ASSAY OF PLASMA ANTIDIURETIC HORMONE IN CASES OF TOXAEMIAS OF PREGNANCY

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The aetiology of toxaemias of pregnancy is a very complex matter. There seems general agreement that hormonal influences play a considerable part in their aetiology although there is no agreement regarding the hormone or the endocrine gland involved. Posterior pituitary has been extensively investigated regarding the aetiology of this condition ever since the time of Harvey Cushing (1934) who described the postmortem appearances of nine cases of eclampsia with particular reference to hyperactivation of neurohypophysis. Many other workers since then have implicated posterior pituitary hormones in the genesis of toxaemias. (Ahlstromm, 1935; Kreiger and Kilvington, 1940; Paterson, 1960). The evidence, however, is by no means conclusive and many others have denied the role of this gland (de Wesselow, 1934; Hurwitz and Bullock, 1935; Kreiger, Kilvington and Butler, 1946). Despite the controversy that has existed regarding the presence or absence of posterior pituitary activity in pre-eclamptic toxaemia, it has been known that these patients exhibited a marked sensitivity to posterior pituitary extracts (Dieckman and Michel, 1937; de Valera and Kellar, 1938; Govan and Mukherjee, 1950). Much of the above work is largely qualitative and, barring Paterson (1960), no one has attempted to assay ADH levels in this condition.

The present communication embodies our findings on the assay of ADH levels in plasma of toxaemic women.

Material and Methods

Forty-four cases of toxaemias of pregnancy were collected from the obstetric wards of the Kamla Nehru Hospital, Allahabad. The patients exhibited the criteria of toxaemia according to the classification of the American Committee for Maternal Welfare.

The general physical and obstetric examinations and routine laboratory investigations were done in all cases. In toxaemic patients, examination of fundus oculi was also carried out.

Collection of Blood

On the day after admission, 10 ml. of antecubital blood was collected at 8 a.m. using a heparinised and saline washed syringe and glassware to prevent haemolysis. The blood was centrifuged soon after collection, the plasma carefully separated, sediment washed again with normal saline and centrifuged for 10 minutes, the supernatant collected and mixed to the plasma.

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The plasma was extracted according to the method described by Bisset, Hilton and Poisner (1967).

The assay was performed on alcohol anaesthetised water loaded rats according to the method described by Boura and Dicker (1953). Three point assays were done. The antidiuretic response was measured as the percentage reduction in urine flow during a five minute period beginning 2 minutes after the injection of known ADH or the sample to be tested. Lysine-8-vasopressin was used as the reference. Recovery experiments were performed in the beginning of the series.

In order to confirm that the material which has been assayed is posterior pituitary ADH and not other interfering substances, inactivation by thioglycollate and persistence of response after cyproheptadine was determined. Quantities of ADH in plasma were extrapolated from log dose-response curves.

Observations and Results

The findings of plasma ADH levels in different groups of patients are given in Table I below:

From the above Table it will be seen that detectable amounts i.e. more than three units per ml were not found in the blood taken from the antecubital vein of the normal pregnant women. The above data show that the elevated levels of plasma ADH are statistically significant; the probability being less than 0.001. The representative kymographic records of the different grades of toxaemias are to be seen in figures 1, 2, 3 & 4. Graph 'E' gives a scatter idea of the severity of toxaemia, range of plasma ADH concentration and standard error in the selected cases.

An attempt has also been made to correlate the severity of oedema, blood pressure levels (systolic and diastolic) and ADH titres in toxaemia patients. The comparative figures are to be seen in Table II. A more intimate relationship has been observed between the severity of oedema and plasma ADH concentrations as compared to those of B.P. levels and ADH titres in toxaemia patients in the present series.

Results

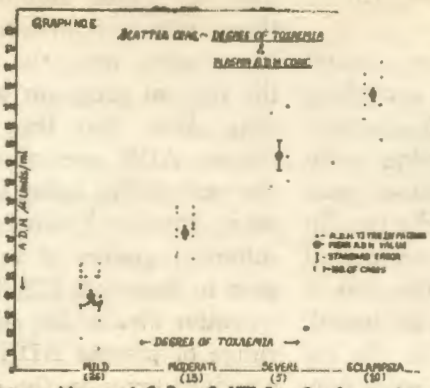
It has not been possible to detect any

TABLE I

The Plasma ADH Levels in Different Groups

Sl. No.	Degree of toxaemia	No. of cases	Percentage of cases	Average & range ADH titre per ml. of plasma ± S.E.	'Z' Standard normal veriate	Probability less
1.	Normal pregnancy	15	—	Not measurable	—	—
2.	Severe toxaemia	5	11.36	118.54 ± 8.125 (91 ± 139.7)	13.5	0.002
3.	Moderate Toxaemia	13	29.34	72.64 ± 3.46 (56-90)	20.3	0.001
4.	Mild toxaemia	26	59.09	39.3 ± 3.34 (11.2-55.9)	15.7	0.001

Fig. 1



Showing scatter and S.D. of ADH levels in various grades of toxaemia

FIGURE - II

Showing antidiuresis with Serum of moderate toxaemia

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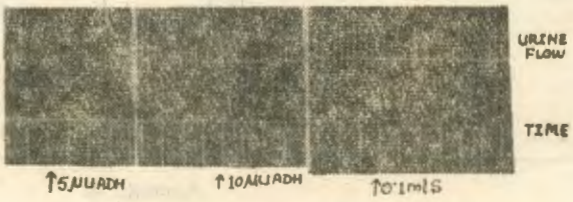
FIGURE - III



Showing antidiuresis with Serum of severe toxaemia

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Showing antidiuresis with Serum of mild toxaemia

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ADH like activity in the plasma of normal pregnant women. Plasma of all cases of pre-eclamptic toxaemia showed ADH like activity which is abolished by incubation with thioglycollate and not abolished by pre-treatment with cyproheptadine. No attempt could be made to establish the chemical identity of the substance responsible for antidiuretic activity in the maternal blood. On the basis of thioglycollate inactivation and inability of 5-HT antagonist to block this effect, it is safe to assume that this substance is akin to or indentical with posterior pituitary antidiuretic hormone.

Discussion

An attempt has been made in the present study to assay plasma levels of ADH in patients of toxaemia of pregnancy. These assays were performed in concentrated plasma of patients using alcohol anaesthetized water loaded rats as the assay preparation. This is one of the best preparations available for assaying small quantities of ADH. It has been possible to obtain a sensitivity paralleling that achieved by Dettlebach (1958). With sensitivity of our method being 3 micro units per 100 G of rat, it has not been possible to detect any ADH in plasma of normal women.

These findings are in consonance with those of Gupta *et al,* (1967) who have reported 0.41 micro units ± 0.06 units per ml in males and 0.30 ± 0.07 micro units in pregnant women within the 7th month of normal pregnancy. Most other workers have been unable to measure ADH in antecubital blood of normal pregnant women.

It has been possible to obtain measurable quantities of ADH from patients of toxaemias of pregnancy. We are not aware of any previous work where quantitative estimation of ADH has been attempted in cases of toxaemias of pregnancy. In this context it is pertinent to consider the comparatively high values obtained in the present series. There is no obvious reason which could account for this, except the fact that most of these assays were carried out during the months of summer. Seasonal variation altering the assays of ADH has been reported earlier. Heller *et al,* (1957), Chaudhury *et al,* (1961) have also reported high values in normal Indian subjects and the reason advanced by them for such high figures is the high temperature which prevailed when the assays were done.

There is a direct correlation between the severity of toxaemia and the levels of ADH in plasma have been obtained in this series in cases of mild toxaemia and highest i.e., 91 to 139.7 micro units per ml of plasma in cases of severe toxaemia. On the basis of the observations made in the present series, a direct and close relationship has been found between the severity of oedema and plasma ADH levels. This is evident from Table II and graph 'E'. Although this series of study is small to give any conclusive data, yet it seems to be reasonable to presume that increased concentration of plasma ADH are responsible for salt and water retention which leads to oedema, the first to appear among three cardinal symptoms of toxaemias of pregnancy.

The major scientific evidence has recently been presented by Paterson (1960). He investigated a large number of patients of toxaemia by using modern methods and succeeded in demonstrating qualitatively the presence of ADH in blood of toxaemic women. He considered the substance in such blood to be identical with posterior pituitary antidiuretic hormone. For establishing this identity,

TABLE II

Correlation of Severity of Oedema, Blood Pressure Levels (Systolic and Diastolic) and Plasma ADH Titres of Toxaemia Cases

Oedema			Blood Pressure mm. Hg.						ADH Micro units ml. Plasma Average ± S.E.		
			Systolic			Diastolic					
Grades	No. of cases	Percentage of cases	Grades	No. of cases	Percentage of cases	Grades	No. of cases	Percentage of cases	Grades	No. of cases	Percentage of cases
Mild	24	54.54	Mild 130–150	28	63.6	Mild 90.100	30	68.18	Mild 39.3 ± 3.34	26	59.09
Moderate	15	34.01	Moderate 150–160	8	18.18	Moderate 100.110	6	13.63	Moderate 72.64 ± 3.46	13	29.54
Severe	5	11.3	Severe above 160	8	18.18	Severe above 110	8	18.18	Severe 118.54 ± 8.125	5	11.36

reliance has been placed by him on chromatography and establishment of antidiuresis on rats. Unfortunately, Paterson has not presented quantitative data.

The findings of the present series indicate that an antidiuretic substance circulates in the maternal blood in toxaemic women and this substance gives biological responses parallel with those of known vasopressin. There is no doubt that several other substances circulating in the maternal blood could also produce antidiuretic response; for example, Kinins will inhibit diuresis but in very large doses (Bisset and Lewis, 1962). Sympathomimetic amines also produce an antidiuresis, but this response is of much shorter duration than that produced by ADH and hence the two responses can be easily distinguished (Oconnor and Verney, 1946). 5-HT also produces antidiuretic response. This aspect has been investigated by Judith (1963) who has investigated the possible role of 5-HT in toxaemias of pregnancy. However, the persistence of response after cyproheptadine, a peripheral 5-HT blocker, rules out this substance being the antidiuretic agent in the present series of cases. Finally inactivation by thiolycollate lends further support to the contention that the substance is antidiuretic hormone. It would have been much more convincing if it were possible to run chromatograms of serum against known arginine-8-vasopressin. This, however, could not be done because arginine-8-vasopressin could not be made available inspite of efforts.

On the basis of the present study and considering the biological responses which have been obtained, it is safe to assume that the antidiuretic substance present in the maternal blood is likely to be akin to posterior pituitary ADH. The next question which has to be decided concerns the

origin of this substance. This substance could either be of maternal or may be produced by the products of conception. Hunter and Howard (1961) speculated that the antidiuretic substance may be of placental origin. They named the substance "Hysterotonin". No other worker has however, substantiated the findings of Hunter and Howard and unless more convincing evidence to the contrary is presented, it would be safe to presume that the antidiuretic substance is maternal in origin.

Summary

Fifteen cases of normal pregnancy and forty-four cases of various degrees of toxaemia of pregnancy formed the basis of this study. Plasma ADH levels were assayed in these cases. No ADH could be demonstrated in the antecubital vein blood of normal pregnant women. ADH levels in mild cases of toxaemia 39.3 ± 3.34 units, 72.64 ± 3.46 units in moderate cases and 118.54 ± 8.125 units in severe cases of toxaemia. These values are statistically significant.

A correlation has been demonstrated between the levels of ADH and severity of oedema but not between the degree of hypertension and ADH levels.

The significant change demonstrated is that the more severe the toxaemia the higher are the levels of plasma ADH.

Acknowledgement

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