stainless steel pillars to which leads were soldered as shown in the illustration. These stainless steel electrodes have given uniformly good results with clear separation of the protein fractions and they have been used in this study.

Drying, Staining and Washing

When the papers were removed from the apparatus they were hung in a hot air oven at 100°C. until completely dry. This method of drying helps in the fixation of the protein bars on the paper strip.

Following the drying the papers were completely immersed for exactly five minutes in a porcelain dish containing a dye. The dyes most commonly used for this purpose are Bromphenol blue (Kunkel and Tiselius, 1951), Amidoblack 10B (Grassmann and Hannig, 1950, 1952) and Azocarmine B (Plückthun and Götting, 1951). The Bromphenol blue method was found to be satisfactory and a one per cent. solution of the dye in 95 per cent. ethanol saturated with mercuric chloride was used on all our paper strips. Following five minutes in the dye, the papers were transferred to a large flat dish through which a gentle continuous flow of tap water was maintained for forty minutes. The water-washing was completed when all dye had left the protein-free areas of the white paper strip and forty minutes, in our experience, proved to be an adequate duration for this operation. The papers, after washing were once more dried in the hot air oven and were then ready for quantitative analysis.

Quantitative Analysis

A densitometer modified from that described by Griffiths (1952) was used for measuring the optical density of the dye-containing protein strips of the various fractions on each paper. Each protein fraction

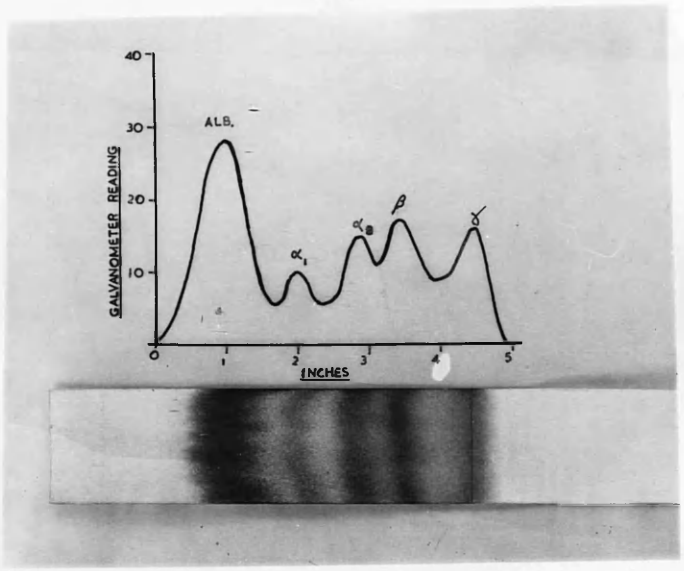


FIG. 9.

appears as a blue bar of varying intensity and width across the white of the paper strip. The paper to be analysed quantitatively was cleared by immersing it in liquid paraffin and it was then placed between two fine glass plates. The densitometer was set by adjusting the potentiometer control until a zero reading was obtained over the undyed area beyond the albumin bar. The glass enclosed paper was then moved along under the light source of the apparatus and a galvanometer reading was taken. Each tenth of an inch of dyed area on the paper was similarly assessed and a galvanometer reading obtained each time. From the resultant readings a graph was drawn by plotting the galvanometer readings obtained against each tenth of an inch of length of the dyed paper. The graph paper used was squared in tenth of an inch divisions so that (Fig.9) the resultant graph could be compared in scale with the paper from which it was obtained.

Each fraction of the protein complex is represented by a peak or curve in the graph. The triangulated area of each fraction peak was measured with a planimeter (Fig.10) and the amount of protein comprising each fraction was calculated from the total protein content of the serum, previously estimated by the "salting-out"

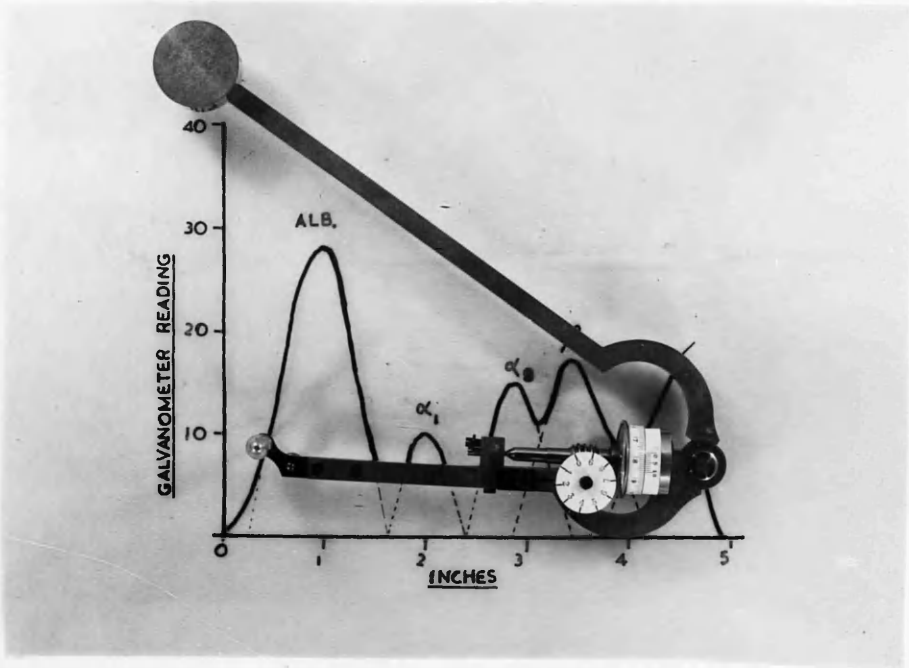

GALVANOMETER READING
40
30
20
10
0
ALB.
α_1
α_2
1
2
3
4
5
INCHES

FIG. 10.

Biuret method.

The "scanning" device or "densitometer" which was used in this investigation is prepared commercially by Evans Electro Selenium Ltd., Harlow, Essex.

Setting up the Electrophoretic Unit

After putting an equal amount of buffer solution (180 ml.) in each dish the paper strips measuring 40 cm. in length and 4 cm. in breadth were prepared. An exact amount of serum (.04 ml. in our case) was applied with a micropipette transversely in an evenly distributed line across a thin pencil line which marked the middle of the paper's length. The papers were then gently bent at the site of application of the protein and draped over the glass rods of the apparatus so that each end dipped to a distance of 1.5 cm. into the buffer solutions. The sera investigated in this study have been obtained from patients attending clinics at, or admitted to the wards of The Glasgow Royal Infirmary, The Glasgow Royal Maternity and Women's Hospital and Lennox Castle Maternity and Gynaecological Unit. The serum was separated as soon as possible after collection of the blood, and then spun in a centrifuge to get rid of all red cells. All samples of serum were subjected to electrophoretic separation

within three hours of collection. In the early trial phases of this investigation it was found that if sera were allowed to stand for any length of time a sediment forms and the total protein content of the supernatant fluid, measured chemically, diminishes. This is most marked with sera from pregnant women. Some observers (Longsworth et al., 1945) have preserved sera by freezing but we have found that the sedimentation at room temperature is equally marked in sera preserved by refrigeration.

3. SERUM PROTEIN FRACTIONS IN THE ANTE-PARTUM PERIOD.

A considerable amount of investigation has been carried out on the protein concentration of the blood in pregnancy, in view of its importance in relation to nutritional disturbances in pregnancy, both maternal and foetal. Most of this work relates to the total protein concentration of the blood and reliable data are available for the variations which occur in normal and in complicated pregnancy. It is obviously an important study in view of the marked protein anabolism which occurs throughout pregnancy. This is partly related to foetal growth and partly to the increase in uterine, placental and breast tissue. In addition there appears to be a storage of protein in the organs in preparation for lactation.

While a certain amount of knowledge has been gained by these studies of total protein levels, they shed little light on the more intimate changes associated with maternal complications of pregnancy such as anaemia, hyperemesis gravidarum, and toxaemia of pregnancy, or on foetal pathology. More recently attention has been turned to the changes in absolute and relative values of the various protein fractions.

Chemical methods, which have hitherto been used in the study of blood protein values, may give rise to fallacies, and more recently electrophoretic analysis of serum proteins has been widely employed in the study of disease states in the non-pregnant subject. Although there are occasional reports of electrophoretic studies of the serum proteins in pregnant patients in the literature no detailed analysis has so far been undertaken. The position is clearly demonstrated by the fact that the main reference to this subject quoted by Dieckmann (1952) is that of Longsworth et al (1939, 1945). In one of these papers (1939) Longsworth reports, among specimens from patients suffering from various conditions, the results of analysis of a serum from one pregnant individual. In the other communication Longsworth deals with four sera from patients in the post-partum state. It is known that considerable change occurs in these protein fractions in certain diseases particularly in those associated with the liver, kidney and reticulo-endothelial system and in view of theories currently held regarding the importance of these organs in diseases peculiar to pregnancy it is surprising to find so little of a factual nature in the literature.

Blood-protein values for the non-pregnant individual are not acceptable as normal standards for pregnancy. There is a progressive haemo-dilution in pregnancy and the nitrogen anabolism which occurs may well influence the protein content of the blood. In addition the rate of foetal growth varies throughout the period of gestation and the demands made upon the maternal metabolism must similarly vary. According to Dieckmann (1952) the values for protein fractions obtained by electrophoretic analysis do not differ from those found by chemical methods but this view is subject to some modification in view of his references which are quoted above. By chemical analysis Dieckmann finds that the average serum protein concentration in normal pregnant patients is 6.5 gms. per cent. and this is 1 gm.per cent. less than the average serum protein level of 7.5 gms.per cent.for non-pregnant individuals. A decrease in total serum protein and serum albumin occurs in normal pregnancy. The albumin-globulin ratio falls from the normal non-pregnant value to a lower value in pregnancy. Dieckmann gives an average value for non-pregnant subjects of 1.6 and an average value in pregnancy of 1.3. He does not attempt to give values for the various globulin fractions. Since pregnancy lasts

TABLES FROM WHICH AVERAGE TRIMESTER PROTEIN VALUES WERE OBTAINED.

Amounts are in g. per 100 ml.

Sera	Alb.	α1	α2	β	γ	Total
		1st Trimester				
1	4.37	.82	.62	.8	1.08	7.69
2	4.1	.9	.6	1.	.7	7.3
3	3.7	.2	.73	1.1	1.05	6.78
4	4.4	.4	.5	.9	.9	7.1
5	4.7	.47	.47	1.66	.65	7.95
6	4.75	.25	.7	.9	1.1	7.7
7	3.67	.63	1.	1.2	.9	7.4
8	5.25	.9	.25	.5	1.	7.9
9	3.9	.4	.5	.6	.5	5.9
10	4.44	.54	.85	.98	.64	7.45
Totals	43.28	5.51	6.22	9.64	8.52	73.17
Averages	4.328	.551	.622	.964	.852	7.317
Alb./Glob. = 1.4		Glob. = 2.989				
		18.4%	20.8%	32.3%	28.5%	

Sera	Alb.	α1	α2	β	γ	Total
		2nd Trimester				
1	3.63	.7	.86	.86	1.38	7.43
2	2.7	.4	.9	1.2	.9	6.1
3	3.38	.58	.82	1.06	.86	6.7
4	3.89	.69	1.	1.06	.51	7.15
5	4.7	.5	.7	1.1	.5	7.5
6	3.84	.61	.56	.72	.67	6.4
7	3.51	.48	.59	.71	.71	6.
8	4.09	.36	.73	1.14	.68	7.
9	4.08	.51	.51	1.02	.48	6.6
10	3.8	.4	.9	1.4	.9	7.4
Totals	37.62	5.23	7.57	10.27	7.59	68.28
Averages	3.762	.523	.757	1.027	.759	6.828
Alb./Glob. = 1.2		Glob. = 3.066				
		17.%	24.7%	33.5%	24.8%	

Sera	Alb.	α1	α2	β	γ	Total
		3rd Trimester				
1	2.3	.8	1.5	1.2	.7	6.5
2	3.6	.6	1.2	1.5	.8	7.7
3	3.5	.8	1.45	1.45	.4	7.6
4	2.3	.7	1.	1.8	.7	6.5
5	2.6	.7	1.3	1.6	.8	7.
6	3.31	.75	.78	.81	.35	6.
7	2.75	.24	.96	.98	.77	5.7
8	2.5	.32	.68	1.4	.8	5.7
9	3.3	.66	1.11	.87	.46	6.4
10	2.66	.35	.35	1.54	.8	5.7
Totals	28.82	5.92	10.33	13.15	6.58	64.8
Averages	2.882	.592	1.033	1.315	.658	6.48
Alb./Glob. = .8		Glob. = 3.598				
		16.5%	28.7%	36.5%	18.3%	

for a fairly long period and since many of its important complications arise at different stages of pregnancy, normal values for the three trimesters are essential before any reasonable interpretation can be made of the levels found in the abnormalities of pregnancy.

The present investigation is an attempt to establish reliable standard values for serum proteins during the various trimesters of normal pregnancy using the electrophoresis technique.

Material and Methods

Serum was collected from 30 patients, ten being in each trimester of pregnancy. All patients were healthy normal women with no sign of oedema and free from albuminuria. The patients' blood pressures were within normal limits; the highest level of normality being taken as 120 mm. systolic and 70 mm. diastolic. The sera were subjected to electrophoresis within two hours of collection.

In addition to the thirty sera already mentioned a further five were obtained from healthy non-pregnant females in the reproductive age-group. All were subjected to analysis by the above technique.

Results

Table 1.

Plasma and Serum Protein Values for Normal Adults.

Author	Total protein gm./ 100 ml.	Albumin	Total Globulin	α1	α2	β	γ	Alb./ Glob. Ratio	Method
Levin et al (?single serum) (?sex)	7.02	3.18	3.84	1 + 2 =	1.65	1.17	1.02	.83	Electrophoresis
Dole & Braun Findings from 15 adult male plasmas	6.38	4.04	2.34	.31	.48	.81	.74	1.53	Electrophoresis
Dieckmann	7.1	4.4	2.7	No figures given for fractions				1.6	?Chemical
Present investigation 5 adult non-pregnant females	7.1	4.186	2.89	.358	.689	.995	.848	1.5	Electrophoresis

The average values for the normal non-pregnant patients are shown in Table 1 together with results from normal adults given by several other authors. In the case of Dole and Braun (1944) the figures relate to adult males, fifteen in number. Levin et al (1950) do not state the sex and their values relate to a single case only. Dieckmann (1952) gives values for the non-pregnant female, but in this case the estimation of the proteins appears to have been carried out chemically. This is the only reference which we could find in the literature which definitely refers to the non-pregnant female.

From the table it will be seen that our results for total protein agree fairly closely with those of Dieckmann and Levin et al. Dole and Braun's figure of 6.38 is very low, particularly for males. According to Bradshaw (1950) who analysed serum protein values in 2500 adults the total protein values for the majority of males lies within the range of 7.0 to 7.9 g.per cent., and in females it is slightly higher. Considerable variation exists between the values given by various observers for the individual protein fractions. The value for albumin in our series is reasonably close to that given by Dieckmann and also that reported by

Dole and Braun. Level et al give a very low figure for albumin. Similarly the total amount of globulin in our series is comparable with that reported by Dieckmann. The lower figure quoted by Dole and Braun is a reflection of the abnormally low value which they give for total protein. Levin et al give an abnormally high reading for total globulin due obviously to their low estimate of albumin. These variations are to some extent indicated by the albumin, globulin ratios. Our ratio of 1.5 is similar to that reported by Dieckmann and also that of Dole and Braun. The ratio in the case quoted by Levin et al. is abnormally low. Sufficient work has been done to allow us to accept the values for albumin and globulin obtained by chemical methods as accurate. Since our values for these components agree closely with those given by Dieckmann, it seems reasonable to accept the figures we have quoted for the various globulin fractions. It will be noted that they are similar to those quoted by Dole and Braun, being only slightly higher in each instance. As already pointed out, the value given for total protein by these authors is abnormally low and this would be reflected in the absolute figures calculated from the relative values

Table 2.

Serum Protein Values in Pregnancy.

Author	Total protein gm./ 100 ml.	Albumin	Total Globulin	α1 + α2	β	γ	Alb./ Glob. Ratio	Method
Longsworth et al(1939) ?single serum	6.65	4.29	2.36	= .6	1.03	.73	1.81	Electrophoresis
Dieckmann	6.6	3.7	2.9	No figures given for fractions			1.3	?Chemical
Present investigation 30 pregnancy sera	6.9	3.7	3.21	.555	.804	1.102	.75	1.15	Electrophoresis
Longsworth et al(1945) average of 4 sera (about 1 hour postpartum)	7.83	3.84	3.99	.46	.76	1.82	.95	.96	Electrophoresis

given by electrophoretic analysis.

The average results found in this investigation of serum protein in 30 pregnant women, 10 in each trimester, are shown in Table 2. Findings reported by Dieckmann, Longsworth and co-workers (1939, 1945) are recorded.

From Table 2 it will be seen that there is some difference between the average figures for the various fractions in our series and those quoted by the other authors. Part of this may possibly be due to the fact that we have included sera from patients at several different stages of pregnancy. It must be pointed out, however, before any comparison can be made, that Longsworth et al (1939) refer to one case only and Longsworth, Curtis and Pembroke (1945) deal with 4 cases after delivery. Once more, however, there is fair general agreement between our values for total protein, albumin and total globulin and those given by Dieckmann. This provides considerable assurance that our values are reasonably accurate. Dieckmann does not relate the duration of pregnancy to the values he gives and this is obviously necessary before any assessment of results, and the trends they show, can be made. In Table 3 we have separated the values for

Table 3.

	Total serum Protein	Albumin	Total glob-ulin	α1	α2	β	γ	Albumin/ Globulin Ratio
Non-pregnant	7.1	4.186	2.89	.358	.689	.995	.848	1.5
1st trimester Average of 10 sera.	7.317	4.328	2.989	.551	.622	.964	.852	1.4
2nd trimester Average of 10 sera.	6.828	3.762	3.066	.523	.757	1.027	.759	1.2
3rd trimester Average of 10 sera.	6.48	2.882	3.598	.592	1.033	1.315	.658	.8

individual patients into three groups, in accordance with the particular trimester during which the examination was made.

In the first trimester there is a slight rise in the total serum proteins from the non-pregnant value of 7.1 gm. per 100 cc. The rise affects both albumin and globulin but thereafter there is a progressive fall in the total serum proteins from 7.317 gm. through a level of 6.828 gm. to 6.48 gm. per 100 cc. in the third trimester. The fall in amount of total serum protein represents a drop of 8.8% from the non-pregnant level to the level in the third trimester and a fall of 11.5% from first to third trimester values.

The fall in serum albumin values from early to late pregnancy is approximately 32% and the increase in serum globulin over the same period amounts to 20%.

The fractions of the serum globulin, other than the gamma fraction, increase by varying amounts from early to late pregnancy. α_1 increases by 7%, α_2 increases by 66%, and β by 37½%. The gamma fraction decreases by approximately 22%.

The albumin/globulin ratio of 1.5 in the non-pregnant state falls by stages of 1.4 and 1.2 to a value of 0.8 in late pregnancy.

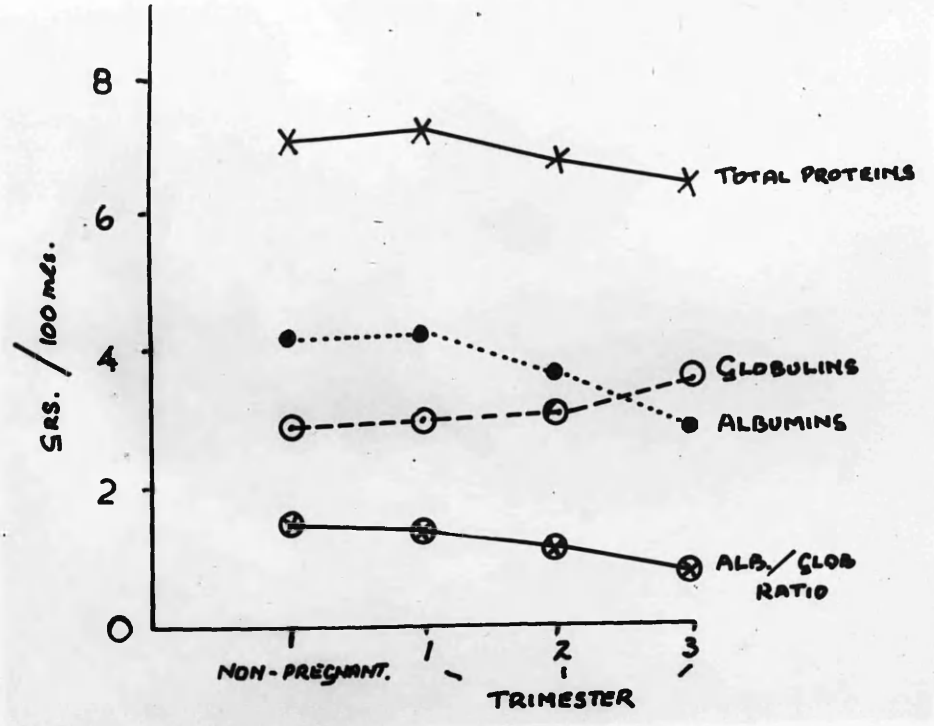

8
6
4
2
0
GRS. / 100 MLS.
TOTAL PROTEINS
GLOBULINS
ALBUMINS
ALB./GLOB RATIO
NON-PREGNANT.
1
2
3
TRIMESTER

FIG. 1.

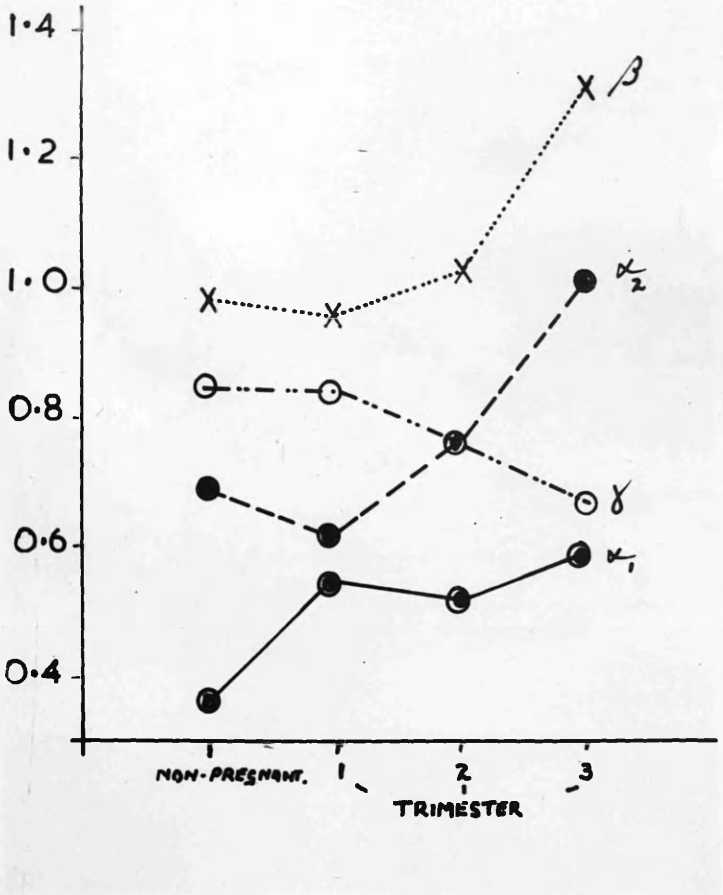


FIG. 2.

The trends of the various fractions can most clearly be demonstrated by plotting the values in graphic form.

Figure 1 shows the values of total protein, albumin, globulin and the albumin globulin ratio in the non-pregnant state and in each trimester of pregnancy. After a slight rise from the non-pregnant state to the first trimester of pregnancy both the total protein and the albumin values show a steady fall. The globulin gradient rises fairly regularly as pregnancy is established and advances towards term. The gradient of the albumin/globulin ratio follows inversely the trend of the serum globulin.

The graph presented in Figure 11 shows the values of the globulin fractions before and throughout pregnancy. The $\alpha 1$ fraction shows a slight increase from the non-pregnant value to that in the first trimester, falls slightly in the second trimester and then regains its first trimester value late in pregnancy. The $\alpha 2$ and β fractions form almost parallel curves. After a slight fall in the first trimester they rise progressively, showing their steepest gradient in the third trimester. The γ fraction is unaltered in amount until after the first trimester

when it decreases steadily to its late pregnancy value. It is interesting to note that the α and β fractions follow a curve similar to that shown by the total globulins. The γ fraction on the other hand behaves in the same way as albumin.

Discussion

We have been unable to find in the literature any values for the various protein fractions relating definitely to the non-pregnant female. Values have been quoted for pregnancy by several authors but no details are available regarding the stage of pregnancy. So far as we are aware the present communication represents the first attempt to provide values for these fractions by the electrophoretic method. Values are available for the total albumin and globulin determined by chemical methods. Bradshaw (1950) in an analysis of sera from 300 pregnant women found that in 90 per cent. of these patients the value for total proteins lay within the range of 6.0 to 7.4 g. Dieckmann quotes a figure of 6.6 g. for total protein, 3.7 g. for albumin, and 2.9 g. for globulin. It can be seen from the tables that our figures for total protein, albumin and globulin agree fairly closely with those given by the authors quoted above and it seems

reasonable to assume that our methods have been accurate.

The most significant feature is the progressive fall in total protein throughout pregnancy. At first sight this might be thought to be due to the haemodilution which is also a progressive phenomenon during pregnancy, but reference to the values for the various fractions shows that no simple explanation can be offered for this change. If haemodilution were the whole explanation one would expect a fairly equal fall in the values for all the protein fractions. The only fractions, however, which behave in a similar fashion to the values for total proteins, are the albumin and γ globulins. It is apparent, therefore, that the main cause of the reduction in the proteins of the blood in pregnancy is the change in these fractions and obviously albumin is the more important. During the second trimester the α_1, component also takes part in the fall in protein values. It is also to be noted that at this period the rising curve for α_2 and β is rather flat compared with the third trimester and it may be that during this time haemodilution is an important factor. This is also suggested by the fact that the albumin/globulin ratio alters only slightly

in the progress from first to second trimesters. During the third trimester, however, it is apparent that marked changes occur in both albumin and globulin fractions. The albumin shows a more marked fall and there is a steep rise in the α_2 and β globulins. There is also a pronounced fall in the γ globulin but the α_1 particle shows little change. These alterations are reflected in the albumin/globulin ratio which drops to a very low level.

It is important to establish the values for these protein fractions in the trimesters. Without them it would be impossible to undertake a study of the changes in abnormal pregnancy and even more impossible to compare the findings in disease states in pregnancy with those in diseases of the non-pregnant individual. For example, according to Lewis and Page (1947) malignant hypertension is characterised by a fall in albumin and a pronounced rise in β globulin. This is also characteristic of the third trimester of normal pregnancy and, since hypertensive conditions are common at this period of pregnancy, it is necessary for comparative purposes to know the normal pregnancy levels of these fractions.

No explanation is available at the present moment

for the changes in these fractions. Tiselius (1937) has shown that immune bodies migrate with the γ globulins and it is possible that the fall in this fraction during the third trimester is associated in some way with the transfer of immunity to the foetus.

Commentary

A study has been made of the electrophoretic pattern of the serum proteins in pregnancy. For this purpose sera from 35 women have been analysed. Five were not pregnant and the remaining 30 consisted of 3 groups, 10 in each trimester of pregnancy.

It has been shown that during pregnancy there is a progressive decline in the serum protein values. This is due almost entirely to diminution of the albumin fraction. There is also, however, a marked fall in γ globulin. β globulin and the second fraction of the α complex show a continuous rise; α_1 scarcely alters. The results suggest that up to the second trimester the changes are modified to some extent by haemodilution but thereafter they become absolute. The variations in the main components of the serum proteins are reflected in the albumin/globulin ratios which fall steadily from a non-pregnant level of 1.5 to 0.8 in the last trimester.

4. EFFECT OF PARTURITION IN NORMAL PREGNANCY ON SERUM PROTEIN FRACTIONS.

Introduction

Following delivery there is usually a dramatic clinical improvement in the condition of toxaemic patients. The urinary output increases, oedema diminishes, blood pressure gradually falls and albuminuria clears up. This clinical improvement may be associated with changes in serum protein values and, if so, it may be possible to correlate these changes with the improvement in signs and symptoms. Interpretation however is difficult, if not impossible, unless standard values are obtained for this period. Many cases show immediate improvement within 24 hours of confinement, but in others the improvement is more gradual and takes place over a period of some days. It was therefore considered necessary to study the serum proteins on several occasions during this period. As a first step towards this it was decided to analyse the serum proteins before and after labour in a chosen group of patients. Patients were chosen carefully from the point of view of normality. All had remained normal throughout their ante-natal periods. There had been no albuminuria, rise of blood pressure

Table 1.

Values of protein fractions obtained by electrophoretic analysis of sera from 8 normal pregnancy cases before labour.

Values of fractions are recorded in g./100 ml.

Serum	Albumin	α1	α2	β	γ	Total Protein
1	2.96	.339	1.575	1.502	.884	7.26
2	1.83	.85	1.54	.85	.99	6.06
3	2.85	.23	1.09	1.32	.53	6.02
4	1.88	.27	.94	1.86	1.2	6.15
5	2.5	.35	.8	1.09	1.13	5.87
6	3.576	.423	.761	1.128	.804	6.692
7	2.529	.529	1.66	1.504	.765	6.987
8	2.76	.345	.843	1.233	1.119	6.3
Totals	20.885	3.336	9.209	10.487	7.422	51.339
Averages	2.61	.417	1.151	1.311	.927	6.417

Average Total Globulin = 3.806 g./100 ml.

Albumin/Globulin ratio = 0.69.

Table 2.

Values of protein fractions obtained by electrophoretic analysis of sera from 8 normal pregnancy cases after labour.

Values of fractions are recorded in g./100 ml.

Serum	Albumin	α1	α2	β	γ	Total Protein
1	2.67	.52	.91	1.5	1.08	6.68
2	2.15	.4	1.42	1.55	1.28	6.8
3	2.42	.32	.9	1.29	1.16	6.09
4	2.25	.345	.88	1.67	1.315	6.46
5	2.9	.3	.8	1.34	1.91	7.25
6	3.23	.41	.87	1.31	1.02	6.84
7	2.45	.44	1.48	1.59	1.03	6.99
8	2.17	.4	.99	1.56	.93	6.05
Totals	20.24	3.135	8.25	11.81	9.725	53.16
Averages	2.53	.392	1.031	1.476	1.216	6.645

Average Total Globulin = 4.115 g./100 ml.

Albumin/Globulin Ratio = .615.

or evidence of oedema in this series. The haemoglobin levels had remained within normal limits and the albours, occurring at the expected date of delivery (or within seven days of that date), had terminated in spontaneous vertex deliveries within twenty four hours.

Serum was obtained from a large number of normal patients at term and, from these, eight have been chosen in whom labour commenced within twelve hours of obtaining the specimen. A further specimen from each of the chosen normal cases was obtained twenty-four hours after confinement. The sera, taken before and after confinement, were subjected to the same technique of analysis detailed in the section on methods and materials.

Results

The results obtained from the sera taken before labour appear in Table 1. The results from sera of the same patients after labour appear in Table 2. In Table 3 for comparison are reproduced the average values for serum protein fractions of sera taken from 10 normal pregnancies in the third trimester of pregnancy.

On comparing the tables of results it will be

Table 3.

	Total serum Protein	Albumin	Total glob-ulin	$\alpha 1$	$\alpha 2$	β	γ	Albumin/ Globulin Ratio
Non-pregnant	7.1	4.186	2.89	.358	.689	.995	.848	1.5
1st trimester Average of 10 sera.	7.317	4.328	2.989	.551	.622	.964	.852	1.4
2nd trimester Average of 10 sera.	6.828	3.762	3.066	.523	.757	1.027	.759	1.2
3rd trimester Average of 10 sera.	6.48	2.882	3.598	.592	1.033	1.315	.658	.8

noted that there is little change in the average total protein values during the third trimester (6.48 g./100 ml.) and just before labour (6.417 g./100 ml.). There is however a definite fall in the albumin/globulin ratio from 0.8 to 0.69. This is due, on further inspection of the tables, partly to a fall in albumin but there is also a rise in total globulin and mainly in the γ fraction which increases by 0.269 g./100 ml. A slight increase (0.118 g.) also takes place in the α_2 fraction, the α_1 moiety decreases slightly (0.175 g.) while the β complex is practically static. Twenty four hours following labour (Table 2) the total protein concentration of the serum rises slightly, from a level of 6.4 g. before labour to 6.6 g./100 ml. but the albumin/globulin ratio shows a further fall, from 0.69 to a value of 0.61. Albumin is decreased from 2.61 to 2.53 g./100 ml. while the globulin is increased by .309 g./100 ml. The main change noted in the globulin complex occurs, once more, in the γ fraction which increases from 0.927 to 1.216 g./100 ml. A slight rise in the β fraction occurs but the α_2, increased just before labour, returns to its third trimester value. The α_1 factor continues to show a slight fall amounting to .025 g.

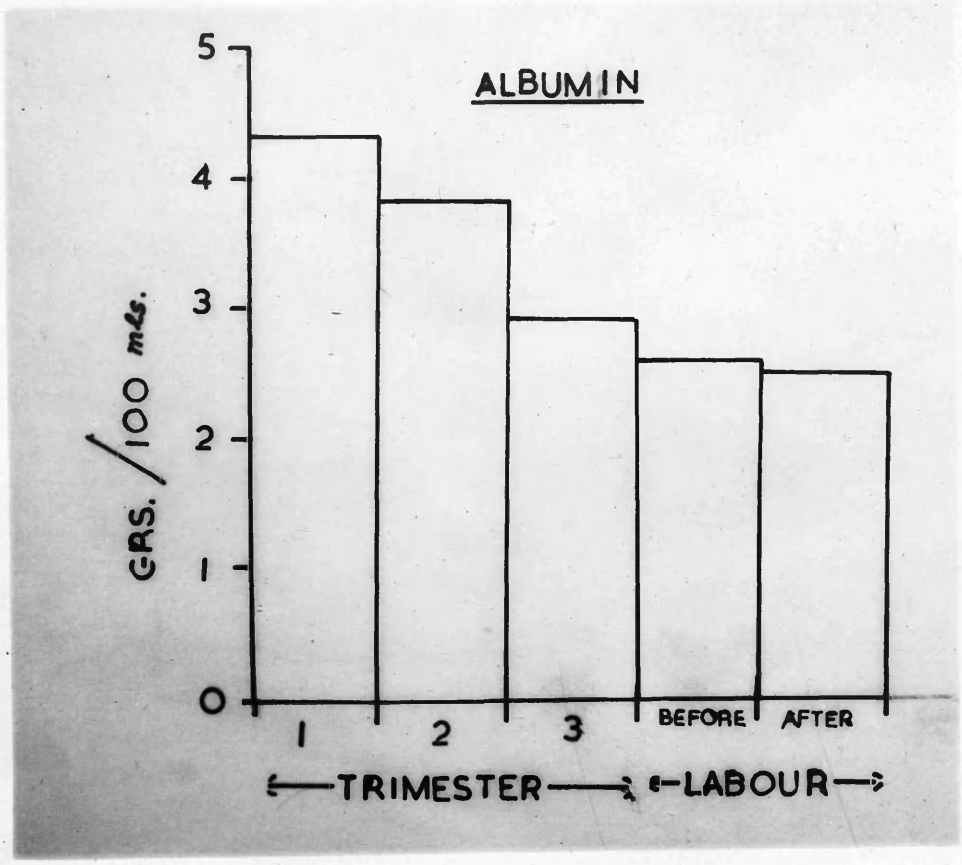
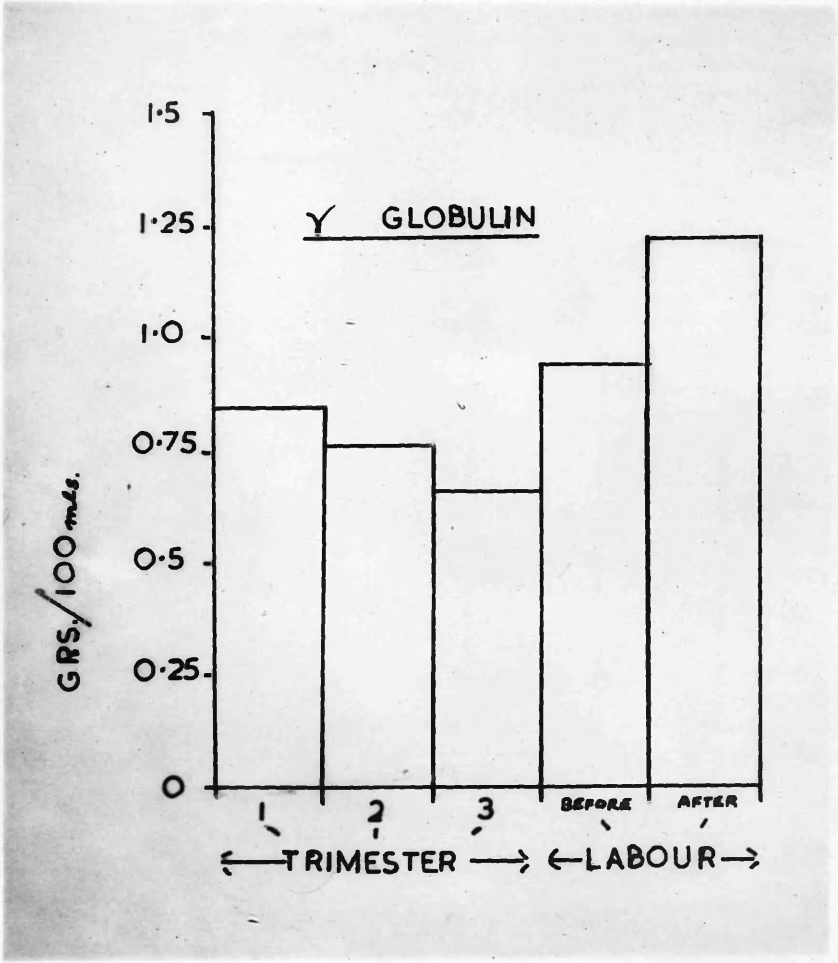

ALBUMIN
GRS. / 100 mls.
5
4
3
2
1
0
1
2
3
BEFORE
AFTER
TRIMESTER
LABOUR

FIG. 4.


1·5
1·25
1·0
0·75
0·5
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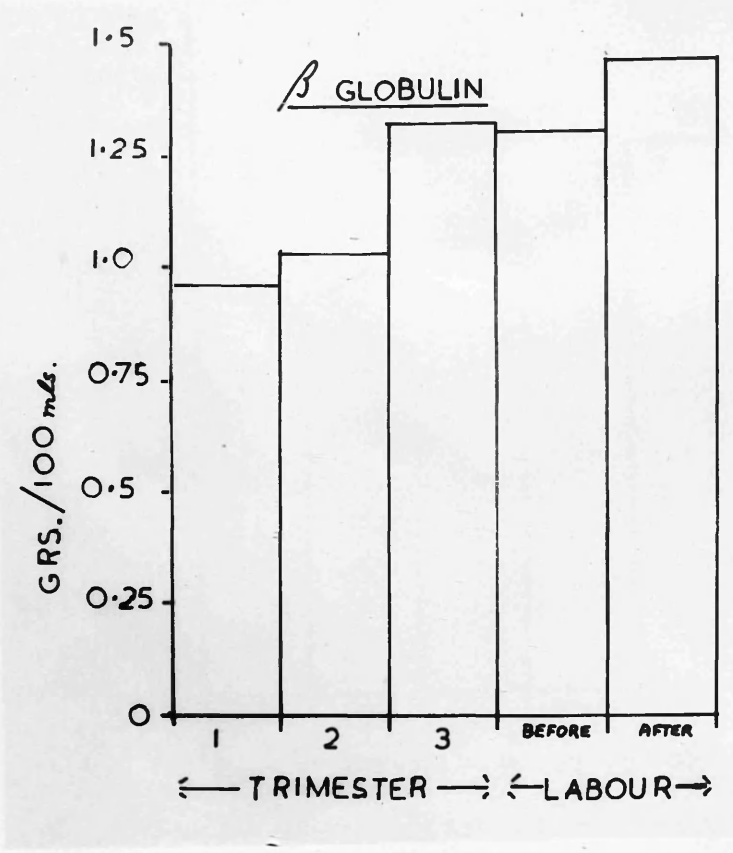
0
γ GLOBULIN
GRS./100 mls.
1
2
3
BEFORE
AFTER
TRIMESTER
LABOUR

FIG. 5.


β GLOBULIN
1·5
1·25
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0·25
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1
2
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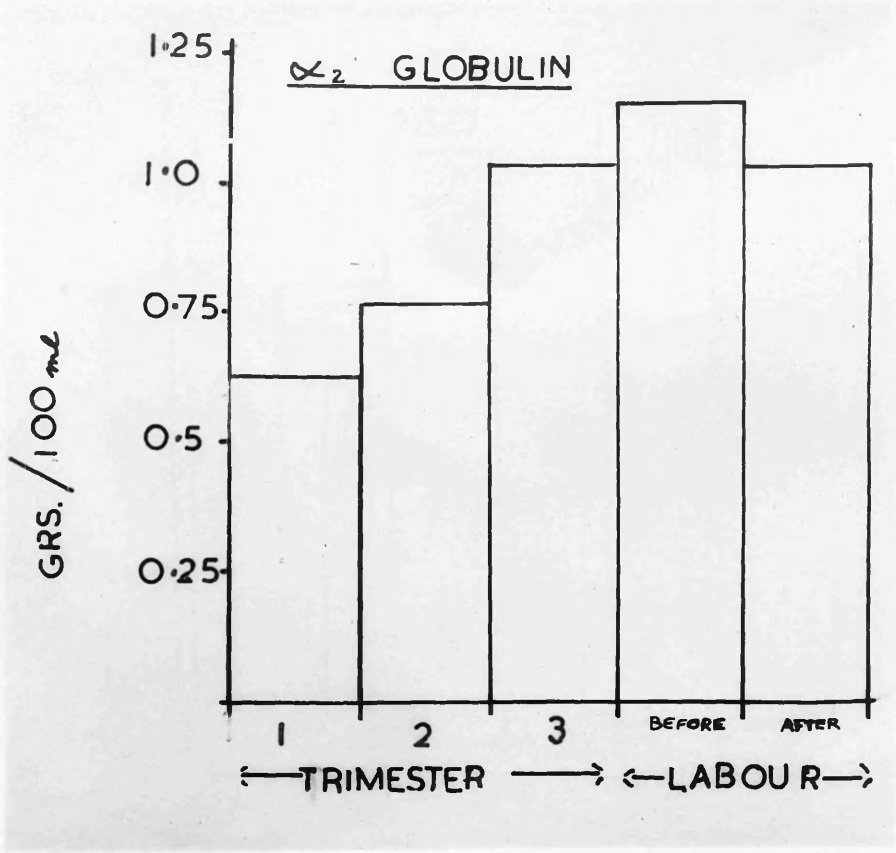
⟵ TRIMESTER ⟶
⟵LABOUR⟶

FIG. 6.


1·25
1·0
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GRS./100 ml
α2 GLOBULIN
1
2
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BEFORE
AFTER
←TRIMESTER→
←LABOUR→

FIG. 7.

however, changes in the values of certain fractions and in the relationship of the main fractions. The albumin, which shows a fall during the antenatal trimesters, continues to diminish whereas the globulin shows a further increase thus resulting in a further fall in the albumin/globulin ratio. The increase in globulin however, previously due to increases in α_2 and β fractions, is now mainly caused by a rise in the γ factor. Following confinement, the total protein is increased but the reversal of the main component values is even more marked and the albumin/globulin ratio is further diminished. The fall in albumin and rise in globulin continue. The increase in the globulin is once more mainly due to the rise in the γ factor.

While these changes may be of primary nature there is the possibility that they are secondary to events occurring at this time. During any obstetric delivery there is always a certain amount of unavoidable blood loss. In addition there are said to be changes in blood volume, probably in part of a compensatory nature due to the haemorrhage. Around this time, too, there is an active secretion of colostrum which has a relatively high protein content (2.0 - 2.6 g.per cent.

according to Holt, Courtney and Fales (1915). If these changes were due to blood loss or readjustment of blood volume alone, one would expect any change in serum protein concentration to be reflected equally in the various protein fractions. It has been shown that such is not the case. There is a quite distinct change in the pattern of these fractions which can only be explained on the basis of a more vital mechanism.

The possibility that these changes may be related to the mammary secretion must be considered and this has been done in the following section.

5. SERUM PROTEIN VALUES IN ESTABLISHED LACTATION.

As stated in the previous section, it is necessary to study the effect of mammary secretion on the serum protein fraction values before an interpretation of the changes occurring at this time can be made. According to Holt, Courtney and Fales (1915) human milk contains a considerable amount of protein. During the period with which we are dealing two types of breast secretion occurs. Colostrum is secreted in the first few days of the puerperium and thereafter true milk secretion is established. Holt et al found that colostrum contains 2.0 - 2.6 g. per cent., and that true milk contains 1.1 - 2.0 g. per cent., of protein. This loss of protein may well influence the level of serum proteins of the patient and it must be taken into consideration when comparing normal and abnormal patients. In addition, it has already been noted that many cases of toxaemia show a gradual improvement in symptoms and signs during the puerperium and it is necessary to obtain figures for the normal mid-puerperium for comparison. It was felt that both these studies could be combined by analysing sera from normal patients in whom lactation was

Table 1.

Lactation Series

Results of protein analysis of 10 sera from normal patients with established lactation.

	Albumin	α 1	α 2	β	γ	Total protein
1	2.78	.36	1.24	1.84	0.67	6.89
2	2.81	.41	1.22	1.22	.74	6.4
3	2.38	.42	1.08	1.39	.93	6.2
4	2.43	.45	1.49	1.39	1.22	6.98
5	3.07	.3	.7	1.49	1.14	6.7
6	2.45	.42	1.31	1.07	1.34	6.58
7	3.12	.44	.57	1.25	1.06	6.44
8	3.36	.4	1.11	1.0	.83	6.7
9	2.7	.52	1.18	1.12	.54	6.06
10	2.81	.4	.76	1.68	1.6	7.25
	27.91	4.12	10.66	13.45	10.07	66.2
	2.79	.41	1.066	1.345	1.007	6.62

Total Globulin (from Average) = 3.828

Albumin/Globulin ratio = 0.73

established. For this purpose it was decided to study sera from normal patients on the fifth day of the puerperium. Only patients who had been considered normal, from a clinical viewpoint, before and throughout delivery and thereafter were included in this series. In each case lactation was properly established.

Specimens of serum were obtained from ten patients who fulfilled the requirements already stated. The sera were subjected to electrophoretic analysis by the technique described in Section 2 of this study.

The results of analysis with values for each fraction of the protein complex, and the albumin globulin ratio, appear in Table 1. For the purpose of comparison Table 2 shows the values for the serum proteins and the albumin/globulin ratio before and after labour and includes the results of Table 1.

Reference to the results shows that the average total serum protein value for the lactation sera of 6.62 g. does not differ greatly from the value of 6.65 g. per cent. found twenty four hours after labour. The albumin value of 2.79 g. has risen slightly from a level of 2.53 g. per cent. in patients after labour. The total globulin value of 3.828 g. is

Table 2.

	Before Labour	After Labour	5th day of Puerperium: Lactation established
Total Serum Proteins	6.42	6.65	6.62 g./100 ml.
Albumin	2.61	2.53	2.79 "
Total globulin	3.81	4.12	3.83 "
Globulin fractions			
α 1	.42	.39	.41 "
α 2	1.15	1.03	1.07 "
β	1.31	1.48	1.35 "
γ	.93	1.22	1.01 "
Albumin/ Globulin Ratio	0.69	0.61	0.73 "

lower than that of 4.12 g. per cent. after labour.

The gain in albumin from the early to mid-puerperium values (0.26 g.) and the fall in total globulin (0.29 g.) is reflected in the albumin globulin ratio, which rises from 0.61 to 0.73. This rise in albumin-globulin ratio reverses the fall which has continued since the early months of pregnancy.

The globulin fractions show a very slight change in the $\alpha 1$ and $\alpha 2$ moieties which increase by .02 g. and 0.04 g. from their values in the early puerperium. The β and γ fractions both decrease more markedly, by 0.13 g. and 0.21 g. respectively and are therefore mainly responsible for the fall in amount of total globulin.

Commentary

The total protein value appears to remain fairly static following labour and shows but little difference from that found before labour. It is however slightly higher than before labour having appreciated by 3.1% over the pre-labour value. This slight increase in total serum protein does very little to restore its value to that found in the early months of pregnancy (7.32 g. per cent.) from which it has fallen by almost 9.6%. The rise in total serum protein occurring after

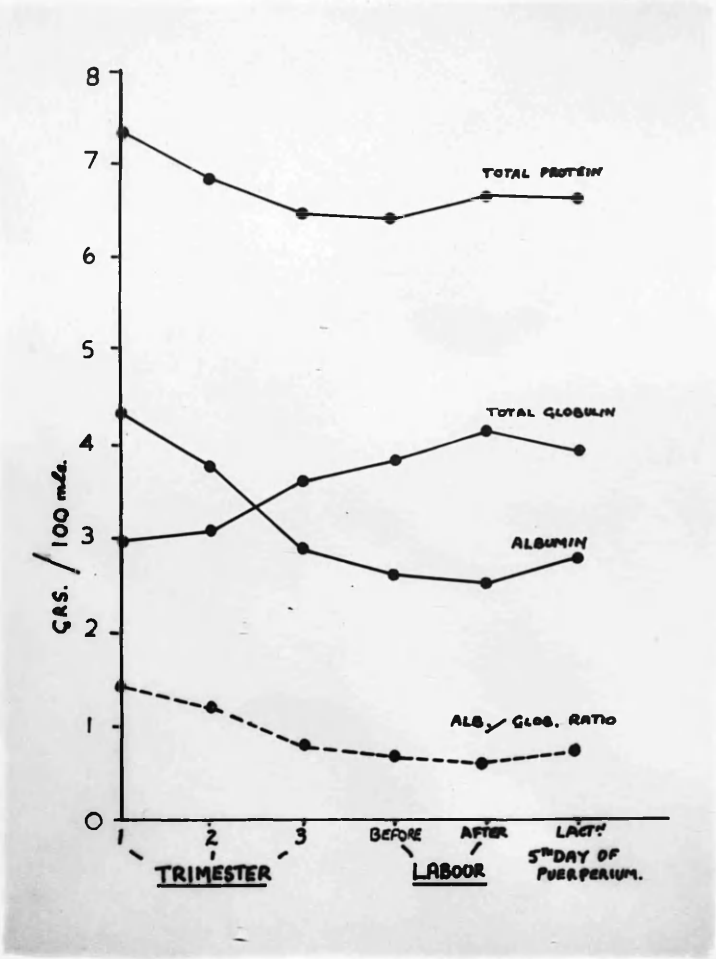
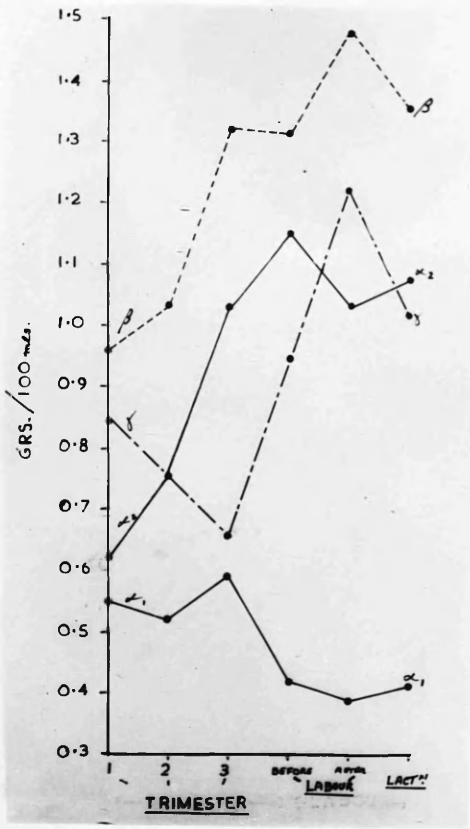

8
7
6
5
4
3
2
1
0
GRS. / 100 mls.
TOTAL PROTEIN
TOTAL GLOBULIN
ALBUMIN
ALB. / GLOB. RATIO
1
2
3
BEFORE
AFTER
LACT.
TRIMESTER
LABOUR
5TH DAY OF PUERPERIUM.

FIG. 1.

labour, however, is worth noting since it is the first time that the progressive fall operative since early pregnancy has been reversed. The albumin value, like the total protein value, increases over the level found before labour but differs from the behaviour of total protein, in that it falls very slightly during labour. At the fifth day of the puerperium the serum albumin has gained 7% from the level found before labour but is still below the average value of 4.33 g. per cent. in the early months of pregnancy.

Serum globulin, which continued its ante-partum progressive increase until after labour, appears to fall when lactation is established. The average value found at this time is however 28% above that found in the first three months of pregnancy.

The albumin globulin ratio of 0.73 has risen, like serum albumin, from its value of 0.61 after labour. The behaviour of the main constituents of the serum protein thus far can best be appreciated by graphic representation. Figure 1 shows the concentrations of total protein, albumin, globulin and the varying values of the albumin/globulin ratio throughout pregnancy, before and after labour, and on the fifth day of the puerperium.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GRS./100 mls.
β
β
γ
α2
α2
α1
α1
1
2
3
BEFORE
LABOUR
LACT.
TRIMESTER

FIG. 2.

The variations in the total serum protein value are almost paralleled by the changes in the serum albumin and in the albumin/globulin ratio, which one might expect. The levels of serum albumin and globulin seem to follow inverse tendencies.

The fall in globulin from the level found after labour is mainly due to the β and γ fractions which appear to decrease when lactation is established. The β fraction value appeared to change little during the later months of pregnancy, to rise during labour, and then to fall to a level almost exactly like that found before labour started. The γ fraction which increased progressively from the third trimester of pregnancy after an initial fall in the early months, diminished in value following labour. It is obvious that during the puerperium there is a trend towards the establishment of the normal non-pregnant values. Fig.11 illustrates, in graphic form, the behaviour of the fractions of the globulin complex from early pregnancy until lactation is established.

Reference to Fig.11 emphasises the fact that changes in the volume of circulating blood whether associated with the physiological haemo-dilution of the ante-partum stages of pregnancy, incidental to the

normal blood loss of parturition, or compensatory thereto, cannot adequately explain the variations in amount of the serum protein fractions which vary in diverse fashion. It is interesting to note that, apart from a rise in total protein mainly related to serum albumin and a consequent rise in albumin/globulin ratio, lactation has no particular effect on the level of the serum proteins. Serum albumin level alone has risen. All fractions of the serum proteins have been subjected to the various volume losses and readjustments incident to labour, but the albumin moiety is the only one which shows a positive change.

SUMMARY OF CHANGES OCCURRING IN THE SERUM PROTEINS THROUGHOUT NORMAL PREGNANCY AND THE PUERPERIUM.

From results obtained in the previous sections the changes occurring in the values of the various serum protein fractions may be reviewed.

In our series the total serum protein level in the non-pregnant subject has an average value of 7.1 gm. per cent. This figure is exactly the same as that quoted by Dieckmann (1952) and is in agreement with reports by other authors such as Bradshaw 1950. As pregnancy advances in its first trimester there is little significant change in the total protein content of the serum but there is a diminution in the middle trimester of pregnancy and this fall continues until labour is over. Following labour there is little change in the total serum protein level which remains fairly constant until true lactation is established. From non-pregnant to term the total serum protein level is diminished by 9.8 per cent. When lactation is established the serum protein level rises but the mid-puerperium still shows a decrease of 6.8 per cent. compared with the non-pregnant level. The serum albumin value falls from the early months of pregnancy

until labour is over and then rises slightly, decreasing over all by 33.6 per cent. of its non-pregnancy level. The total serum globulin follows an inverse pattern of values and when lactation is established it has increased, in its value in the non-pregnant female, by 32 per cent.

The reversal of values of serum albumin and globulin which is found on the fifth day of the puerperium results in a considerable change in the albumin globulin ratio. From a non-pregnant value of 1.5 the ratio gradually falls as pregnancy advances; a value of 1.45 is found in the first trimester, 1.2 in the middle trimester and 0.8 in the third trimester. Twenty four hours after labour, the albumin globulin ratio has fallen to a level of 0.6 but at the fifth post-partum day it has risen to 0.73.

The globulin fractions undergo many variations in amount when investigated at various stages of pregnancy and before and after labour. The α 1 fraction increases in early pregnancy, remains fairly constant in mid pregnancy then rises again in the third trimester. Before labour the α1 fraction decreases slightly and when labour is over it approximates to its value in the non-pregnant state.

At the fifth day post-partum the amount of α1 globulin is little different from the amount found before labour started. From the non-pregnant state to that pertaining when lactation is established, there is an appreciation of 14% in α1 globulin.

The α2 globulin fraction increases in amount steadily from early pregnancy until just before labour, diminishes in amount after labour to the amount found during the third trimester and increases slightly when lactation is established. The increase in amount of α2 globulin, from non-pregnant to 5th day post-partum average values, was 55 per cent. β-globulin increases over a similar period of time, by 35 per cent., its value increasing rapidly until late in pregnancy, falling slightly before labour, rising in the course of labour and falling to a level which approximates to its late pregnancy value on the 5th day after delivery.

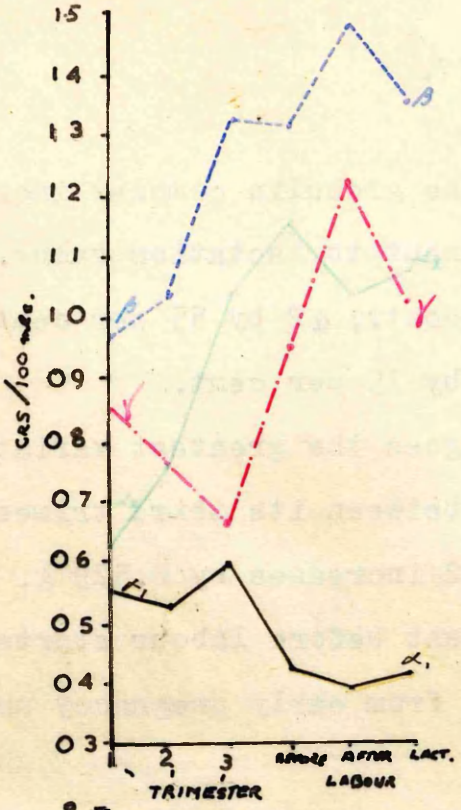
γ globulin differs from all the other globulin fractions in that its value decreases steadily from early to late pregnancy then increases until after labour. Following labour it decreases to, approximately, the same value found before the onset of labour.

Each fraction of the globulin complex increases in amount from non-pregnant to lactation value. $\alpha 1$ increases by 14 per cent., $\alpha 2$ by 55 per cent., β by 35 per cent. and γ by 19 per cent.

The γ factor undergoes the greatest variation, increasing by 0.558 g. between its third trimester and post-partum values. $\alpha 2$ increases by 0.529 g. from early pregnancy until just before labour starts. β increases by 0.512 g. from early pregnancy until labour is over.

Both α factors diminish in the course of labour and increase thereafter. β and γ fractions increase in the course of labour and diminish thereafter.

The fall in total serum protein may in part be related to the hydraemia occurring in pregnancy. The anabolic effects of pregnancy on protein metabolism are reflected in the positive nitrogen balance reported by many workers Hoffström (1909-10), Wilson (1916), Murlin (1916-17), Seegers (1937) found that the positive nitrogen balance increases as pregnancy advances. These findings corroborate our findings of a steady fall in total serum protein values which is not checked until labour is over. Coons and Blunt (1930) observed that storage of protein tended to fall


GRS/100 mls.
1·5
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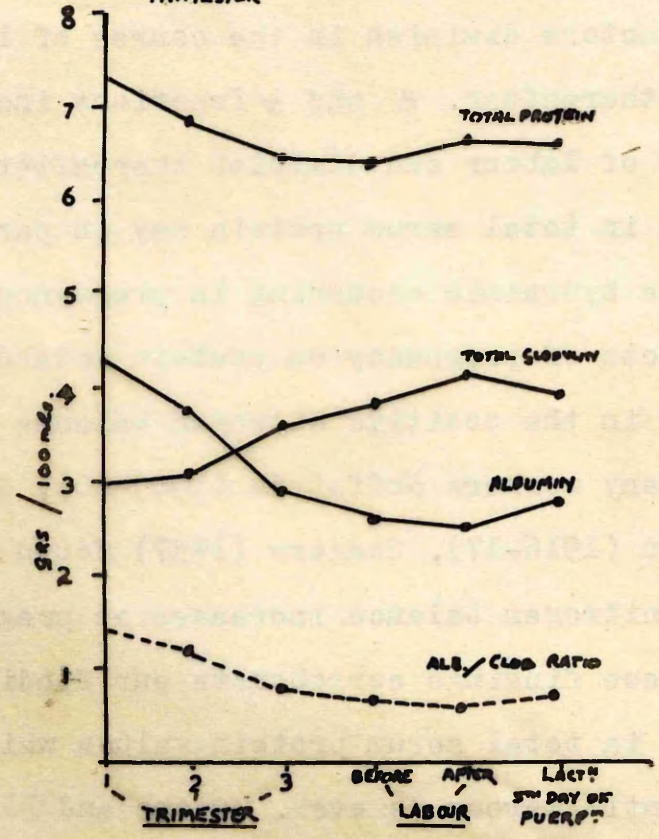
β
γ
α1
1
2
3
TRIMESTER
BEFORE
AFTER
LABOUR
LACT.

FIG. 1.


GRS/100 mls.
8
7
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1
TOTAL PROTEIN
TOTAL GLOBULIN
ALBUMIN
ALB./GLOB RATIO
1
2
3
TRIMESTER
BEFORE
AFTER
LABOUR
LACT.
5TH DAY OF PUERP.

FIG. 2.

off towards the end of pregnancy. Our findings suggest a similar trend (Figs.1 and II).

Many authors have reported a similar fall in serum proteins during pregnancy(Dieckmann and Wegner, 1934; Eastman, 1930; Strauss, 1935 and Plass and Bogert, 1924), and it has been repeatedly shown that the fall is mainly due to reduction in the albumin fraction (Dieckmann, 1952). It has also been shown that the globulin fraction increases with a consequent reversal of the albumin globulin ratio. What has not been clear hitherto is that the change in the globulin is not due to the same fraction at all stages of pregnancy. During the ante-natal period the α and β fractions are responsible for the rise in globulin protein but at term the γ moiety shows a sudden rise. These changes in the various globulin fractions must have some vital significance which is not at present apparent. It is possible that the rise in these fractions is related to events at the time of confinement. At delivery there is always a certain amount of unavoidable blood loss. It has been shown that there is an increase in the coagulability of blood at this time (Glass, G.B.J. 1950, Amer.J.Med. 8, 745) and since many of the factors associated with coagulation of blood are contained in the globulins especially

the β fraction it may be that the rise in globulin is partly related to this phenomen of increased coagulability. No doubt the increased activity of blood clotting is a protective mechanism related to the shedding of the placenta. In addition, many of the immune bodies, such as diphtheria anti-toxin etc., are passed to the foetus either by blood stream or in the milk. Tiselius and Kabat (1939) have shown by electrophoretic fractionation, that in animals, antibodies are in general mainly associated with the γ moiety. Using similar methods Moore, Van der Scheer and Wyckoff (1940) have found that anti-pneumococcal antibodies were associated with the γ fraction. Kekwick and Record (1941) have shown that two fractions of the diphtheria anti-toxin exist, one of which migrates with the β complex and the other with the γ . It is possible that the rise in γ globulin is related to the passage of immune bodies to the foetus during the later stages of pregnancy.

Further work along these lines will require to be done in order to determine the nature of these changes. It may be possible to relate the levels of these globulin fractions to the amount of antibody present in the maternal blood. This is one of the

projects on which a start has been made in relation to rhesus immunisation.

The tendency for reversal of these changes to occur during the puerperium has been noted. Many factors such as lactation, involution of uterus, and loss of fluid may all play their part. Harding, Allin and van Wyck (1924) and Harding and Montgomery (1927) have noted that the nitrogen balance, previously positive, becomes negative in the puerperium. The retention of nitrogen during pregnancy is almost certainly due to hormonal influences and it may be that the storage of nitrogen occurs at the expense of the plasma proteins, particularly albumin. During the puerperium this influence is obviously removed and nitrogen may then be used once more in the restoration of the albumin fraction.

Whatever the cause of these variations, this study provides a basis for comparison with the changes in serum proteins which may occur in diseased states during pregnancy. They also supply standards which may be used in further physiological studies.

6. A STUDY OF THE EFFECT OF PRE-ECLAMPTIC TOXAEMIA ON THE SERUM PROTEIN FRACTIONS.

Pre-eclampsia is a complication of the late months of pregnancy. It is sometimes referred to as "the late toxaemia" of pregnancy. As stated in the introduction to this thesis it is a disease of theories. Many divergent views have been stated on the aetiological importance of various factors related to diet, toxins of various origins, anaphylaxis, excessive water retention, hormone upset and to many other agents. It is not within the scope of this section to deal with the aetiology of pre-eclamptic toxaemia nor further to burden the literature with our personal views on aetiology. We wish to investigate the effects, if any, produced by pre-eclamptic toxaemia on the serum of a patient. The classical signs of pre-eclampsia are usually taken to be (1) hypertension, (2) oedema, (3) albuminuria. These signs may present in any order, not necessarily in the sequence noted. It is not necessary to find all three symptoms present in equal degree to permit a diagnosis of pre-eclampsia. In fact, quite frequently, this complication exists in a severe degree with one or other of the 'classical triad' of signs

absent, or present, at most, in minimal degree.

The cases which we have chosen for our survey were all, by clinical standards, acceptable as cases of pre-eclamptic toxaemia. The patients, forming the series, were all in the last 10 weeks of pregnancy and presented hypertension, oedema, and albuminuria in varying degree. The incidence of pre-eclamptic toxaemia is generally accepted as having some relationship to geographical and climatic factors. Even within the confines of the British Isles there are, consequently, variations in incidence and in severity of pre-eclamptic toxaemia. Standards of normality of blood pressure vary accordingly and that accepted as the upper limit of normal blood pressure in the present series, drawn from the South-West of Scotland, is 140 mm. of mercury Systolic pressure and 90 mm. mercury Diastolic. All our patients had blood pressures above this standard. Oedema was present in all cases and it was a generalised oedema not, in any case, related to local vascular or other pathology. Albuminuria occurred in each patient and the degree is indicated in the clinical memoranda presented as an appendix to this study. The appendix contains a short description of each

individual case with relevant clinical data and information regarding the eventual developments, treatment and termination of the pregnancy. Patients with twin pregnancy are included in the series since they are more commonly affected with pre-eclampsia than are single pregnancies. There has not been any selection regarding parity of the patients in this series.

Table 1.

Pre-eclamptic toxaemia.

Serum Analysis

Serum	Albumin	α1	α2	β	γ	Globulin	Total
1	1.9	.36	1.05	1.28	.8	3.49	5.39
2	2.32	.71	1.01	1.3	.96	3.98	6.3
3	2.45	.4	.73	1.13	1.09	3.35	5.8
4	1.96	.7	1.33	1.42	.98	4.43	6.39
5	3.07	.31	.52	.92	.18	1.93	5.0
6	2.07	.64	1.56	1.1	.22	3.52	5.59
7	2.2	.7	1.34	1.56	.7	4.3	6.5
8	2.65	.15	.67	1.4	.73	2.95	5.6
9	2.67	.51	.69	1.3	.83	3.33	6.0
10	2.18	.76	1.35	1.07	.33	3.51	5.69
Totals	23.47	5.24	10.25	12.48	6.82	34.79	58.26
Averages	2.35	.52	1.03	1.25	.68	3.48	5.83
		15%	29.6%	35.9%	19.5%		

6. TOXAEMIA AND THE SERUM PROTEINS.

Results

It will be seen from Table 1 which contains the results of serum protein analysis in ten cases of pre-eclamptic toxaemia that there is a marked resemblance to the findings, reproduced in Table 2, in the analysis of sera from normal pregnancy cases in the last trimester.

The average total serum protein level of 5.83 gm./100 ml. in the toxaemic series is low and is 0.65 gm. (10%) lower than the level found in the third trimester of normal pregnancy.

The serum albumin value in toxaemic women was found to be 2.35 gm. and this is 0.54 gm., almost 19% lower than the value found in cases of normal pregnancy in the later months.

Total globulin has an average value of 3.48 gm./100 ml. in the toxaemic series which is little different from the 3.6 gm./100 ml. in our normal late-pregnancy series.

With a fall in serum albumin and little change in the globulin content of the serum during pre-eclampsia we find that the albumin-globulin ratio is lower than it is in normal cases.

The factors of the globulin complex, when reviewed individually, vary little in amount when toxaemic cases are contrasted with normal cases, at approximately, the same stage of pregnancy. The α 1 value of 0.52 gm. is little different from the normal 0.59 gm./100 ml. α 2 values, 1.03 gm./100 ml., are exactly alike in each series; β amounts to 1.25 gm. in toxaemia and 1.32 gm./100 ml. in the normal series; the γ values are comparable, 0.68 gm. in the toxaemia series and 0.66 gm./100 ml. in normal cases.

As with the absolute figures little change can be found on comparing the relative values of the toxaemic and normal cases. In toxaemia α 1 is 15 per cent., α 2 29.6 per cent., β 35.9 per cent. and γ 19.5 per cent. of the total globulin. Our normal third trimester cases provided values as follows: α 1 16.5 per cent., α 2 28.7 per cent., β 36.5 per cent., and γ 18.3 per cent. The total globulin and its separate components found in the serum of toxaemic patients vary little in amount from those found during the latter part of normal pregnancy.

Commentary

When the results of anlaysis of a series of pre-eclamptic cases are compared with those of a series

of normal pregnancy in its later months we find that the total serum protein level is lower in the toxaemic series. Harden (1936) supports the view that hypoproteinaemia occurs in pre-eclampsia and gives a level of 5.6 gm./100 ml. as the amount of serum protein found. This figure is comparable with our findings of 5.8 gm./100 ml. Dieckmann (1952) states that "Various investigators have studied the total serum protein and the fractions by various methods in pregnant and toxaemic patients. All agree that there is a decrease in the total serum protein and serum albumin in normal pregnancy, with even lower values in pre-eclampsia, except during the phase of haemo-concentration." Beach, Coryell, Moyer, Robinson, Schoeb, Wiseman, Macy and Mack (1952) using electrophoretic technique found that the total protein of maternal venous blood was lower in complicated than in normal pregnancy.

The albumin value of 2.35 gm./100 ml. found in our "toxaemic series" is considerably lower than that of 3.2 - 3.3 gm. reported by Dieckmann and our value for serum globulin of 3.48 gm./100 ml. is higher than his value of 3.1 gm.

SERUM PROTEINS IN RELATION TO SEVERITY OF TOXAEMIA.

Pre-eclamptic toxaemia varies in degree and in severity in individual patients. It is, therefore, of interest to study individual cases and to analyse the serum protein content of the blood at different stages in the disease process.

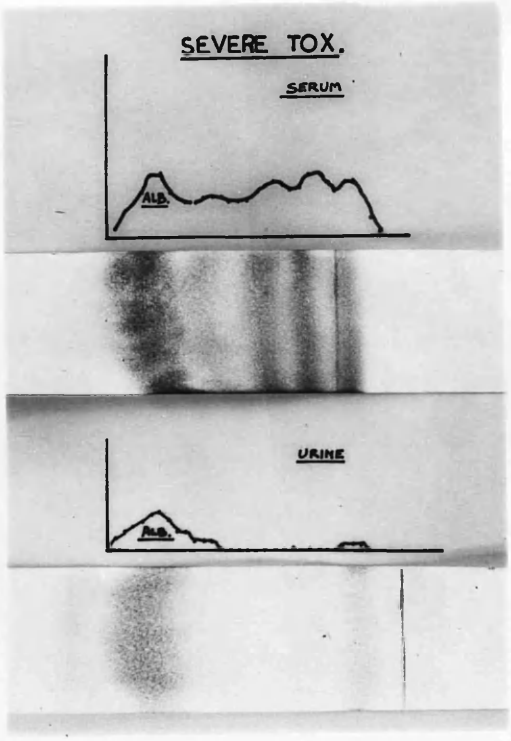
Certain of the cases may present features which one might consider atypical when compared with the average results in the preceding section, but it is seldom that any illness or disease presents an exactly "classical" or "textbook" picture in every particular. It would appear that pre-eclampsia is no exception.

Our cases of toxaemia were studied over a period of several weeks. There was a certain similarity in the findings of all patients but there were certain individual cases which showed considerable variation in the serum protein patterns with the progress of the disease. The average findings for the group tended to obscure these changes in individual cases. In order to illustrate the influence of increasing severity the findings in a typical case are given in detail below. This patient is an example of a case

in which the disease progressively increased in severity resulting in the death of the foetus in utero.

Patient No.1

A woman of 29 years of age, 36 weeks advanced in her first pregnancy was admitted for hospital treatment as a case of pre-eclamptic toxaemia. Her ante-natal care had been conducted by her family doctor who said prior to admission that she had been resting in bed and on a light diet for two weeks because she had developed albuminuria and some oedema when 30 weeks pregnant. On admission the patients blood pressure reading was 150/95 mm.mercury. There was a marked degree of generalised oedema (++) and the urine contained albumin (3 parts Esbach). On examination of the patients abdomen it was found that the size of the uterus corresponded to that of a 36 weeks pregnancy and the vertex presented free above the brim. On ausculation, no foetal heart sounds were heard. The patient had not felt any foetal movements for 4 days although, previously, she had felt the baby moving actively. It was decided to treat the patient conservatively. Two days later the patient's general condition was deteriorating. The blood pressure had risen to 170/105 mm.of mercury,



GRAPHS AND PAPERS FROM

SPEC. 2.

the degree of oedema had increased to ±++++ and the urine contained a large amount of albumin (2 g./100 ml. when estimated by Biuret method).

Surgical induction of labour was performed and, in 5½ hours the patient delivered herself spontaneously of a macerated stillborn male child weighing 4 lb. 14 ozs. Following delivery the patient's general condition gradually improved, urinary output increased and oedema diminished. On the tenth day of the puerperium the patient was dismissed to her own home. Prior to dismissal the blood pressure was 140/90 mm. of mercury, the urine was free from albumen and there was no clinical oedema. Six weeks after delivery, the patient felt well, her blood pressure was 140/85 mm. of mercury, the urine was free from albumin and no evidence of oedema was detected.

Specimens of blood and urine were collected for electrophoretic analysis when the patient was admitted (Spec.1). The urinary protein (3 g./1000 ml.) failed to record electrophoretically since there was an insufficient amount. Further specimens of blood and urine were collected when the toxaemic process seemed to be at its height (Spec.2). Both serum and urine were fractionated by electrophoresis. Spec.3 was taken before dismissal at which time there

Table 1.

Patient No.1.

	Total Protein	Albumin	Total Globulin	α 1	α 2	β	γ	A/G.
Spec.1 Serum	3.6	1.22	2.38	.46	.6	.86	.46	.5
Spec.2 Serum	5.4	1.21	3.19	.78	1.25	1.3	.86	.38
Urine	2	1.88	0.12					15.6
Spec.3 Serum 10 days post-partum	6.2	1.86	4.34	.88	1.08	1.52	0.86	.43
Spec.4 Serum 6 weeks post-partum	7.29	3.49	3.8	.22	1.33	1.45	0.8	.92
Average Toxaemic Case	5.83	2.35	3.48	.52	1.03	1.25	0.68	0.67

was no oedema, the blood pressure was 140/90 mm. of mercury and the urine was normal. A final specimen of blood and of urine were taken at the post-natal clinic (Spec.4). The urine did not contain any protein.

The analyses of the serum and urinary proteins are presented in tabular form. (Table 1).

From the table it is seen that the total serum protein in the first specimen was very low indeed (3.6 g./100 ml.). The serum albumen is similarly very low in amount and the globulin high in comparison, which results in a value for albumin/ globulin ratio of 0.5. The fractions of the globulin complex vary greatly in amount; α 1 and γ being of equal amount (0.46 g./100 ml.) and each representing 19.3% of the total globulin complex. β globulin is the largest factor, having a value of 0.86 g./100 ml., which is 36.1 per cent. of the total globulin. α 2 globulin (0.6 g./100 ml.) represents 25.2 per cent. of total globulin.

The second specimen, taken when the pre-eclamptic process was at its height shows a marked change in the protein pattern. The total protein content has increased by 1.8 g./100 ml., and the increase is entirely due to the globulin fraction which appreciates

by 1.81 g./100 ml. Serum albumin is virtually unchanged in amount. The albumin/globulin ratio was exceptionally low in consequence of the increased globulin content of the serum. Each fraction of the globulin complex was increased in amount as the severity of the disease increased in this individual case. The α 1 and α 2 values of 0.78 g. and 1.25 g./100 ml. increased by 0.32 g. and 0.65 g. respectively from the values found in the first specimen. β globulin increased by 0.44 g. and γ by 0.4 g./100 ml. to values of 1.3 g. and 0.86 g./100 ml. respectively.

The urine contained a large amount of protein and this was analysed at the same time as the patient's serum. Two protein fractions were isolated, the larger, identified from its electrophoretic mobility as albumin, representing 1.88 g. and the lesser, apparently globulin, representing 0.12 g. of the urinary content of 2 g. protein per hundred millilitres. An Esbach reading, obtained by dilution of the urine, showed 22 parts of albumin per litre. These values for the urine gave an extraordinary reading of 15.6 for the albumin, globulin ratio. A series of readings of average values for protein fraction content in our series of pre-eclamptic patients appears at the bottom

of Table 1. Comparison of these readings with those of Spec.1 in this severe case of pre-eclamptic toxaemia show that, in Spec.1, all the fractions of the protein complex are lower in amount than in the average undelivered toxaemic case in our series.

Total protein is 2.23 g./100 ml. lower, albumin is 1.13 g./100 ml. and total globulin 1.1 g./100 ml. lower in this case, than in the average pre-eclamptic case. The decrease has been almost equally shared by the major components. This might suggest that a haemo-dilution of the blood has been responsible for the variation between these contrasted figures; but, when we compare the fractions of the globulin complex similarly, we find a more selective change in their values. In the individual case we have described the values for the fractions α 1, α 2, β, and γ are respectively 0.06 g., 0.43 g., 0.39 g., and 0.22 g. per hundred ml. lower than the values found for their counterparts in our series of pre-eclamptic patients (Section 6).

Consideration of the findings of analysis of Specimens 3 and 4 will be deferred until later in this section of the thesis. The influence of delivery must be considered.

Commentary

It would appear that in severe toxaemia there is progressive diminution in the serum protein concentration. The fact that all fractions suffer this decrease suggests that there is some interference with their production. Since the various fractions are elaborated in different parts of the body this means that any process interfering with their production must be of a systemic nature.

This reduction in the serum protein concentration with increasing severity of the disease is only true up to a point. This is obvious from the values recorded for the second specimen in this patient. Despite a gross increase in albuminuria the serum protein concentration was raised and it is significant that the value for serum albumin remained static although the major portion of the urinary protein was of this type. Her urinary output on these two occasions was 21 ozs. and 23 ozs. on the respective days and it can be seen that the increase in the concentration of urinary protein was not due entirely to oliguria and that the total output of protein must have been greatly augmented. At the same time there was a marked increase in the degree of oedema

and it is likely that the rise in serum protein concentration was partly due to haemo-concentration which is known to occur in severe toxaemia (Dieckmann, 1952). That this was not the only factor is obvious from the selective increases in the globulin fractions. If the changes were entirely due to haemoconcentration one would expect the percentage increase in each fraction to be similar. The actual percentage increases however, were α1, 70 per cent., α 2 108 per cent., β 51 per cent. and γ 87 per cent. This may be of some interest in view of the work of Smith and Smith (1948) who report the presence of a toxic englobulin in large amounts in severe toxaemia.

One other interesting feature may be noted at this point. According to Reiner (1952) the urinary protein pattern is usually normal even if the serum proteins are grossly upset. In other words the albumin globulin ratio in the urine is normal. In severe toxaemia it is apparent that this does not hold. The question however may be taken a little further. In renal disease, with which Reiner was dealing, the serum albumin is reduced and the globulin relatively increased. The urine proteins were the reverse of this. Since both types of protein must filter through

the glomerulus, these findings suggest that there is a selective excretion of proteins and that globulins are held back either at the glomerulus or by reabsorption in the tubule. In severe toxaemia it appears that this selective retention of globulin is even more pronounced than in nephritis. The urinary proteins in one other case, less severe, gave an albumin globulin ratio of 2.5.

The Influence of delivery on the serum proteins in pre-eclamptic toxaemia.

A considerable number of cases of pre-eclamptic toxaemia have been studied over periods of varying duration. Hospital beds are needed for urgent cases and one is restricted in this, and in other respects, from long term study of cases. In some instances cases have been studied over periods of eight weeks and in others, of necessity, the duration of the study was curtailed. It was however possible to obtain values for our cases in the first stage of labour and immediately after delivery. Table 2 provides the average readings for this group. Included are the average results found in these cases before labour commenced and the results, before and after delivery in normal pregnant patients.

The average results of analysis of toxaemic cases before labour shown in Table 2 give a value for total serum protein of 6.02 g./100 ml. The serum albumin level is 2.21 g. and serum globulin 3.8 g. which gives an albumin globulin ratio of 0.58. α 1 globulin forms .44 g., α 2 .91 g., β globulin 1.65 g. and γ globulin .82 g. of the total serum globulin. The β globulin is large in amount, accounting for just over 43 per cent. of the serum globulin.

The results of analysis of sera taken from pre-eclamptic patients, after delivery do not show any marked changes. The total protein of the serum decreases by 0.15 g. to a value of 5.87 g./100 ml., albumin decreases by .05 g. to a level of 2.16 g./100 ml. and serum globulin decreases by 0.1 g. to 2.16 g./100 ml. The albumin/globulin ratio, in effect, remains unaltered.

The globulin fraction most affected is the β fraction which decreases by 0.27 g. to a level of 1.38 g./100 ml. The other fractions increase. γ globulin increases by 0.1 g. to a level of 0.92 g./100 ml.; α 1 increases by .01 g. to 0.45 g./100 ml. and α 2 increases by 0.05 g. to a value of 0.96 g./100 ml.

Table 2.

Toxaemias: Before & After Labour.

	Total	Albumin	Globulin	α 1	α 2	β	γ	A/G Ratio
Before	6.02	2.21	3.8	.44	.91	1.65	.82	.58
After	5.87	2.16	3.7	.45	.96	1.38	.92	.58
Increase	-	-	-	.01 (2.3%)	.05 (5.5%)	-	.1 (12%)	
Decrease	.15 (2.5%)	.05 (2.26%)	.1 (2.6%)			.27 (16%)		

Normal Cases: Before & After Labour.

Before	6.42	2.61	3.81	.42	1.15	1.31	.93	.68
After	6.65	2.53	4.12	.39	1.03	1.48	1.22	.61
Increase	0.23 (3.6%)	-	.31 (8%)	-	-	.17 (13%)	.29 (31%)	
Decrease	-	.08 (3%)	-	.03 (7%)	.12 (10.4%)	-	-	

Antepartum P.E.T. Average results from Section 6.

Series	5.83	2.35	3.48	.52	1.03	1.25	0.68	.67

Average readings from ante-partum pre-eclamptic sera (Section 6) are reproduced at the bottom of Table 2. Comparison shows that the total protein of the serum is higher before delivery, mainly due to an increase in globulin of 0.32 g./100 ml. Serum albumin decreases by 0.14 g./100 ml. and the albumin/globulin ratio decreases from 0.67 to a value of 0.58 before delivery. β globulin is the fraction of the globulin complex which alters most, increasing 0.4 g./100 ml. before labour. γ globulin increases by 0.14 g., α 1 and α 2 globulin decrease by .08 g. and 0.12 g./100 ml. respectively before delivery.

Having reviewed the changes which occur in the serum proteins of toxaemic patients in the course of labour and having compared the sera of pre-eclamptic patients in the ante-partum state with sera from patients prior to delivery it may prove interesting to compare the changes occurring in complicated labour with those occurring in normal labour.

It may be seen from Table 2 that the total protein of the serum increases by 3.6 per cent. in the course of labour in normal cases, whereas there is a decrease of 2.5 per cent. in the toxaemic cases.

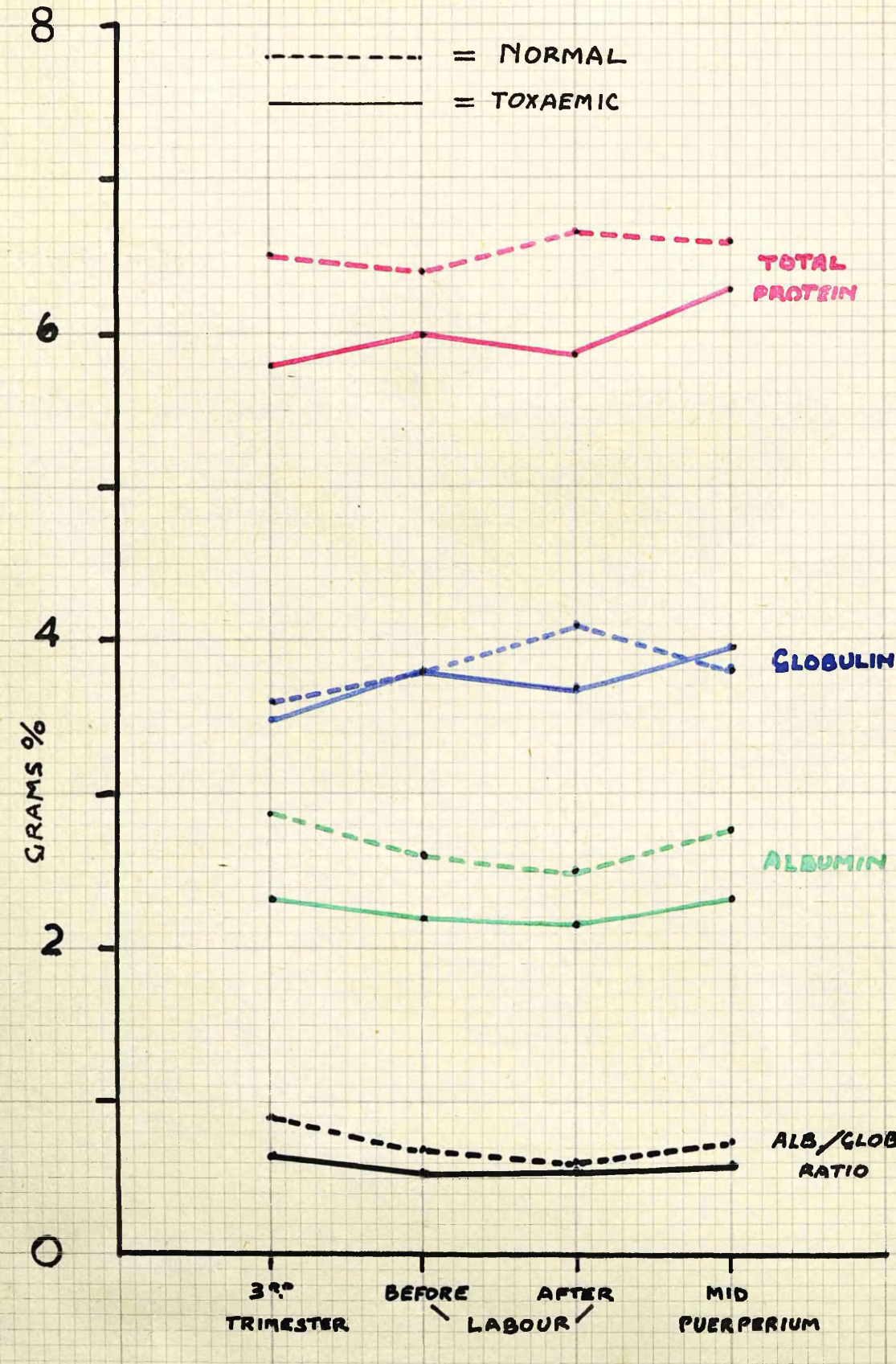
Serum albumin decreases during labour in both

normal cases (3 per cent.) and toxaemic patients (2.26 per cent.).

The total serum globulin, increased in the process of labour by 8 per cent. in normal cases, decreases by 2.6 per cent. in the pre-eclamptic patients.

Consideration of the fractions of the globulin complex shows that while α 1 and α 2 globulins decrease by 7 per cent. and 10.4 per cent. respectively in normal cases during labour, they increase by 2.3 per cent. and 5.5 per cent. respectively in our pre-eclamptic series. β globulin is observed to increase by 13 per cent. in the course of labour in normal patients and to decrease by 16 per cent. in the complicated cases. The fraction which undergoes the most significant change in the normal series is γ globulin which increases by 31 per cent. of its value during labour. In the toxaemic patients γ globulin, in the course of labour, increases by only 12 per cent. and the factor showing the most significant change is therefore the β globulin which decreases by 16 per cent.

GRAPH 1.


8
6
4
2
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GRAMS %
= NORMAL
= TOXAEMIC
TOTAL PROTEIN
GLOBULIN
ALBUMIN
ALB./GLOB. RATIO
3RD TRIMESTER
BEFORE LABOUR
AFTER LABOUR
MID PUERPERIUM

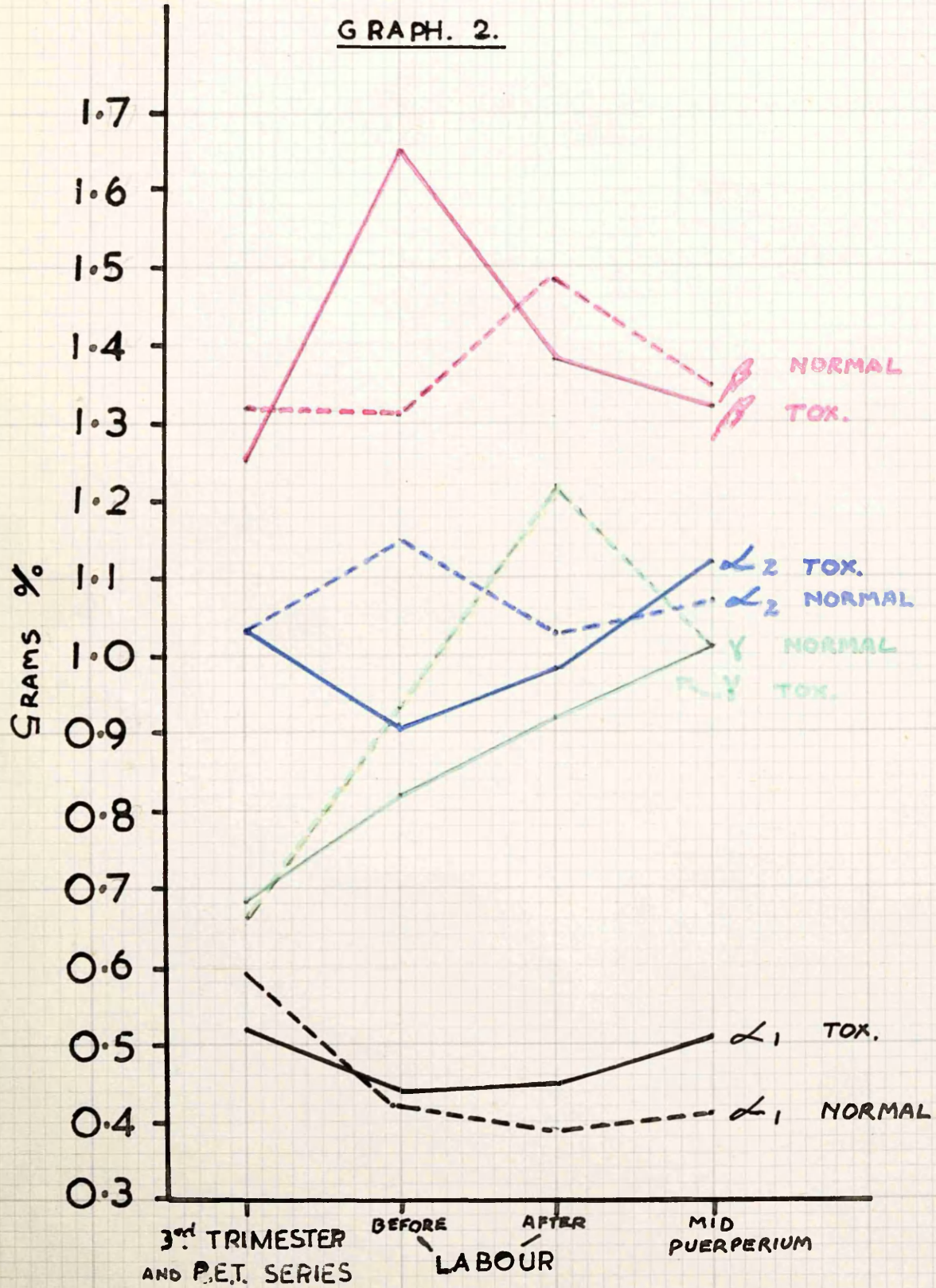
SUMMARY OF THE CHANGE OCCURRING IN THE SERUM PROTEINS IN PRE-ECLAMPTIC TOXAEMIA

In pre-eclamptic toxaemia there is a fall in total serum protein as compared with the level found in normal pregnancy. The fall in total serum protein is mainly evidenced in the albumin portion, but serum globulin also diminishes. In a very severe case of pre-eclampsia the total serum protein tends to increase, probably, largely due to haemo-concentration, but there is an absolute increase in the γ fraction.

Graphs I and II show the trends in the normal and toxaemic cases. The remarkable feature is that, during the puerperium, the values for nearly all components approximate in both the normal and toxaemic series. Certain complex changes occur in both series at the time of delivery. In the normal cases there is an increase of the $\alpha 2$ component whereas in toxaemia this fraction tends to diminish. The reverse is true of the β complex, in normal cases there is a slight diminution at the time of confinement but in pre-eclamptic toxaemia it is greatly increased. The trend of the other factors of the globulin complex is similar in both the normal and toxaemic series.

Although there may be distinctive features in the

GRAPH. 2.


1·7
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GRAMS %
β NORMAL
β TOX.
α2 TOX.
α2 NORMAL
γ NORMAL
γ TOX.
α1 TOX.
α1 NORMAL
3rd TRIMESTER AND P.E.T. SERIES
BEFORE
AFTER
LABOUR
MID PUERPERIUM

toxaemic cases it would appear that, in the antenatal period, the protein fractions behave in a manner essentially similar to that found in normal pregnancy except that the values are at a lower level. It has been shown that pregnancy itself is associated with a fall in serum protein and this might suggest that the changes which occur in pre-eclamptic toxaemia are merely an exaggeration of those found in normal pregnancy.

The fact that the low protein values in toxaemia are mainly due to diminution in the albumin component brings this disease into line with others in which there is hypoproteinaemia. It has been shown by Iversen (1932) and Iversen and Nakazawa (1927) that hypoproteinaemia in a large range of diseases is due to a fall in the albumin concentration of the blood. The relatively high total globulin may be of little significance. Salvesen (1935) has found hyperglobulinaemia in cases of Kala-azar and Boeck's sarcoid where the bone-marrow has been invaded. It has also been recorded as a finding in diseases of the central nervous system by Bing and Neel (1936). In such cases however the hyperglobulinaemia is absolute and not relative as in toxaemia. Unfortunately values are not available

for the various globulin fractions in these conditions so that comparison with toxaemia is not possible.

7. RELATIONSHIP OF SERUM PROTEIN VALUES TO OEDEMA.

In pre-eclamptic toxaemia there is a fall in the total protein content of the serum and this fall is mainly noted in the albumin moiety. There is in addition a relative increase in serum globulin with a consequent lowering of the albumin/globulin ratio.

The increase in globulin is mainly due to a rise in its β complex although the γ complex rises markedly in very severe cases.

While these results are interesting when compared with the findings in cases of normal pregnancy their significance is not apparent. Questions which arise are: (1) Do the results of serum protein analysis have any relation to symptoms? (2) Are the results of diagnostic value? (3) Do the results indicate any aetiological factor?

The first question, regarding symptoms, may be considered initially with respect to oedema. For this purpose our findings are compared with those in other complications of pregnancy which are associated with oedema. It was necessary to chose the cases for study carefully to eliminate a renal factor which may have caused oedema.

Table 1.

Serum protein values in untreated, oedematous cases of anaemia, and in cardiac disease complicating pregnancy.

Serum	Total protein	Albumin	Globulin	$\alpha 1$	$\alpha 2$	β	γ	A/G ratio
1	4.79	1.55	3.24	0.54	0.6	1.14	0.96	0.47
2	6.25	2.73	3.52	0.41	1.02	1.34	0.75	0.77
Average	5.52	2.14	3.38	0.47	0.81	1.24	0.85	0.63
Cardiac Case	6.16	2.35	3.81	.35	.82	1.23	1.41	0.6

The cases chosen were two in which anaemia complicated pregnancy. The patients had no other abnormality and each had a moderate degree of anaemia. One case of cardiac disease (Grade III) was included in the study. This patient was in the last trimester of her second pregnancy. In the cases of anaemia it was found that oedema disappeared with treatment of the anaemia. One is therefore able to contrast the findings before and after treatment. The results in the cardiac case after delivery are, unfortunately, not available. Oedema is not a striking feature of cardiac failure in pregnancy and it is therefore difficult to obtain suitable cases for study.

Results

Table 1 contains the values obtained from serum analysis in two anaemic patients prior to treatment, while oedema was present. The main features are low albumin and high globulin levels. The β fraction is especially high and the albumin globulin ratio is low.

Table 11 shows the results of serum protein analysis in the same patients, after treatment, when the oedema had gone. The albumin has risen in amount and the globulin is static or slightly lower. β globulin has decreased in amount. The albumin/

Table 2.

Serum protein values in treated cases of anaemia complicating pregnancy after oedema had disappeared.

Serum	Total protein	Albumin	Globulin	α 1	α 2	β	γ	A/G ratio
1	5.79	2.15	3.64	0.54	0.68	1.14	1.28	0.6
2	6.3	3.24	3.06	0.22	0.78	1.16	0.9	1.05
Average	6.04	2.69	3.35	0.38	0.73	1.15	1.09	0.8

Table 3.

	Total protein	Albumin	Globulin	α 1	α 2	β	γ	A/G ratio
Normal 3rd Trimester pregnancy	6.48	2.89	3.6	0.59	1.03	1.32	0.66	0.8
Toxaemia series	5.83	2.35	3.58	0.52	1.03	1.25	0.68	0.67
Anaemia	5.52	2.14	3.38	0.47	0.81	1.24	0.85	0.63
Anaemia treated	6.04	2.69	3.35	0.38	0.73	1.15	1.09	.8

globulin ratio has risen.

In the last line of Table 1 appear the results of serum protein analysis the case of cardiac disease previously mentioned. The values are very similar to those found in anaemia. Albumin is low in amount and serum globulin is high in value. The β fraction of the globulin complex is of a low value and the γ fraction appears to be increased.

In Table 3 for comparison are shown average values for the components of the serum protein complex in the third trimester of normal pregnancy, our series of toxaemic patients, and the anaemic patients before and after treatment.

It can be seen that the values in toxaemia bear certain resemblances to those found in anaemia complicating pregnancy. The main feature in both is a diminution of albumin and globulin with a resultant low albumin/globulin ratio. The values for the various fractions of the serum globulin are very similar with the exception of the γ moiety which is increased in anaemia. This increase is even more marked after treatment of the anaemic condition.

Commentary

Attention has been drawn to the similarity existing

between pre-eclamptic toxaemia and certain cases of anaemia in pregnancy (Naegeli, 1912; Beckman, 1921; Callender, 1944; Scott and Govan, 1949). Scott and Govan recorded 13 cases of this type showing hypertension, oedema and marked anaemia. One of the features which distinguishes these cases from true toxaemia is the absence, or the presence of at most a trace, of albuminuria. It is also interesting to note that these authors found low values for plasma proteins. Rest in bed soon brought the blood pressure down, and the albuminuria, if present, disappeared within a few days, but the oedema persisted for longer periods. This would indicate that oedema was not related to renal or vascular failure. The possibility that the fall in serum albumin is related to the oedema must be considered. This is obvious also from the findings in the case of cardiac disease. It is unlikely however, that the relationship is a direct one. Scott and Govan record that, after treatment, the plasma proteins in their anaemic cases fell to an even lower level. It seems likely that oedema and low serum protein values are both the result of upset in the function of some other organ which is closely linked with protein metabolism.

8. SERUM PROTEINS AND HYPERTENSION.

While changes in serum protein levels in toxaemia are distinctive enough when compared with normal pregnancy it remains to be seen whether they have any diagnostic value. It is always difficult to distinguish a case of hypertensive toxaemia from one of essential hypertension in pregnancy. If the patient is seen early in pregnancy the diagnosis of essential hypertension is obvious but later in pregnancy the similarity to toxaemia may be pronounced. Both types of case may show oedema and albuminuria may occur in the hypertensive case. The difficulty is increased if the hypertensive case enters a malignant phase.

In view of this we have analysed the serum protein values in known cases of essential hypertension during pregnancy. At the same time it is interesting to note whether the trend of the various protein fraction values has any relationship to the hypertension of toxaemia. Since pregnancy might tend to obscure the changes associated particularly with the occurrence of hypertension we have included an analysis of serum proteins in non-pregnant cases of essential and malignant hypertension.

We have considered the findings obtained in non-pregnant hypertensive cases before those obtained from pregnant patients.

The non-pregnant cases have been studied in two groups. Firstly the essential hypertension group. The sera from three patients suffering from essential hypertension were obtained from the medical wards of a general hospital. The diagnosis was arrived at by the physicians in charge of the wards, in each case. Patient No.1, a female aged 42, had a blood pressure of 240/140 mm. mercury when the serum specimen was obtained. The urine was albumin free and the physicians were sure that her condition was one of essential hypertension.

Patient No.2 was a man of 66 years of age. The blood pressure was 210/110 at the time of collection of his serum. The renal function was said to be good and the urine was free from all but a trace of albumin. The physicians diagnosis was one of essential hypertension.

Patient No.3 was a woman aged 36, whose blood pressure, when first seen by the physicians had been 260/190 mm. of mercury. At the time when her serum was obtained the blood pressure was 240/130. A

Table 1.

Results of serum protein analysis in Non-pregnant patients - ESSENTIAL HYPERTENSION

Patient	Total Protein	Alb-umin	Glob-ulin	α 1	α 2	β	γ	A/G Ratio
No.1	6.85	4.75	2.1	0.24	0.31	0.71	0.87	2.26
No.2	5.6	3.07	2.53	0.27	0.48	0.75	1.03	1.2
No.3	5.7	2.52	3.18	0.35	0.93	1.08	0.83	0.78
Average values	6.05	3.44	2.6	0.28	.57	.84	.91	1.32
Normal non-pregnant averages	7.1	4.19	2.9	.36	.69	.99	.85	1.5

marked degree of anaemia was present. Hb. 8.5 gm.per cent. This patient's general condition, urinary findings and persistent hypertension had resulted in a diagnosis of essential hypertension.

Table 1 shows the results of serum protein analysis in each case with the average results for the series.

For purpose of comparison the average results of analysis in normal non-pregnant patients are included in Table 1.

On comparing the results the albumin is lower in the hypertensive group than it is in the normal non-pregnant series. This may be mainly due to the fact that patient No.3 was anaemic and it has been shown in Section 7 that anaemia is associated with low levels for serum albumin. The albumin/globulin ratio, consequent to the lowered serum albumin is below the normal series level. There is no gross difference between the values for the various fractions of the globulin complex in the "essential hypertension" and "normal" groups of non-pregnant patients.

The second group of non-pregnant hypertensive patients were examples of malignant hypertension. The specimens of sera, once again, were obtained from

the medical wards of a city general hospital. Sera from six patients were analysed. The patients were diagnosed in each case as suffering from malignant hypertension.

Patient No.1, a male aged 44, had a blood pressure of 235/150 mm. of mercury. The heart was enlarged and papillaedema was found. Headache, dizziness and visual failure were symptoms. The blood urea was 42 mgm. per cent. The urine contained albumin.

Patient No.2, a man of 70 years of age, had a blood pressure of 190/120 mm. of mercury. The blood urea level was 45 mgm. per cent. The heart was slightly enlarged and was he said to be in a state of incipient cardiac failure. The patient suffered from violent headaches and breathlessness. The urine contained albumin.

Patient No.3, was a man of 63 years of age. The blood pressure reading was 170/110 mm. of Mercury. Cardiac enlargement was present. The blood urea was 60 mgm. per cent. and the urine contained albumin.

Patient No.4, this man was 60 years of age. His blood pressure was 160/100 mm. of mercury and was said to have been 210/130 mm. of mercury when he was first seen by the physicians. The patient had headaches and

Table 2.

Average values for serum protein analysis in 6 cases of malignant hypertension. Average values for non-pregnant cases appended.

Patient	Total Protein	Alb-umin	Glob-ulin	α 1	α 2	β	γ	A/G Ratio
No.1	7.3	2.9	4.4	0.44	0.74	1.4	1.83	0.66
No.2	7.15	2.81	4.34	0.27	0.81	1.35	1.89	0.64
No.3	7.05	2.89	4.16	0.33	0.87	1.15	1.87	0.69
No.4	5.95	1.95	4.0	0.27	0.75	1.05	1.94	0.48
No.5	6.17	2.03	4.14	0.33	1.3	1.3	1.18	0.49
No.6	6.8	2.45	4.35	0.33	1.06	1.13	1.83	0.56
Averages	6.73	2.51	4.23	0.33	0.92	1.23	1.75	0.6
Average normal non-pregnant values	7.1	4.19	2.9	0.36	0.69	0.99	0.85	1.5

dyspnoea. The blood urea level was 108 mgm.per cent. and the urine contained 2 g. albumin per litre.

Patient No.5, This male patient was 69 years of age and he had a blood pressure of 160/95 mm.of mercury. He had suffered from attacks of "angina" for three years and he was dyspnoeic. The blood urea level was 50 mgm.per cent.

Patient No.6, was a man, 32 years old, with a blood pressure of 160/100 mm.of mercury. The blood urea level was 54 mgm.per cent. and the urine contained albumin. He suffered from prostrating attacks of headache and was, occasionally, short of breath.

Table 2 contains the results of serum protein analysis, with average values, of the six patients mentioned who have been diagnosed as cases of malignant hypertension by the physicians in charge of them. The average values for our non-pregnant normal cases are included.

Comparison of the results in these analyses shows that the total serum protein, serum albumin and albumin/globulin ratios are lower, and the serum globulin higher, in the 'malignant hypertensive' patients.

The serum albumin isdecreased under normal

standards by 40 per cent. and the serum globulin is increased by 46 per cent. The increase in serum globulin is mainly due to the $\alpha 2$, β, and γ fractions which are increased over normal values by 33.3 per cent., 24 per cent., and 10.6 per cent. respectively. The $\alpha 1$ fraction values, in each group, are comparable. It would appear therefore that the most significant change occurring in the serum proteins in malignant hypertension is a marked increase in γ globulin.

Having considered the effects of hypertensive disease in non-pregnant subjects we proceed to the study of pregnant women whose pregnancies have been complicated by hypertension.

Hypertensive disease in pregnancy.

It is a well known clinical fact that in many cases seen in advanced pregnancy, it is difficult to differentiate pre-eclamptic toxaemia from essential hypertension or chronic nephritis. It is necessary for a correct diagnosis that the patient be seen and examined either before pregnancy or very soon thereafter. In the normal course of events patients are rarely seen at hospital ante-natal clinics until pregnancy is fairly well advanced and therefore our choice of suitable cases for study has been limited.

Over the past three years we have found three patients whose history and clinical findings satisfied our criteria with respect to hypertensive disease. It was impossible to obtain a history of high blood pressure prior to pregnancy. All were apparently healthy and had not consulted a physician for some considerable time. All were seen early in pregnancy and all had a severe degree of high blood pressure. Each case represents a separate facet of hypertension complicating pregnancy and each case has been studied, and is presented separately.

Patient No.1 was a young woman of 28 years of age who was admitted to hospital when twelve weeks advanced in her first pregnancy. This patient's doctor had found the patient's blood pressure to be 210/125 mm. of mercury. The patient had previously led a normal life, free from any symptom, and she had no complaints. The urine contained only a trace of albumin and there was no evidence of oedema. Apart from the exceptionally high blood pressure there were no significant abnormalities on examination of this woman. The size of the uterus corresponded to the duration of amenorrhoea. The patient's treatment consisted essentially of rest in bed and she was observed over

a period of three weeks. The blood pressure did not fall to any great extent and remained very high, a level of 160/100 mm. of mercury being the lowest recorded in that period of time. In view of the albuminuria the patient was seen by a urologist who could not find a primary renal defect. It was decided to terminate the pregnancy and this was done by abdominal hysterotomy when the pregnancy was 15 weeks advanced. Under general anaesthesia the blood pressure had a transient fall to a level of 130/100 mm. of mercury. On the first day after operation the blood pressure rose to 200/120 mm. of mercury and remained high until the patient was dismissed to her own home, for further rest in bed, on the eleventh day after operation. The lowest level recorded after operation was 180/110 mm. of mercury and the level on dismissal was 195/115 mm. of mercury. This woman, when seen 8 weeks after operation, had a blood pressure of 170/105 mm. of mercury but she had no complaint of any symptoms. The urine was albumin-free at this point and there was no oedema. The results of serum protein analysis of a specimen of blood taken when this patient was admitted are as follows:

Total serum protein	5.8 g./100 ml.
Serum albumin	2.22 g./100 ml.
Serum globulin	3.58 g./100 ml.
Globulin fractions, α 1	0.25 g./100 ml.
α 2	1.04 g./100 ml.
β	1.6 g./100 ml.
γ	0.64 g./100 ml.

Albumin/globulin ration = 0.62.

Since this patient was first seen at the beginning of the second trimester one might reasonably compare the values with those found during this period of pregnancy. The average values found in our normal series at this time were as follows:- Total protein 6.828 g.; albumin 3.762 g.; globulin 3.066 g.; α 1 0.523 g.; α 2 0.757 g.; β 1.027 g.; γ 0.759 g.; A/G ratio 1.2. From this it will be seen that in the hypertensive patient there was a decrease in total protein, albumin, globulin and albumin globulin ratio. Of the globulin fractions α 1 and γ are decreased, but α 2 and β are much increased. In this respect the values are more akin to those found in the third trimester of normal pregnancy. It does not appear however that at this stage of pregnancy there is any resemblance to the pattern found in our non-pregnant

cases of malignant hypertension.

The second patient was 32 weeks advanced in her 5th pregnancy when admitted to hospital. Her own doctor had found her to be grossly anaemic and hypertensive. She was known to have been hypertensive in her previous pregnancies and according to her doctor her blood pressure had been raised since the beginning of this pregnancy. There was sign of albumin in the urine, her blood urea was normal and on admission her blood pressure was 150/95 mm. of mercury. On examination, a twin pregnancy was found and the abdominal size was in accord with the estimated duration of the pregnancy. Treatment for anaemia was instituted and the patient's haemoglobin level quickly rose to normal. At this point the only abnormality presenting was a high blood pressure. The patient remained in hospital for 11 weeks. The patient was delivered vaginally of healthy twins.

When seen eight weeks after delivery the patient's general and local condition was satisfactory but the blood pressure remained at 145/105 mm. of mercury. The urine was free from abnormal constituent. A specimen of blood was taken after the patient's anaemia had been treated and the blood picture was normal. Serum

protein analysis gave the following result:

Total serum protein	6.39 g./100 ml.
serum albumin	1.96 g./100 ml.
serum globulin	4.43 g./100 ml.
Globulin fractions:	
α 1	0.7 g./100 ml.
α 2	1.33 g./100 ml.
β	1.42 g./100 ml.
γ	0.98 g./100 ml.

Albumin/globulin ratio = 0.44.

Normal third trimester values are:

Total serum protein	6.48 g./100 ml.
serum albumin	2.88 g./100 ml.
serum globulin	3.6 g./100 ml.
Globulin fractions:	
α 1	.59 g./100 ml.
α 2	1.03 g./100 ml.
β	1.32 g./100 ml.
γ	.66 g./100 ml.

Albumin/globulin ratio = 0.8.

Comparison of the serum protein fractionation results in the case of the patient described above with the average normal results for pregnancy in its last trimester show that total serum protein falls slightly,

as does serum albumin and the albumin globulin ratio is lower in the patient described.

Serum globulin is greater in the hypertensive patient above as are all the globulin fractions. Further comparison shows that in the case described total protein falls by 1.4 per cent. and serum albumin by 32 per cent. Serum globulin, on the other hand, increases by 23 per cent. All the serum globulin fractions increase as follows $\alpha 1$ by 18.6 per cent., $\alpha 2$ by 29 per cent., β by 7.6 per cent. and γ by 48.5 per cent.

The major alteration in the serum proteins in case number two is therefore an increase of γ globulin of 48.5 per cent. over its normal average value at a comparable stage in pregnancy.

The third patient to be considered in this section was a young woman, aged 30, admitted to hospital in the last week of her first pregnancy. She had a twin pregnancy and was obese and grossly oedematous. The blood pressure on admission was 160/110 mm. of mercury and the urine contained albumin (8 g./1000 ml.). This patient was known to have had hypertension since early in her pregnancy but the oedema and albuminuria had first appeared two weeks before admission. Multiple

foetal parts could be palpated and two foetal hearts were heard on abdominal examination. This case was treated, after observation over twenty-four hours without evidence of improvement, by digitally separating the membranes from the lower uterine segment during vaginal examination. When this examination was prformed the pelvis was found to be normal in shape and size, the cervix was taken up and soft and admitted two finger tips and a vertex was engaged in the pelvis. Within eight hours of induction of labour the patient was delivered, by low forceps of a lively male child weighing 5 lb. 7½ ozs. The second baby, a girl weighing 5 lb. 10 ozs. presented as a breech and was delivered manually twenty minutes after the first child. The third stage of labour presented no abnormality. Following delivery this patient became rapidly less oedematous and her albuminuria disappeared within five days, but a degree of hypertension remained. The children and their mother were dismissed well on the fourteenth day after delivery. When dismissed from hospital the patient's blood pressure was still high, measuring 145/98 mm. of mercury. Eight weeks later the patient's blood pressure was 140/90 mm. of mercury.

The serum proteins of the above named patient were

analysed from a sample of blood taken after admission. The results of analysis were as follows:

Total serum protein	5.8 g./100 ml.	(6.48)
serum albumin	2.45 g./100 ml.	(2.88)
serum globulin	3.35 g./100 ml.	(3.6)
Globulin fractions:		
$\alpha 1$	0.4 g./100 ml.	(0.59)
$\alpha 2$	0.73 g./100 ml.	(1.03)
β	1.13 g./100 ml.	(0.66)
γ	1.09 g./100 ml.	(0.8)

Albumin/globulin ratio = 0.73.

Values for serum protein fractions found in late normal pregnancy appear in brackets above.

When the results of analysis in the case described are compared the average findings in a series of normal patients in late pregnancy we find that all the fractions of the protein complex other than γ globulin are lower in value than normal in the patient whose history is given. Total serum protein is 10.5 per cent., albumin 15 per cent., and globulin 7 per cent. below normal levels. The albumin globulin ratio is below the normal value. On examination of the globulin fractions we find that $\alpha 1$ is 32.2 per cent., α 2 29 per cent., and β 14.4 per cent. less than the average

Table 3.

	Total Protein	Alb-umin	Glob-ulin	α1	α2	β	γ	A/G Ratio
Normal non-pregnant	7.1	4.186	2.89	0.36	0.69	0.99	0.84	1.5
Normal late pregnant (3rd trimester	6.48	2.89	3.6	0.59	1.03	1.32	0.66	0.8
Toxaemic pregnancy series	5.83	2.35	3.48	0.52	1.03	1.25	0.68	0.67
Very severe pre-eclampsia (Table 1) (page)	5.4	1.21	3.19	0.78	1.25	1.3	0.86	0.38
Non-pregnant Essential Hyper-tension	6.05	3.44	2.6	0.28	0.57	0.84	0.91	1.41
Non-pregnant malignant hyper-tension	6.72	2.51	4.21	0.32	0.89	1.25	1.74	0.59

normal values. γ globulin alone is above normal average value, and we found it to be increased by 65 per cent.

The changes in these three cases can best be understood by reference to Table 3.

Commentary

From Table 3 it can be seen that essential hypertension does not materially alter the serum protein pattern of the non-pregnant individual. In malignant hypertension albumin is decreased and globulin increased. The globulin increase is most marked in the β and γ globulin fractions in our series. Lewis and Page (1947), using electrophoresis, found that plasma proteins in essential hypertension did not materially vary from normal and that the most significant variation from normal in malignant hypertension was an increase in β globulin and a decrease in albumin, but they do not mention any alteration in the γ component.

With the onset of pregnancy, as evidenced in the first of the three individual pregnancy cases described above, α2 and β fractions are increased. Reference to our results in normal pregnancy will show that this increase in α 2 and β globulin is a normal

accompaniment of pregnancy and first shows itself in the second trimester. It is likely therefore that the changes in the serum protein in this case are due to pregnancy and not to hypertension. Perhaps however the increase in the β fraction may be a true increase related to vascular disease.

As pregnancy proceeds to term as seen in the second case described the only change from normal serum protein values is a rise in the γ globulin but the value for this fraction is practically the same as that found in the non-pregnant cases of essential hypertension.

From this it would appear that pregnancy scarcely alters the serum protein picture in essential hypertension.

In the third individual case described, the values for the various serum protein fractions are greatly changed from normal. There is a certain resemblance to the values found in malignant hypertension but in addition the values are similar to those found in severe pre-eclampsia. The clinical signs found in this case resembled more those of pre-eclampsia than those of malignant hypertension and it seems likely that this was a case of essential hypertension with

superimposed pre-eclampsia. Since the values found in this case and those found in very severe pre-eclampsia resemble in some degree those found in malignant hypertension it would suggest that there may be some connecting link between the three types of patient. It may be that when toxaemia becomes very severe some mechanism similar to that operating in malignant hypertension supervenes.

Differential Diagnosis

As previously stated it is often very difficult to differentiate pre-eclampsia from essential hypertension complicating pregnancy. Comparison of the values of Case 3 with those given for moderate toxaemia in Table 3 shows that some degree of differentiation may be possible on the basis of serum protein analysis. In essential hypertension all fractions of the globulin complex are increased whereas in toxaemia these values scarcely differ from those of normal pregnancy. If, however, the toxaemia is very severe the values approximate more and more to those found in the hypertensive case.

TOXAEMIA AND RENAL DISEASE.

Since first protein was demonstrated in the urine of pre-eclamptic patients theories relating to the aetiology of the condition to renal disease have been a recurring phenomenon. These theories gained some credence through the studies of Schmorl (1902) and Fahr (1920, 1924). Many writers have noted the similarity which exists between nephritis and pre-eclampsia. In both conditions the main features are oedema, albuminuria and hypertension and it is not surprising that observers have tried to link the two conditions aetiologically. These attempts have met with varying success. In most instances workers have studied cases of toxaemia of pregnancy in retrospect. For instance, according to Dieckmann (1952) approximately 12 per cent. of eclamptic patients are subsequently shown to have a renal defect. Meyer and Weiz (1904), in a pathological study of fatal cases of eclamptia, showed that chronic nephritis was present in 8 per cent. Acosta Sison (1931) on the other hand, in a similar study, put the figure at 38 per cent. Teel and Reid (1937) in a follow-up of eclamptic patients found that 28 per cent. had a residual high blood pressure, 9 per cent. had

albuminuria but only 6 per cent. showed any renal deficiency as measured by the urea clearance test. Lewis (1940, Osman (1934) and Page and Cox (1938), the latter in a very large series, also found that renal insufficiency seldom followed upon pregnancy toxaemia. Browne and Dodds (1939) studied 400 toxaemic patients over a long period and stated that chronic nephritis complicating pregnancy was rare and occurred in only 17 of these patients. Nevertheless, the idea of a renal basis for toxaemia of pregnancy keeps recurring in the literature and for this reason it was decided to compare the serum protein pattern in the two conditions. It was impossible to find a case of known nephritis complicating pregnancy and we were relectantly compelled to use values for the non-pregnant individual.

Sera from three cases of chronic nephritis were obtained from the medical wards in a general hospital, and these sera were submitted to electrophoretic protein analysis. All three cases had been diagnosed by the physicians as cases of true chronic nephritis in view of their histories of repeated attacks of typical acute nephritis. At the time of examination of their sera, the patients were suffering

Table

Serum protein fractions in chronic nephritis.

	Total Protein	Alb-umin	Glob-ulin	α 1	α 2	β	γ	A/G Ratio
Serum 1	6.0	3.4	2.6	0.34	0.75	0.9	0.6	1.3
Serum 2	8.0	5.35	2.65	0.57	0.66	0.73	0.68	2.0
Serum 3	7.2	4.16	3.04	0.35	0.35	0.96	1.39	1.3
Average values	7.06	4.3	2.76	0.42	0.58	0.86	0.55	1.56
Toxaemia series	5.83	2.35	3.48	0.52	1.03	1.25	0.68	0.67
Third Trimester Normal Pregnancy	6.48	2.89	3.6	0.59	1.03	1.32	0.66	0.8
Normal Non-Pregnant	7.1	4.186	2.89	0.36	0.69	0.99	0.84	1.5

from oedema, poor renal function as assessed by various excretion tests, slight albuminuria and moderate hypertension. In addition, the patients complained of mild uraemic symptoms such as headache. The blood urea level was raised in each case.

The table shows the individual and average values for the various protein constituents of the sera from these patients. In addition, the average values for serum proteins in our normal non-pregnant and pregnant patients and in our toxaemic series are included.

Commentary

It can be seen that, in chronic nephritis, the values for the various fractions are very similar to those found in the normal non-pregnant individual although the γ fraction is somewhat lower in the nephritic group. This has also been noted by Squire (1953) in a recent electrophoretic study of blood proteins in nephritis. There does not appear to be any resemblance between the average values for the nephritic group and those of pregnancy toxaemia. Even in our case of very severe toxaemia the values bear no relationship to those in the nephritic cases, even allowing for the effects which pregnancy might

bring about in the latter. This point however might require modification if cases of known nephritis in pregnancy became available for investigation. In general, however, if renal insufficiency of a nephritic type were associated with typical changes in serum protein pattern one might reasonably expect these changes to show in the cases under review, that is those cases with symptoms of incipient uraemia. Similarly, if pregnancy toxaemia is aetiologically related to nephritis the serum protein pattern in severe toxaemia would resemble that found in nephritis, or at least the association would show in the changes related to one or other particular fraction. No evidence of this kind can be found in the present study.

HEPATIC FUNCTION AND TOXAEMIA.

Since Jürgens (1886) first reported the occurrence of haemorrhagic lesions in liver in eclampsia numerous writers have attempted to relate the symptomatology and etiology of toxaemia to functional deficiency of this organ. Even in normal pregnancy opinion appears to suggest that the liver function is defective. Hofbauer (1907) described changes in the liver histology in normal pregnancy. Seitz (1927) later showed that urobilinuria occurs in 64 per cent. of normal pregnant patients and both Kauffmann (1932) and Soffer (1933) found evidence of increased bilirubin retention in pregnancy. Numerous observers have reported evidence of hepatic deficiency in toxaemia, particularly when employing over-loading tests (Herrmann 1929; Eufinger and Bader 1926; Cantarow, Stuckert and Gartman 1935).

Apart from a primary liver defect in toxaemia there is the possibility that liver deficiency may develop during the course of the disease. Elman and Heifetz (1941) have found that if hypoproteinaemia is prolonged liver function is upset, and experimentally Himsworth and Glynn (1944) have shown that hepatic necrosis occurs when protein is deficient. In view

Table 1.

Hepatic Function and Toxaemia.

Cirrhosis	Total Protein	Alb-umin	Glob-ulin	α1	α2	β	γ	A/G Ratio
Serum 1	6.06	1.33	4.73	0.53	0.71	1.11	2.38	0.28
Serum 2	5.01	1.35	3.66	0.3	1.0	1.24	1.12	0.36
Averages	5.53	1.34	4.19	0.41	0.85	1.17	1.75	0.32
Secondary Cirrhosis								
Serum 1	7.0	3.12	3.88	0.27	0.59	1.41	1.61	0.8
Toxaemic Series	5.83	2.35	3.48	0.52	1.03	1.25	0.68	0.67
Severe Toxaemia	5.4	1.21	3.19	0.78	1.25	1.3	0.86	0.38
Normal pregnancy	6.48	2.89	3.6	0.59	1.03	1.32	0.66	0.8
Non-pregnant	7.1	4.186	2.89	0.388	0.689	0.998	0.848	1.5

of the marked loss of protein in the urine in toxaemia it is possible that hepatic deficiency may develop as a result of this.

For purposes of comparison it was decided to analyse the serum protein fractions in known cases of hepatic deficiency. Two cases of jaundice secondary to hepatitis were studied in this way. At the same time since any change in hepatic function during the course of toxaemia may be of secondary nature serum from another case of hepatic deficiency secondary to cardiac failure was analysed.

Results

Table 1 shows the results of analysis of the sera from our cases of primary and secondary hepatic dysfunction. For comparison the average results found in toxaemia, severe toxaemia, and normal cases, pregnant and non-pregnant, are also shown.

It can be seen that in both types of hepatic deficiency there is a diminution in the serum albumen but this is most marked in the cases due to hepatitis. Globulin is increased in both but once more the change is less in the case of hepatic deficiency secondary to cardiac disease. Despite the gross increase of globulin in the cases of primary liver disease the

Table 2.

Comparison of Globulin Fractions.

	$\alpha 1$	$\alpha 2$	β	γ
Severe Toxaemia compared with normal pregnancy	+32%	+21%	-1.5%	+23%
Primary Hepatic disease compared with normal non-pregnant values	+17%	+23%	+18%	+108%
Secondary Hepatic disease compared with normal non-pregnant values	-25%	-14%	+40%	+89%

reduction in the albumin is so great that the total protein values are also diminished. Comparison of the globulin fractions in these cases with those of the normal non-pregnant subject shows that in primary liver disease all components of the globulin complex are increased. This is most marked in the γ fraction. In secondary liver disease the α fractions are diminished but both β and γ fractions are increased. It seems likely from this that the main effect of liver disease is on albumin and the γ component. A similar comparison of toxaemic cases with normal pregnant subjects shows that in the average case of toxaemia the main feature is a diminution in serum albumin. No marked change occurs in the globulins unless the disease becomes severe. When this occurs there is an increase in both α fractions and in the γ moiety. The β globulin is diminished. The comparison can probably best be made by referring to Table 2 which shows the percentage increase in both severe toxaemia and hepatic deficiency when compared respectively with normal pregnant and non-pregnant subjects.

Commentary

Many observers have drawn attention to the tendency to hepatic upset during pregnancy and it is

an accepted fact that acute yellow atrophy is most commonly associated with pregnancy. There has been a considerable amount of vague speculation in the past regarding the etiology of this condition and there has been thought to be a certain degree of similarity between the pathological changes found in acute yellow atrophy and those of phosphorus poisoning, chloroform poisoning (Wells 1914), mushroom poisoning (Frey 1912) and eclampsia (Wells and Bassoe 1904). In other words toxaemia has always been thought to be associated with diminished liver function and one might reasonably expect changes in the serum proteins of a toxaemic patient to resemble those found in known hepatic disease. According to Foster and Whipple (1921) the first change in serum protein values in hepatic dysfunction is a fall in albumin. This has also been noted by Popper and Schaffner (1952) and in addition the albumin globulin ratio is decreased. In the later stages of hepatic disease the γ globulin increased, and according to Popper and Schaffner this is most marked in cirrhosis. This has been confirmed by Reiner (1952) in electrophoretic studies. According to this author hepatic disease is associated with a marked increase in γ globulin, less frequently a rise

in β globulin and a decrease in albumin. From the first table it can be seen that our results confirm this finding. It is obvious, however, that even in severe toxaemia the pattern of the serum proteins does not resemble that found in advanced hepatic disease in any way. Nevertheless, in view of the statements of Foster and Whipple (1921) and Popper and Schaffner (1952) it may be that the fall in albumin is an indication or forewarning of commencing hepatic dysfunction.

Another possibility must be explored. It has been shown that in toxaemia there is an increased output of ammonia (Zweifel and Lockmann, 1906 and 1909), a decrease in alkalinity of the blood due to organic acids (Zangmeister 1911; Zweifel 1905), and an increase in the elimination of sulphur in an unoxidised form (Wells 1914). All of these facts suggest that oxidation within the body is impaired. This is also indicated in the work of Mukherjee and Govan (1950) who found a retention of organic acids and phosphorus in the tissue fluid in cases of toxaemia. It is possible therefore that hepatic dysfunction, if present, may be related to lack of oxidation. If this were so one might reasonably expect the changes

in serum proteins to resemble those in hepatic dysfunction secondary to cardiac disease. Reference to Table 1 shows that in our case of jaundice secondary to cardiac disease the main feature is an increase in β globulin with little or no change in the γ fraction or albumin. No resemblance to the toxaemic pattern exists. From this one is forced to conclude that hepatic dysfunction, even in severe toxaemia, must be of mild degree. The fall in serum albumin is the only evidence we have in this study that the liver is upset, and one must take into consideration that part of the reduction in this fraction may be related to the albuminuria.

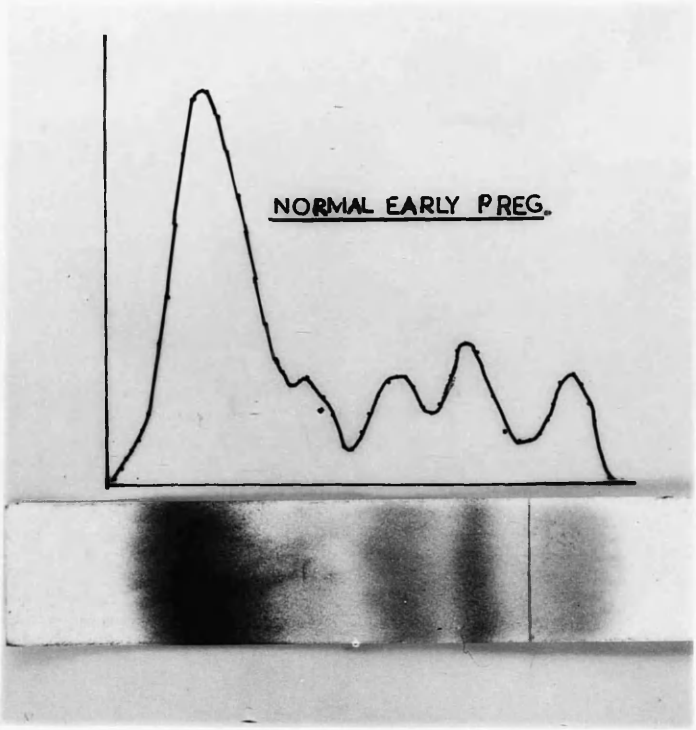

FIG. 1.

Final Commentary

The present thesis is in the main a basic preliminary investigation into the behaviour of the serum protein fractions in pregnancy. It represents primarily an attempt to provide average values in normal pregnancy and to show the trend of change in these fractions as pregnancy advances.

It has been shown that there is a fairly constant and progressive diminution in the concentration of total serum protein and in the albumin fraction thereof. This fall in total protein concentration is only relative. It has been demonstrated repeatedly by various methods that the plasma volume is increased in pregnancy (Dieckmann and Wegner, 1934; Richter, Meyer and Bennett, 1934; Bethell, 1936; MacLennan, 1943; White, 1950), and it is apparent that there is an active increase in the total circulating protein of the blood. At first glance it might be thought that the changes in the protein concentration of the serum were entirely due to haemodilution, but the alteration in the relative values of the various fractions indicates that this is not so. The total globulin concentration is raised and this is due to increase in the $\alpha 2$ and β fractions. At the same time

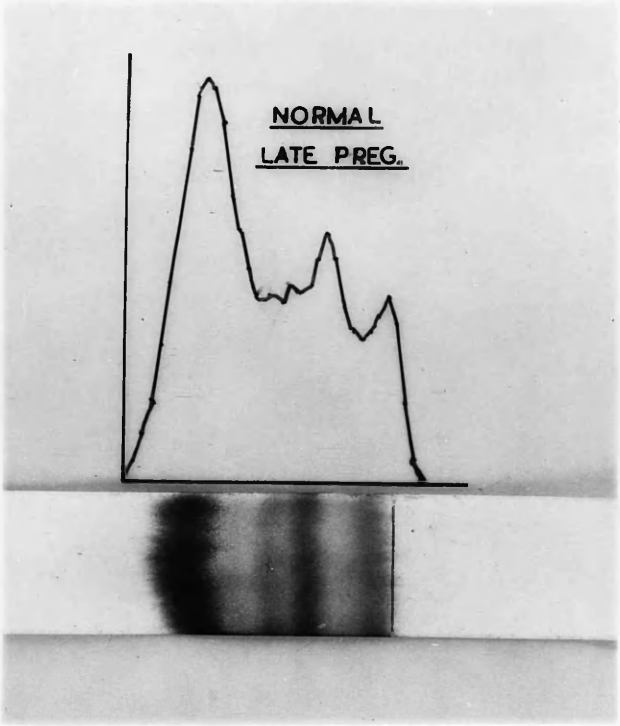

FIG. 2.

it is generally agreed that the plasma volume increases by 20 per cent. during pregnancy but the albumin concentration decreases by 30 to 40 per cent. It would therefore appear that there is a true diminution in the albumin and a gross increase in the $\alpha 2$ and β globulin fractions. Towards term the γ fraction also increases. The effect of these changes on the graphic pattern of serum analysed electrophoretically can be seen in figures 1, 2 and 3. Following delivery these changes are gradually reversed and by the fifth day of the puerperium are approaching the non-pregnant levels. Some tentative suggestions have been made regarding possible physiological interpretation of these changes but more intensive study will be necessary before the true reasons for these changes can become apparent. Such factors as dietary protein, foetal growth, development of foetal organs, breast development and endocrine activity will all have to be taken into consideration.

When toxaemia supervenes there is a further fall in the serum protein concentration, mainly affecting the albumin moiety. This change is even more marked than is evident at first sight since it has been shown that toxaemia is associated with haemoconcentration

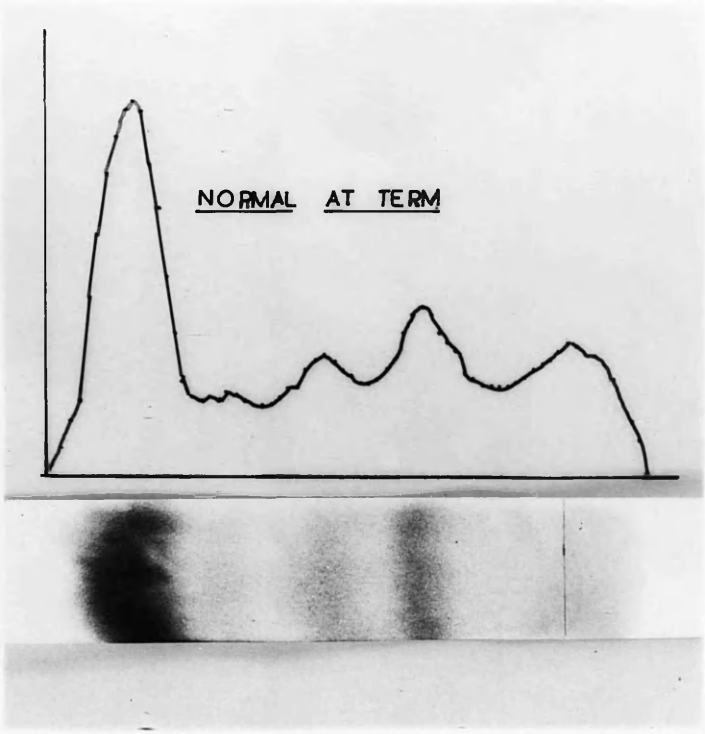

FIG. 3.

(Luisi, 1937; Freis and Kenny, 1948; Dieckmann, 1952). Practically no change occurs in the concentration of any of the globulin factors. It would almost appear that in the average case of toxaemia there is an intensification of the changes which occur in normal pregnancy. There is however, the complicating factor of albuminuria and it might be argued that the loss of albumin in the urine is the cause of the low serum albumin. Until toxaemia becomes very severe, however, the amount of protein lost in the urine is small, the concentration being not more than 0.1 to 0.3 g.per cent. (1 to 3 parts Esbach). It can be seen also that not all of the protein in the urine is albumin. In respect of this it is interesting to note that in both normal and toxaemic subjects the serum albumin increases rapidly in the puerperium, although there is a considerable drain on the protein of the body by milk secretion, which contains 2.0 g. per cent. Again, in the toxaemic case the changes are greater than is generally realised. Following delivery of the toxaemic case albuminuria does not disappear for several days. At the same time these patients, previously haemo-concentrated, show haemodilution. Despite these factors, loss of protein

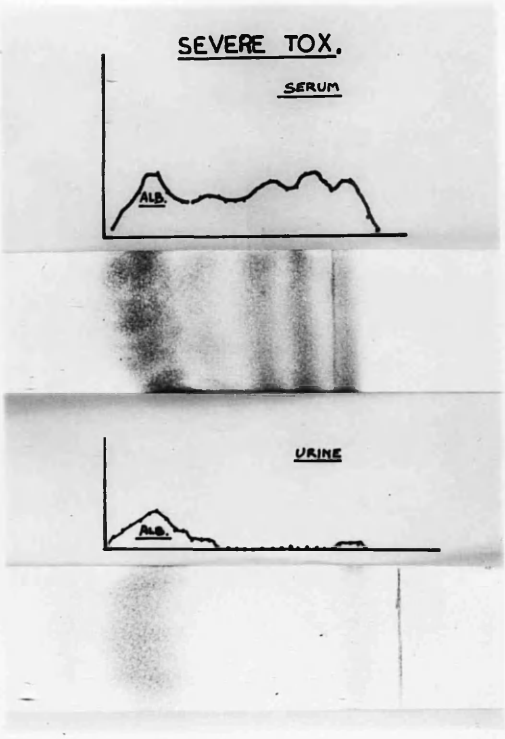

FIG. 4.

in milk and urine, and haemodilution, the serum protein continues to rise. This indicates that the fall in serum albumin during the ante-natal period of these toxaemic cases is not due entirely to loss of protein in the urine. During the puerperium of the toxaemic patient values for all the main constituents of the serum protein approximate to those of the normal puerperium.

No relationship could be found between the serum protein values and the occurrence of oedema in pregnancy. Retention of fluid occurs in normal pregnancy and is sometimes associated with minimal oedema (Dieckmann, 1952). This however, appears to be due mainly to retention of sodium (Black, Platt and Stanbury 1950; Freyberg, Reckie and Folsome, 1938). Frank oedema is common to many conditions in pregnancy and it is remarkable for the ease with which it may occur. In some of these conditions there is also a diminution in the serum albumin but no direct relationship can be shown to exist between this finding and the occurrence of oedema. The protein concentration rarely if ever falls to a critical level and as has been shown in the case of anaemia of pregnancy, improvement in the oedema may take place

when the serum proteins show a further fall (Scott and Govan, 1949). In toxaemia too, the serum proteins are always well above the critical level and it has been shown that the oedema, as in normal pregnancy, is more related to retention of sodium (Taylor, Warner and Welsh, 1939; Rossenbeck, 1931; Mukherjee and Govan, 1950). It would appear that oedema and fall in serum protein are related only in that both are the result of some other metabolic upset. It may be noted that in the puerperium of toxaemic cases the oedema frequently disappears before the protein values regain the normal levels.

The significance of hypertension during pregnancy is a vexed question. Patients, especially if multiparous, are seldom seen at ante-natal clinic until pregnancy is well advanced. At this stage hypertension may be a manifestation of toxaemia, or it may be of chronic nature and merely incidental to the pregnancy, or it may accompany anaemia (Scott and Govan, 1949). This problem was approached by comparing the serum protein pattern in essential and malignant hypertension with that in normal non-pregnant subjects, and the pattern in pregnant subjects known to have chronic hypertension with that in normal and toxaemic

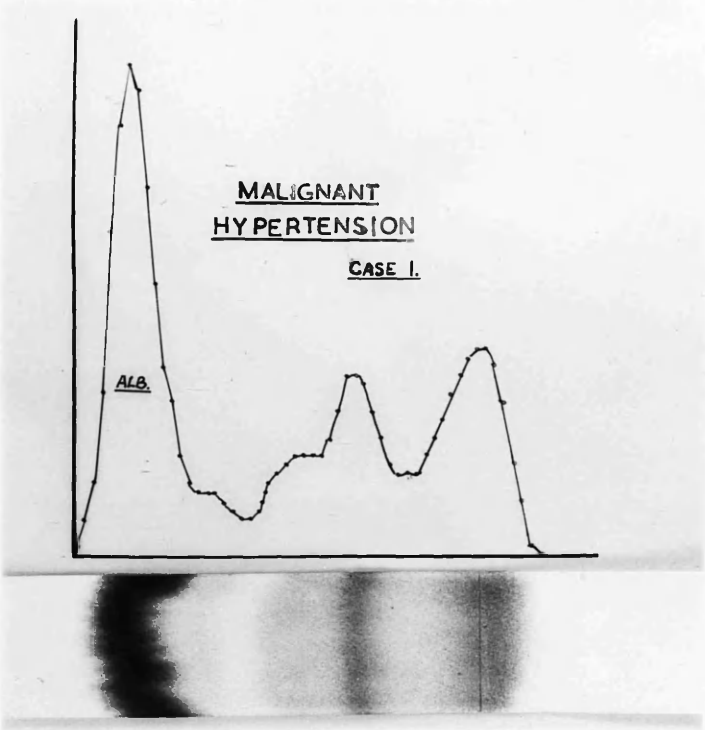

FIG. 5.

pregnant patients. The analysis has shown that in the non-pregnant subject with essential hypertension there is a variable decrease in albumin and a slight increase in the γ fraction. Pregnancy makes little difference to this pattern. The values for the various fractions are similar to those in normal pregnancy apart from the slightly raised $\beta + \gamma$ values, and the greater depression of the albumin. In the average case of toxaemia the only features are a diminution in the serum albumin and a very slight decrease in the β globulin. There does not appear to be any similarity between the patterns in the two diseases. When, however, toxaemia becomes severe the pattern begins to resemble that of chronic hypertension more closely (Figs.4 and 5). This relationship is brought out clearly by a study of the pattern in malignant hypertension in the non-pregnant individual and comparing it with that found in our third case of essential hypertension complicated by a superimposed severe toxaemia of pregnancy. The values in both instances are almost indistinguishable. If the serum protein pattern can be taken as a guide it is obvious that initially toxaemia has little in common with chronic hypertension, but as the disease increases in severity

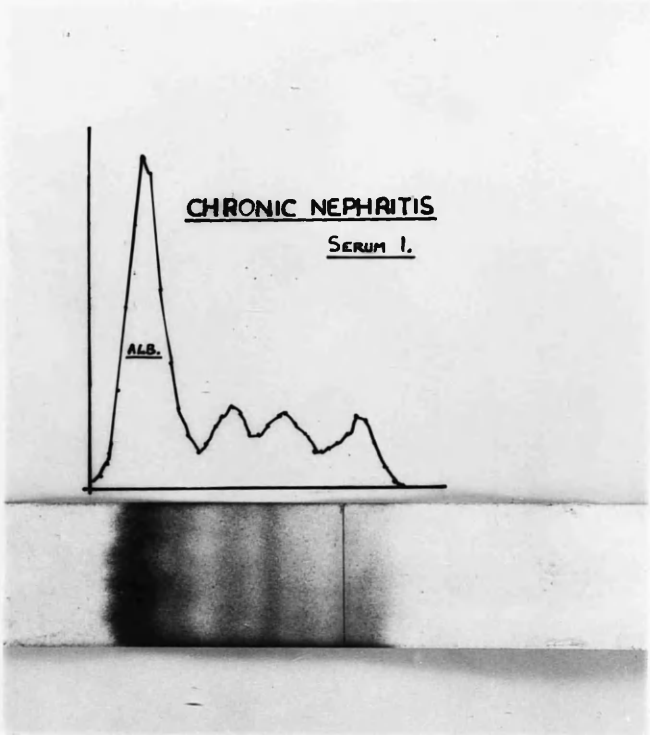

FIG. 6.

a certain resemblance can be traced and it may be that at this stage in toxaemic syndrome a new mechanism begins to operate.

From time to time the renal theory of toxaemia is resuscitated and attempts are made to prove an association with nephritis. So far as serum protein values are concerned no resemblance could be found between toxaemia and nephritis. This opinion, however, may require to be altered however, since all our nephritic cases were non-pregnant and we were unable to study a proved case of this condition in pregnancy. It has been shown, however that it is unlikely that any relationship between the two conditions can exist (Fig.6).

Another problem which is aired in the literature from time to time is the relationship of hepatic function to pre-eclampsia. Many attempts have been made, with some degree of success, to show that in the toxaemic patient the reserve capacity of the liver is limited. In the present thesis a study was made of the serum protein patterns in primary and secondary liver disease of advanced degree. It has been demonstrated that in both there is a diminution in the albumin fraction and a gross increase in the

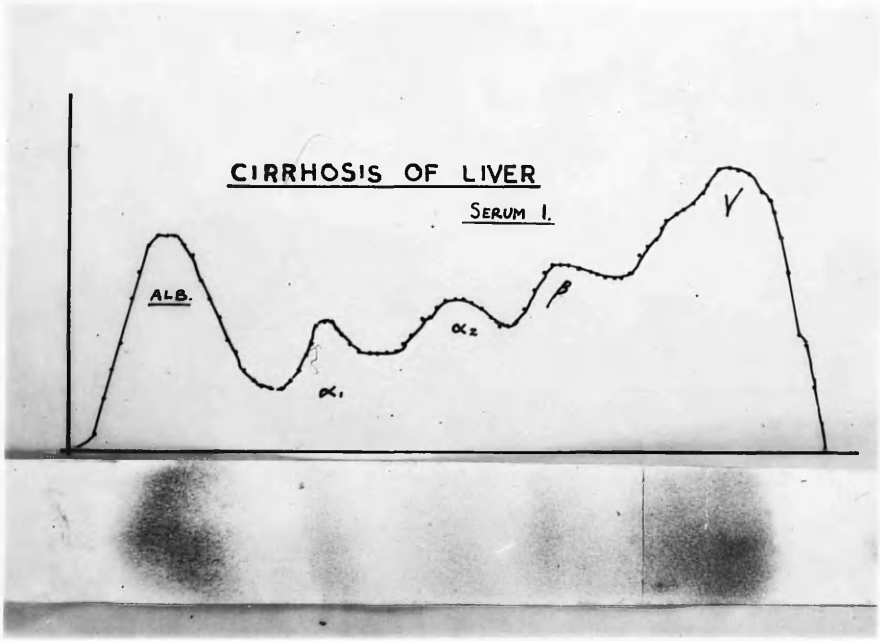

FIG. 7.

γ globulin. These changes are most marked in a primary liver defect (Fig.7). Similar results have been reported by other observers and it has been pointed out that the first change is a fall in the albumin. A similar fall in albumin occurs in toxaemia and as the disease increases in severity a slight rise in γ globulin occurs. It is unlikely, however, that this increase in γ globulin is related to failure of hepatic function. In known liver disease the γ fraction only increases when the condition has reached the cirrhotic phase and this does not occur in pre-eclampsia.

Summing up, it appears that in the average case of pre-eclampsia the changes in the serum protein pattern resemble those found in normal pregnancy but they are more marked. This agrees with Govan (personal communication) who is of the opinion that many of the changes in toxaemia are largely of hormonal origin and are to be viewed as an exaggeration of those occurring in normal pregnancy. In severe toxaemia secondary changes occur which may have some relationship to malignant hypertension.

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A P P E N D I X

CLINICAL NOTES REGARDING PATIENTS WHOSE SERUM PROTEIN ANALYSIS RESULTS APPEAR IN SECTION 6.

Patient No.1.

A young woman, aged 27, admitted to hospital three days before her expected date of confinement in her first pregnancy. Her blood pressure on admission was 155/90 mm. of mercury and she had slight generalised oedema. The urine contained a trace of albumin. On examination the uterus was of a size corresponding to the calculated maturity of the pregnancy. A single foetus, with vertex engaged, could be palpated. The foetal heart was clearly audible. The treatment given was conservative. This patient went into labour and, after a labour lasting $11\frac{3}{4}$ hours, she delivered herself spontaneously of a live male child weighing 9 lbs. The third stage of labour was uneventful as was the puerperium. The mother breast fed her child successfully and was dismissed home on the tenth day after delivery, at which time her blood pressure was 110/80 mm. of mercury, she had no oedema, and the urine was free from albumin. The serum was analysed following admission.

Patient No.2.

A primigravida woman of 29 years of age, 36 weeks pregnant, was admitted to hospital with a blood pressure of 150/95 mm. of mercury. The urine contained three parts (Esbach) of albumin and oedema was extensive. Abdominal examination revealed that a single foetus was present with the vertex at the pelvic brim. No foetal movements were felt and no foetal heart sounds could be heard. The patient's condition did not improve and, two days later, labour was induced by artificial rupture of the membranes. Following a short uneventful labour (5½ hours) a stillborn male child of 4¾ lbs. was delivered spontaneously. Gradually the patient improved, oedema and albuminuria diminished and she was allowed home on her tenth day post-partum. The blood pressure on dismissal was 140/90 mm. of mercury, the urine was free from albumin, and the patient had no oedema. The serum was analysed following the patient's admission to hospital.

Six weeks after delivery the urine was free from albumin, there was no oedema and the blood pressure was 140/85 mm. of mercury.

Patient No. 3.

A primigravida of 30 years of age, was admitted to hospital when 39 weeks pregnant. This patient had a twin pregnancy and she was grossly oedematous. On admission her blood pressure was 160/110 mm. of mercury and her urine contained albumin (8 parts Esbach). Digital separation of the membranes from the cervix resulted in the delivery, some 32 hours after admission, of a live male child weighing 5½ lbs. (vertex) and a live female child weighing 5 lb. 10 ozs. (breech). Labour was completed without mishap and the patient improved thereafter. Oedema and albuminuria gradually disappeared over five days. The patient was dismissed home, with her two children, fourteen days after delivery. The blood pressure was 145/98 mm. of mercury, there was no oedema, and the urine was free from albumin when the patient went home. The serum protein analysis was carried out when the patient was admitted to hospital.

Patient No.4.

This thirty-seven years old woman was 32 weeks advanced in her 5th pregnancy when admitted to hospital. She was anaemic and had a blood pressure of 150/95 mm. of mercury. The urine contained albumin (2 parts Esbach) and she was oedematous. On investigation renal function proved to be normal. The patient's previous deliveries had been conducted in her own home and had been complicated by a degree of hypertension. Examination showed that the patient had a twin pregnancy. A course of rest and treatment for anaemia was instituted. The patient's haemoglobin rose to a normal level quickly and when this occurred a specimen of serum was submitted for protein analysis. At this point the only abnormality present was a raised blood pressure (145/95 mm. of mercury). Seven weeks after admission the patient was delivered vaginally of healthy male twins weighing 5 lb. 11 ozs. and 5 lb. 14 ozs. On dismissal from hospital, with her children, four weeks after delivery the patient felt well and, apart from a blood pressure reading of 145/100 mm. of mercury, presented no abnormality. Eight weeks later the blood pressure reading was 145/105 mm. of mercury, there was no oedema and the

urine was free from albumin.

Patient No.5.

This girl of 25 years of age was admitted to hospital nine days before the expected date of confinement in her first pregnancy. Her blood pressure was 150/90 mm. of mercury, she was moderately oedematous and her urine contained albumin (1 part Esbach). A single live child was present with the vertex engaged in an adequately large pelvis. Serum protein analysis was performed and, since the blood pressure was unchanged twenty-four hours later, labour was induced surgically. Spontaneous delivery of a normal, live, female child (6 lb. 10 ozs.) took place after a labour lasting 11 hours 45 minutes. The blood pressure, which remained at a level above 140/90 mm. of mercury for 13 days, was 110/70 on dismissal twenty-two days after delivery. The mother and her child were both well when dismissed from hospital.

Patient No.6.

This patient was admitted to hospital in the last weeks of her first pregnancy. She was 28 years old and she had felt well throughout her pregnancy. The blood pressure was 160/105 mm. of mercury, she had slight oedema and the urine contained albumin (2 parts Esbach). The serum was analysed at this point. A single child presenting by the vertex was palpated on abdominal examination. Labour was induced surgically and, after 5 hours, delivery was effected by lower-segment caesarean section. Operative delivery was performed since serious foetal distress developed when the cervix was only three fingers dilated. A mature, living, female child of six pounds weight was delivered. The patient's condition was satisfactory following operation and her blood pressure reading was 120/70 mm. of mercury when she went home with her child two weeks after operation. There was no oedema and no albuminuria when the patient was dismissed from hospital.

Patient No.7.

A primigravida woman of 23 years of age was admitted to hospital when 38 weeks pregnant. On admission the blood pressure was 155/105 mm. of mercury, the urine contained albumin (2 g./1000 ml.) and oedema (++) was present. A single, live, child was found on examination with the vertex engaged in the pelvis. The serum was analysed electrophoretically following admission of the patient. Conservative treatment was adopted and the patient's condition improved slightly. One week after admission, following surgical induction and a labour of 26 hours, spontaneous delivery of a live male child weighing 7 lb. took place. Inthe puerperium albuminuria and oedema disappeared quickly but when the patient was dismissed with her child on her tenth post-partum day the blood pressure was still slightly raised - 130/80 mm. of mercury.

Patient No. 8.

On admission to hospital, when 38 weeks pregnant in her second pregnancy, this young woman had a blood pressure of 170/110, oedema (++++) and her urine contained albumin (6 g./1000 ml.). Twin pregnancy was diagnosed on abdominal palpation. A serum protein analysis was carried out and, next day, labour started spontaneously. After a short labour, live twin girls (5 lb. and 5¼ lb.) were delivered spontaneously. The patient's signs and symptoms of pre-eclampsia gradually disappeared in the early puerperium and, fourteen days after delivery, she went home with her children. On dismissal the urine was free from albumin, there was no oedema, and the blood pressure was 130/90 mm. of mercury.

Patient No.9.

This young woman was admitted to hospital two weeks before her first pregnancy was at term. Aged 25, she had never been seriously ill nor had she had any operations. On admission the blood pressure was 150/95 mm. of mercury, the urine contained albumin (1 g./1000 ml.) and she was oedematous (++). On abdominal examination a large baby could be palpated with the vertex engaged. The foetal heart was clearly audible. A sample of blood was analysed for protein content. Surgical induction of labour was performed and a live, male, child of 8 lb. weight was delivered spontaneously after a labour of 29 hours duration. Albuminuria disappeared within 5 days and oedema within one week of delivery. When the patient went home with her child, on the tenth day after delivery, her blood pressure was 125/80 mm. of mercury.

Patient No.10.

A primigravida woman, 25 years old, who became oedematous (+++) and hypertensive when 37 weeks pregnant was admitted to hospital with a urine albumin content of 8 g./1000 ml. The blood pressure on admission was 170/120 and a single live child was palpated with the vertex free over the pelvic inlet. A specimen of blood was obtained for serum protein analysis. Labour was induced surgically two days later when the blood pressure rose to 175/130 mm. of mercury and the urine contained more protein (10 g./ 1000 ml.). Severe headache, visual disturbance and sudden acute foetal distress occurred when labour had been in progress for 5 hours (cervix 3 fingers dilated). Lower segment caesarean section was performed and a live male child weighing 4 lb. 14 ozs. was delivered. The patient's condition rapidly improved following operation. Two weeks following delivery, the mother and her infant were dismissed to the care of their family doctor. On dismissal from hospital the patient's blood pressure was 145/95 mm. of mercury, her urine was free from albumin and she had no oedema. This patient was seen eight weeks after dismissal when her blood pressure was

140/85 mm. of mercury, there was no oedema and the urine was protein free.