MATERIAL & METHODS

MATERIAL

The present study on 'Toxaemias of Pregnancy' was carried out in Eden Hospital, Medical College, Calcutta, during July 1973 to December 1977. Since a significant number of cases were admitted with the fully manifested form of gravest toxaemia i.e. eclampsia, the studies were carried out more on these cases to get better and conclusive results. An attempt was made to evaluate all the cases of eclampsia admitted in the said period along with some cases of non-convulsive toxaemias, non-toxemic hypertension with pregnancy, pregnancy with recent history of epileptic fits for comparative study. Altogether 355 cases (Eclampsia-294; Preeclampsia-36; Hypertension with pregnancy-10; Accidental haemorrhage-10 and Epilepsy with pregnancy-5) were studied. Cases in different stages of pregnancy, labour and puerperium matched to age and socio-economic conditions were also investigated as controls.

CRITERIA FOR SELECTION OF CASES

I. CONTROL GROUP

Normal Pregnancy - First trimester cases were taken from the outpatient abortion clinic (with no past history of major illness) after excluding other systemic diseases. The cases with pregnancy duration of 8-10 weeks were preferred to avoid diagnostic error.

Second trimester cases were selected from those individuals who were waiting for indoor medical termination of pregnancy in between 16-20 weeks of gestation.
Third trimester cases were selected from obstetric ward from the normotensive patients who were admitted for elective caesarean section at 37-38 weeks of gestation.

Twenty (20) cases in each of the above groups were taken at random.

Labour and Puerperium

Ten normal cases in labour were studied in 1st, 2nd and 3rd stage. The same individuals were selected for studies in the puerperium within 48 hours of delivery.

II) STUDY GROUPS

A) ECLAMPSIA - A pregnant mother without any systemic disease in the later half of pregnancy or within 48 hours of last childbirth with any two features of the well known triad of hypertension, oedema, proteinuria alongwith convulsions, was diagnosed as a case of eclampsia. Hypertension (mean arterial pressure 105 or more) though probably the most important factor could not be always kept a criteria for diagnosing eclampsia, as many cases came in a state of shock and hypotension.

While diagnosing eclampsia, other common causes of convulsions such as meningitis, encephalitis, epilepsy and space occupying lesions of brain were always excluded with the help of expert physicians.

These eclampsias were classified conventionally as 'Antepartum' (192 cases), 'Intrapartum' (37 cases) and 'Postpartum' eclampsia (65 cases). Antepartum eclampsia was labelled when the patients came with fits having no sign of labour pains or in a very early stage of labour. Classification of Intrapar-
tum eclampsia posed a little problem as the relatives could not always give a proper history regarding the onset of fits and labour pain. However for uniformity of the study the 'convulsion-delivery interval' was carefully noted in each case and the patients, who delivered within 6-8 hours or earlier from the beginning of convulsion were put into intrapartum group. Similarly for postpartum group, 48 hours time limit following the last child birth were maintained. While most of the cases were admitted as emergency, a few patients developed intrapartum and postpartum eclampsia in hospital.

B) PRE ECLAMPSIA - The pre-eclampsia cases were selected from pregnant women in their later half of gestation at random basis with any one of the following criteria :-

i) A systolic blood pressure level of above 160 m.m. of Hg. or a diastolic pressure level above 110 m.m. of Hg. on at least two occasions while the patient was on bed rest.

ii) Gross proteinuria (++ to ++++).

iii) Cerebral or visual disturbances.

iv) Oliguria (400 ml. or less of urine output in 24 hours).

Usually these patients had associated severe oedema also.

These patients were either admitted from regular antenatal clinic or as emergency cases.

C) PREGNANCY WITH HYPERTENSION - 10 cases were selected at random, when they were admitted routinely or before at 37th weeks of pregnancy with following criteria (Pritchard & Macdonal 1976).
i) A well authenticated history of elevated blood pressure (systolic 140 mm. or above and diastolic 90 mm. or above) before the present pregnancy; ii) the discovery of such hypertension prior to the twentieth week of gestation.

These patients were selected after excluding the following feature of superimposed toxaemia viz. (1) a significant aggravation of the hypertension (rise of 30 mm. systolic and 15 mm. systolic), (2) sustained gross proteinuria (+++ to ++++), (3) significant oedema.

D) ACCIDENTAL HAEMORRHAGE - 10 cases were selected at random in this group in third trimester when features of toxaemia of pregnancy were apparently absent. The following were the criteria for presumptive diagnosis:

(i) Some vaginal bleeding, (ii) Hypertonic uterus, (iii) Uterine tenderness. Diagnosis was confirmed later on after the birth of placenta when definite retoplaental clots were found to be present.

E) PREGNANCY WITH EPILEPSY - During the study period, 5 patients were found who initially reported to the hospital with convulsions in the later half of the pregnancy. They were provisionally diagnosed as pregnancy with convulsions. Final diagnosis was however made as 'pregnancy with epilepsy' on the basis of the following:

i) Known history of epileptic attacks during non-pregnant state.

ii) Absence of all features of toxaemia of pregnancy.

iii) Radical improvement with anti-epileptic drug.
METHODS

HISTORY AND CLINICAL EXAMINATION - The detailed history and clinical examination was carried out as per proforma (vide appendix). The following features were specially noted.

BLOOD PRESSURE - As suggested by Page 1972, the blood pressure was expressed as mean arterial pressure (MAP) which was calculated as follows (Burton 1965): \[ \text{MAP} = \frac{\text{Systolic Pressure} + 2}{3} (\text{Diastolic Pressure}). \] Hypertension was considered when the MAP was found as 105 mm. of Hg. or above, or when there was a rise of 20 mm. Hg. of MAP above the basal level (Page 1972).

OEDEMA - Extent of oedema was graded arbitrarily as Mild (+) - pitting oedema over dependent parts eg. ankles in ambulatory and sacral region in a non-ambulatory patient; Moderate (++) - Gross oedema of legs and fingers with heaviness of eyelids. Severe (+++) - Generalised oedema of whole body.

INVESTIGATIONS (GENERAL):

Blood - a) Haemoglobin estimation - (Sahli's method).
         b) Total leucocyte count in Neuber Counting Chamber.
         c) Differential leucocyte count (Leishmann stain).
         d) Erythrocyte Sedimentation rate - by Westergren tube.

Urine - A catheter specimen in the cases with convulsion and a midstream specimen in others were taken used. Physical, chemical (specially for proteinuria) and microscopic examination particularly for casts, pus cells and microorganisms were carried out.

Culture and Sensitivity - done occasionally, when indicated.
Proteinuria was tested qualitatively by the "Boiling test" as a bedside method and graded as follows (Krupp, A.M. et al, 1964).

- No cloudiness.
+ Cloudiness was barely visible.
++ Definite cloudiness, but no granularity and no flocculations.
+++ Granular cloudiness, but no flocculations. Seen from above, the cloud was dense but not opaque (about 0.1% protein).
++++ Dense opaque cloud; clearly flocculated (about 0.2 to 0.3% protein).
+++++ Very thick precipitation, almost a solid, (0.5% or more protein).

I. ASSESSMENT OF METEOROLOGICAL INFLUENCE ON ECLAMPSIA

The data regarding average monthly temperature, both maximum and minimum as well as the average humidity was collected for 3 consecutive years (January 1973 to December 1975) from the record section of Alipore Meteorological Office of Calcutta and computed.

II. ASSESSMENT OF NUTRITIONAL STATUS AND LIVER FUNCTION

i) By Haemoglobin estimation - done in all cases.

ii) Estimation of total Plasma Proteins with Albumin and Globulin (After Reinhold 1958) by Biuret method. These tests were carried out in 86 cases, selected at random (Normal pregnancy third trimester-20, Preeclampsia-26, Hypertension with pregnancy-10 and Eclampsia-30).
III. ASSESSMENT OF RENAL FUNCTIONS

Apart from the routine urine examination as already mentioned the following studies were undertaken.

1) Measurement of total Urinary output in 24 hours in all cases.
2) Specific Gravity measurement in all cases.
3) Concentration – dilution tests (only in a few selected cases of Preeclampsia and of Pregnancy with essential hypertension when necessary) as follows (Krupp, A.M. et al, 1964):

Principle :- Urine specific gravity is a measure of capacity of the tubules to reabsorb water from glomerular filtrate, thus concentrating the urine.

Concentration Test or the Specific Gravity Test (Fishberg 1954):
The subject was not allowed to take any fluid for 24 hours after the morning meal. The Urine samples during last 12 hours of the period were collected and specific gravity of each measured. A specific gravity of 1025 or more was considered normal.

In the present study for convenience of the patients, a little modification was made that they were not allowed any food and drinks after 6 p.m., and specific gravity was measured in the morning samples.
Dilution Test

a) After the evening 6 P.M. meal, nothing by mouth was allowed.
b) After bladder evacuation at 6 A.M., patient was given 1000 to 1500 ml. water within 45 minutes.
c) She was allowed to void every half hour for four hours and all specimens preserved.

In normal Kidney functions, specific gravity was 1003 or less in at least one specimen and total quantity of the voided specimens was over 80% of the intake (i.e. 800 - 1200 ml.).

4) Biochemical Study

a) Estimation of Blood Urea - Diacetyl Monoxime method (Hatelson 1957).
b) Estimation of Serum Creatine - After Brod and Sirota (1948). The above biochemical studies were undertaken in 107 patients (Normal pregnancy third trimester-10, Pregnancy with hypertension-10, Preeclampsia-36 and Eclampsia-51) at random.

5) Radiological Investigation

These were undertaken in 25 patients (Eclampsia-15, Preeclampsia-5 and Pregnancy with hypertension-5) selected at random within 4 weeks of recent child birth.

a) Straight film of abdomen and pelvis.
b) Intravenous excretory conventional pyelography: By using intravenous injection of 20 to 40 ml. of Conray 420. The films were taken at 5 minute, 15 minutes, 30 minutes, and 45 minutes interval.
IV. ASSESSMENT OF CENTRAL NERVOUS SYSTEM INVOLVEMENT

Carried out in 65 cases (Eclampsia-30, Preeclampsia-10, Hypertension in pregnancy-10, Pregnancy with Epilepsy-5, Normal pregnancy in 3rd trimester-10) selected at random.

i) Study of Cerebrospinal Fluid:

Lumbar punctures were carried out under aseptic measures within 24 hours of convulsions in cases of eclampsia and epilepsy; in others it was done after hospitalisation, prior to delivery. In all the cases the puncture was made in lying position to avoid any alteration of pressure due to posture. The cases with suspected traumatic puncture were excluded from the study. Following examination were undertaken -

a) Measurement of pressure: Manometric reading was taken by promptly connecting a three-way stopcock with the manometer as soon as the fluid appeared.

b) Examination of Colour of C.S.F. and Cell Count:

The cerebrospinal fluid was collected in 3 clean test tubes and colour was noted. Cell count was done from the last test tube to avoid the presence of traumatic red cells for cell count. Unna's polychrome methylene blue was taken up to the "I" mark in 2 r.b.c. counting Pipette and the Pipette was filled to "101" mark with cerebrospinal fluid. With this white cells were stained blue and red cells yellow.

The count was done in a Neubauer hemocytometer grating.

c) Biochemical Examination: Protein - Turbidimetric method (Varley 1976).

Sugar - Folin Wu method (Varley 1976).
Chloride: By Silver nitrate solution using potassium chromate as indicator (Varley 1976).

ii) X-Ray Skull:

Anteroposterior (AP) & Lateral view for evidence of haemorrhage, abnormal calcification and space occupying lesions.

iii) Electroencephalography (EEG):

EEG recordings were made between 7 to 10 days of cessation of convulsions in epilepsy and eclampsia cases; in others EEG was done prior to delivery and within 48 hours of hospitalisation. The EEG of all cases were recorded with the help of Eight Channel Galileo Electroencephalograph (model E & B). 19 routine electrodes were placed at selected sites over the scalp. Studies were made in different combinations employing international montages and 10-20 system. Responses to eye closing and opening were noted. Hyperventilation were carried out for activation. The abnormalities were classified as slow, fast and paroxysmal as was recommended by Gibbs & Gibbs (1962).

iv) Internal Carotid Angiography:

The aim of this investigation was to demonstrate or exclude cerebral tumour or vascular anomalies and as such was done in 2 cases of eclampsia where there were focal changes in EEG.

Patient was kept in supine position with neck extended; after palpating the pulsation of the carotid artery, local
anaesthesia was given with 5% xylocaine and percutaneous puncture was made into the contralateral carotid artery and the injection of the Dye followed. Dye used was conray 280 (Meglumine lothalamate) containing 280 mg. of iodine per ml. AP & Lateral pictures were taken in supine positions immediately (for arterial phase) after instillation of the dye and within 10 seconds (for venous phase).

V) ASSESSMENT OF COAGULATION STATUS

Following tests were undertaken:

i) Whole blood clotting time.

ii) Plasma fibrinogen level.

iii) Eoglobulin clot lysis time (E.C.L.T.).

As it is well known that blood coagulation parameters change during pregnancy it was thought desirable to establish a normal pregnancy standard of the group of patients studied. With this view plasma fibrinogen and E.C.L.T. was measured in 20 patients in each trimester (selected at random). Similarly 10 individuals (randomly selected) were subjected to these tests in different stages (1st, 2nd and 3rd) of labour and puerperium.

In the study group, cases were selected before the onset of labour and data compared with the normal 3rd trimester value; similarly intrapartum study group was compared with the normal labour values at first stage and post partum study group were compared with the values obtained in the puerperium. Antenatal study group consisted of 124 patients (Epilepsy with pregnancy-5, Hypertension with pregnancy-10, Preeclampsia-35 and Antepartum
Eclampsia-64). Intrapartum study group consisted of 20 patients (Intrapartum eclampsia-10 and Accidental haemorrhage-10) and post partum study group consisted of 10 cases of post partum eclampsia.

Collection of Blood Samples (Varley 1976)

Following precautions were taken to avoid haemolysis:

Absolutely dry syringe and needle was used. The blood was allowed to flow slowly and steadily into the syringe and then expelled into the test tubes slowly after withdrawing the needle. About 8 to 10 ml. of blood was withdrawn and collected in two test tubes one of which was dry for noting the clotting time and the other contained anticoagulant (0.5 to 0.8 c.c. of 3.8% sodium citrate solution). Tests were carried out immediately.

Whole Blood Clotting Time (Method of Lee & White)

This was carried out as an immediate bedside procedure. Time required for the blood (in the dry test tube) to clot firmly was noted (Normal range was taken as 5 to 10 minutes). Similarly whether the clot remained firm or resoluted was also carefully noted.

Estimation of Plasma Fibrinogen (Method based on Ratnoff & Menzie, 1951)

Fibrinogen was measured in 244 samples (Normal pregnancy through different states - 100, Eclampsia-84, Preeclampsia-35,
Pregnancy with hypertension-10, Pregnancy with epilepsy-5, Accidental haemorrhage-10). Initially in about 25 cases, fibrinogen was estimated by Ratnoff and Menzie method using both thrombin, as well as Calcium Chloride. Since no statistically significant difference was found between the results obtained by thrombin and calcium chloride, most of the tests were later carried out by using the calcium chloride instead of thrombin.

Reagents:

1) Stock Solution - 4.5 gms. of Potassium Sodium tartrate was dissolved in 40 ml. of 0.2N NaOH. 1.5 gms. of CuSO4 was added. Stirred well, 0.5 gms. of Potassium iodide was added and the mixture was diluted to 100 ml. with 0.2 N NaOH.

2) Dilution Solution - 5 gms. of Potassium iodide was dissolved in 0.2N NaOH and the volume was made upto 1 litre.

3) Biuret reagent for use - 20 ml. of the Stock Solution was diluted to 100 ml. with the dilution solution.

4) NaCl Solution - 0.154N (0.9%).

5) Standard - 10 mg./ml. fibrinogen solution.

The incubation mixture contained 0.5 ml. of 0.9% sodium chloride, 0.5 ml. of 0.25% calcium chloride and 0.1 ml. of plasma. After mixing well, the incubation mixture was kept at 37°C for 30 minutes. The clot formed was removed with a bent glass rod carefully, washed well in normal saline, and dried on a filter paper. 0.5 ml. of 3% sodium hydroxide was added to the washed clot and kept for an hour at room temperature till the clot dissolved completely. To the above solution 0.5 ml. of
Biuret reagent was added and allowed to stand for 30 minutes at room temperature. The resulting solution was again warmed at 37°C for 5 minutes in an incubator and the absorbance was measured at 560 m\(\mu\) in a Zeiss Spectrophotometer PMII against water. A reagent blank without plasma was set up as a control.

Fibrinogen value was obtained directly from a standard curve, made under identical conditions with authentic fibrinogen.

Euglobulin Clot Lysis Time (Von Kaula, 1963)

Principle - The euglobulin fraction of plasma contains plasminogen activator, plasminogen and fibrinogen. The normally occurring inhibitors of the conversion of plasminogen to plasmin are not in the fraction.

The euglobulin fraction was clotted and the time for clot lysis estimated. This test measured predominantly plasminogen activity (Dacie & Lewis, 1974).

E.C.L.T. was measured in 233 cases altogether (Normal pregnancy - 96, Eclampsia-34, Preeclampsia-28, Pregnancy with hypertension-10, Pregnancy with Epilepsy-5 and Accidental haemorrhage-10). It probably deserves no mention that fibrinogen and E.C.L.T. were measured in the same samples.

Method (Monamy Buckell 1958)

Reagents:
1) 0.05M CaCl\(_2\).
2) 1% Acetic Acid.
3) Borate Sol. (9 gm. NaCl & 1 gm. Sodium Borate made up to 1 litre with distilled water to get pH-9.0.
4) 0.1M Ammonium Oxalate.
Procedure:

The blood was centrifuged at 3000 rpm. for 10 minutes and plasma separated. To 0.5 ml. of plasma, 9 ml. of distilled water was added in a centrifuge tube and the pH was brought to 5.3 by adding 0.1 ml. of 1% acetic acid. The tube was kept for half an hour in a refrigerator at 4°C for the Euglobulin fraction to precipitate and was later centrifuged again for 5 minutes. The supernatent was decanted and the tube was drained by inversion on filter paper. 0.5 ml. of the borate solution was added and the tube was placed in 37°C water bath and stirred gently by a glass rod.

0.5 ml. of the 0.025M CaCl₂ was added to the resulting solution Euglobulin in borate and the time at which the mixture clotted was recorded. The tube was left at 37°C and inspected at intervals and the lysis time determined. When the lysis was near completion, the clot was inspected every 5 minutes.

General Outline of Management

On admission, a thorough general and obstetrical examination was carried out and then pulse, respiration, blood pressure and temperature were noted at four hourly intervals. A detailed intake-output chart, degree of proteinuria and number of convulsions were recorded. The further management was done as was advocated by Prof. Krishnamenon (1965) as follows:

On admission 100 mg. Pethidine and 25 mg. Chlorpromazine
was given intravenously in 20 ml. of 5% glucose solution, followed by intramuscular injection of 25 mg. promethazine and 50 mg. Chlorpromazine. An intravenous drip was started with 15 to 20 percent dextrose solution and 1000 c.c. was infused with 200 mg. of pethidine in first 24 hours. After this initial period of treatment, 25 mg. promethazine and 50 mg. Chlorpromazine were given through intramuscular routes alternately at every four hours according to the blood pressure. Induction of labour by separating the membranes and amniotomy was done and second stage was cut short by application of forceps. Caesarean section was also undertaken whenever necessary (for obstetric indications as well as for uncontrolled fits after 8 to 10 hrs. of conservative treatment). During the 3½ year study period as mentioned earlier, there were 30,922 deliveries and 294 cases of Eclampsia were admitted. A variation of the regime was done in a small number of cases, where convulsions could not be controlled by the above regime.