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PLACENTAL SEROTONIN IN ECLAMPSIA & TOXAEMIAS OF PREGNANCY

by

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Extensive research on the placenta in the past and the present has suggested that the state of pregnancy is very largely the result of hormonal factors in which the placenta holds a key position. It is generally conceded that the placenta is the source of oestrogens and progesterone. In toxaemias of pregnancy the premature aging of the syncytiotrophoblast causes marked reduction in the excretion of the oestrogens and pregnandiol. The presence of ACTH substances in the monkey has been confirmed by several experiments and it is believed that the site of production is in the chorionic villi. The collected data, however, serve to high-light many problems which are still unsolved.

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In 1949, Rapport was able to isolate a crystalline substance from the serum, serotonin, with a suggested structure of 5-hydroxytryptamine sulphate with the excretion product (5 HIAA) 5-hydroxyindole acetic acid in the urine. Since then more than a decade of research has been conducted on the role of serotonin in normal and abnormal conditions of the body but the exact role of this hormone is still not clear. Paasonen (1957) reported that the action of serotonin on the human placenta was normally purely constrictive followed occasionally by secondary dilatation. Sjoerdsma (1959) refers to serotonin as a smooth muscle stimulant since isolated arterial rings, uteri and isolated intestinal strips all contracted on exposure to it. Kuriaki and Inoue (1955) reported that serotonin seems to act on the smooth but not on the striated muscle. The role

played by an affected vascular system in the aetiology of toxaemias of pregnancy is well established. Browne (1944 a) suggested that the vascular process is due to the presence of a large amount of vasopressor substance in the circulation of an eclamptic or pre-eclamptic patient.

Edwards (1951) claimed that pre-eclampsia is a pathological reaction of the body to water retention under the influence of the hormonal secretion of the placenta. Erspamer and Ottolenghi (1950) reported that serotonin when given 20 μg./kgm. body weight in rats resulted in anti-diuresis. They further reported that the primary function of serotonin was concerned with the physiological regulation by reducing glomerular filtration through increased tone of the afferent vessels. Browne and Veall (1953) while studying the clearance of Na^{24} in the choriodecidual space found that the clearance was reduced to 1/3 of the normal in toxaemic pregnancy. Hollander et al (1956) showed that intravenous injection of serotonin in man resulted in significant retention of sodium and water. From the above it is fairly obvious that the symptom complex of toxaemia of pregnancy and the properties of serotonin have much in common. Further, in spite of intensive research the aetiology of toxaemias of pregnancy is still a mystery. One of us working on the role of serotonin in eclampsia and severe pre-eclamptic toxaemias of pregnancy found a marked reduction in the urinary 5 HIAA during the acute stage of the disease. The cause of this interesting finding is not clear. Proteinuria per se does not interfere with the determination of 5 HIAA in the urine but albuminuric renal disease may itself lead to diminished excretion of 5 HIAA. On the other hand the low excretion of 5 HIAA in toxaemic cases may be due to poor monoamine oxidase activity of the placenta or kidney lesions causing retention of serotonin in the blood. Estimation of blood serotonin by bioassay in a few cases with eclampsia revealed raised values in the blood, i.e. findings similar to Krupp and Krupp (1960). Blood and urinary levels could however not be correlated individually with the clinical features. It was then decided to estimate the content of the placental serotonin and to try to correlate it with the clinical condition of the patient.

Material

Total number of 78 placentas were taken for study and they were divided into different groups according to the clinical features of the case in order to correlate the serotonin content. The placentas were thus divided into groups A, B, C, D, E and F. Pieces of fresh placenta were taken from 5 different places and acetone extracts prepared. Group A consists essentially of 34 normal cases. Group B represents 5 cases of essential hypertension, i.e. B.P. above 130/90 mm. of Hg. Groups C and D were composed of 14 and 13 cases respectively suffering from pre-eclamptic toxaemia and were divided into mild (C) and severe (D). In the mild category the B.P. was 160/100 mm. of Hg. or below with not more than one plus proteinuria in the urine. Group E consisted of 7 patients with antepartum eclampsia. Group F was made up of 5 patients

with accidental haemorrhage without any marked pre-eclamptic toxaemia.

Method

A. I. *Preparations of Acetone Extracts of Placental Tissue.* The tissue was collected from the fresh placenta in five different places and mopped with filter paper to remove blood and the fluid portion till no colour stain was visible and the tissue was completely dry. One gm. of such tissue was chopped into small pieces and mixed with 10 c.c. of cold acetone homogenised, kept for a few minutes and the upper liquid part decanted off. The residue was again mixed with 10 c.c. of cold acetone and treated as above. The upper liquid portion was then decanted off. The tissue was again mixed with 10 c.c. of cold acetone and the mixture kept below 0°C for 24 to 72 hours. Before the bioassay the liquid part was decanted off. The whole collection of acetone extracts was then kept open in a petri dish, evaporated and the residue obtained was mixed well with 1 to 2 c.c. of Locke's solution. The solution thus obtained was bioassayed against the prepared standard solutions of serotonin creatinine sulphate. The bioassay was carried out on virgin rat's oestrus uterine horn suspended in 2 c.c. tissue bath (modified Erspamer method).

II. *Selection of Rat.* Virgin white rat's vaginal fluid was examined. If non-nucleated cornified cells were found in large number, without any R.B.C. or W.B.C., it was taken that the rat was in oestrus phase. If the specimens were ready for the bioassay and if the rat in oestrus phase was not found, subcutaneous injection of stilboestrol (0.1 mgm. per Kgm. of body weight) in Arachus oil was given to the rat 24 hours prior to the bioassay to produce an artificial oestrus phase. The selected rat was killed by pithing and the uterine horns were separated with great care. The rats selected were between 120-160 gms.

III. *Preparation of Horn and Its Suspension in Bath.* The uterine horns were at once kept in Locke's solution and were freed from all the neighbouring tissues carefully. About 2 cms. of the middle part of one of the horns was suspended in 2 c.c. electrostatic tissue-bath. Temperature of the bath was kept at 29°C. to 30°C. and the oxygen flow was kept constant. The lever with about 1 gm. tension was fixed and the magnification was about 10 (Fig. 1).

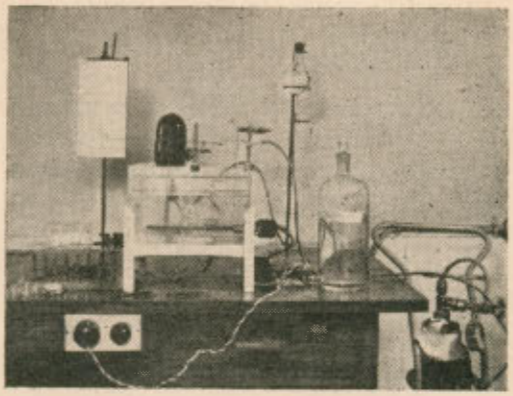

Fig. 1
Bioassay apparatus.

IV. *Bioassay of Serotonin.* After the spontaneous contractions of the suspended uterine horn were over, series of increasing doses of standard

solutions of known concentrations were added and the contractions within one minute recorded. Once the rhythmic contractions were established, the acetone-extract-residue mixed with 1 to 2 c.c. of Locke's solution was added and the contractions within one minute noted. The bath between the doses was changed thrice and the interval given was 3 minutes. If spontaneous contractions appeared, baths were repeated and longer intervals were given. Sometimes rest was given up to 15 minutes for complete relaxation.

V. *Standardisation of Technique.* 1 gm. of the dry tissue was mixed with 10 c.c. of N/10 HCl to see if any blood was left in the tissue but the added HCl remained almost colourless showing absence of acid haematin.

Dose-response curve was drawn to find out the sensitivity of the preparation for bioassay.

B. Specificity of Method of Serotonin Estimation.

I. Erspamer (1954) has shown that serotonin can be extracted quantitatively from tissues even with 95% acetone without substance P. Substance P is insoluble in acetone. The acetone used for these experiments was analar (B.D.H.).

II. The action of acetylcholine is prevented by the atropine 10^{-6} in the bath.

III. The extracts presumably contain salts and also adenosin compounds but they are insoluble in acetone.

IV. Progesterone oestrogen, oestradiol and cholesterol are soluble in acetone but insoluble or almost insoluble in water. Hence these hormones will not be soluble in the Locke's solution.

V. The amount of nor-adrenaline and adrenaline in most organs is correlated with the extent of their adrenergic innervation (Von Euler 1955) and the nerve-free placenta has none at all.

Results

Table I represents the placental serotonin in primiparas and multiparas (Group A). The maximum in primiparas is 49.03 ngm./gm., while the minimum is 28 ngm./gm. of dried placental tissue. In multiparas the maximum reading is 55.59 ngm./gm., while the minimum is only 10 ngm./gm. showing a very much wider variation compared to the primiparas. The significance of this is, however, not clear because the average in primiparas is higher, i.e. 34.13 ngm./gm., compared with the average of 33.73 ngm./gm. in multiparas. This difference is however not significant.

Table II shows the placental serotonin in five cases of essential hypertension (group B). The average of 51.67 ngm./gm. is much higher than that found in normal cases of group A.

Table III or Group C is composed of 14 patients of mild pre-eclamptic toxaemia. These were composed of 5 primiparas and 9 multiparas. The average placental serotonin again was found to rise in both, the average being 55.37 ngm./gm. in primiparas and 56.91 ngm./gm. in multiparas. The maximum reading in

TABLE I

	No.	Age in years	Placental serotonin in ngm./gm.		No.	Age in years	Placental serotonin in ngm./gm.
Primi	1	18	49.03	Multi.	1	29	10.00
	2	18	46.00		2	32	32.14
	3	17	29.57		3	25	40.53
	4	19	31.47		4	24	50.00
	5	21	30.01		5	25	28.07
	6	28	37.22		6	32	25.43
	7	22	31.22		7	32	37.10
	8	25	30.00		8	21	37.10
	9	23	32.23		9	24	31.47
	10	21	28.00		10	23	34.64
	11	20	28.28		11	27	37.22
	12	19	36.56		12	30	38.13
Average		20.9	34.13		13	22	27.29
					14	30	31.22
					15	35	35.38
					16	27	20.00
					17	30	28 80
					18	26	25.08
					19	32	40.00
					20	29	33.39
					21	33	55.59
					22	35	43.50
				Average		28.3	33.73

Total average 33.93 ngm./gm.

TABLE II

	No.	Age in years	Placental serotonin in ngm./gm.
Primi	1	21	41.02
	2	21	53.14
Average		21	47.08
Multi	1	25	49.30
	2	30	64.93
	3	22	50.00
Average		25.6	54.74

Total average 51.67 ngm./gm.

this group is 64.48 ngm./gm. while the minimum is 49.03 ngm./gm., but it is apparent that the variation in the readings is fairly small.

TABLE III

	No.	Age in years	Placental serotonin in ngm./gm.
Primi	1	25	53.29
	2	26	52.42
	3	22	53.14
	4	28	61.49
	5	25	56.52
Average		25.2	55.37
Multi	1	28	50.00
	2	26	60.00
	3	22	60.00
	4	21	64.48
	5	30.0	63.10
	6	23	54.19
	7	21	49.03
	8	20	50.43
	9	22	61.00
Average		23.6	56.91

Total average 56.14 ngm./gm.

Table IV represents the placental serotonin in 13 cases of severe pre-eclamptic toxaemia (group D). This group is composed of 7 primiparas and 6 multiparas. There is wide variation in the primiparas, i.e. from 27.21 ngm./gm. to 67.06 ngm./gm., the average being only 45.63 ngm./gm. On the other hand, the average reading in the multiparas is very much higher, i.e. 72.24 ngm./gm. The range of variation is also much less as seen from the readings.

TABLE IV

	No.	Age in years	Placental serotonin in ngm./gm.
Primi	1	25	31.75
	2	26	60.00
	3	18	40.00
	4	25	67.06
	5	19	50.43
	6	17	27.21
	7	21	48.99
Average		21.5	45.63
Multi	1	25	81.06
	2	27	81.76
	3	35	70.65
	4	32	61.29
	5	21	73.88
	6	31	64.80
Average		28.23	72.24
Total average			58.93 ngm./gm.

Table V represents 7 cases of eclampsia (group E) with 70.47 ngm./gm. of dried placental tissue as the average of the entire group. The difference in the readings in the primiparas and the multiparas is not statistically significant. It will be further observed, that some of the readings in group D are above all these found in group E.

TABLE V

	No.	Age in years	Placental serotonin in ngm./gm.
Primi	1	21	66.49
	2	20	65.03
	3	17	76.14
Average		19.3	69.22
Multi	1	35	75.80
	2	32	79.38
	3	22	70.26
	4	22	60.21
Average		27.7	71.41
Total average			70.47 ngm./gm.

Table VI represents 5 cases of accidental haemorrahage where the average reading shows a marked fall and is comparable to that found in normal cases. There were no cases of accidental haemorrhage in this group where there was any marked pre-eclamptic toxaemia.

TABLE VI

	No.	Age in years	Placental serotonin in ngm./gm.
Primi	1	24	20.00
	2	20	48.48
Average		22	24.24
Multi	1	31	52.42
	2	27	20.00
	3	21	28.28
Average		26.3	33.56
Total average			29.83 ngm./gm.

Table VII represents the average placental serotonin of the groups A, B, C, D, E and F with the standard diviation of ± 6.80, ± 8.28, ± 4.86, ± 5.68, ± 7.18, and ± 4.2 respectively. If group A is taken as 100 then the placental serotonin of group B, C, D, E and F works out as 152.2%, 165.4%, 173.6%, 207.6%, and 71.9% respectively. The above rise in the readings is statistically significant in the groups B, C, D and E.

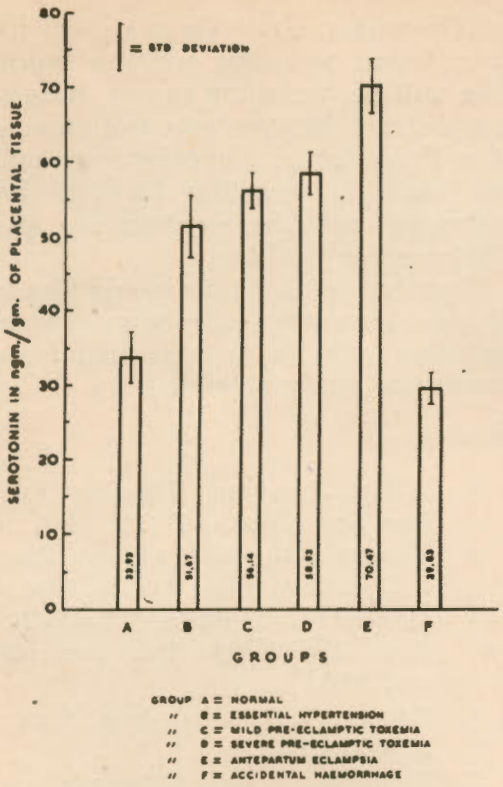

TABLE VII

Discussion and Comments

The foregoing tables and the serotonin concentrations in the placenta of the different groups lead to many interesting speculations. Whereas the concentrations in normal patients and those with accidental haemorrhage are fairly similar 33.93 ngm./gm. and 29.83 ngm./gm. respectively, the patients with severe pre-eclamptic toxamias and eclampsia present markedly raised readings, i.e. 58.93 ngm./gm. and 70.47 ngm./gm. respectively. These findings definitely suggest a correlation between the placental serotonin and the acute toxaemic process. The above data are further strengthened by the consistent finding of increased levels of serotonin in the blood and extremely poor excretion of 5-hydroxyindole acetic acid in the urine in the acute phase in those cases (unpublished work done by one of us). A study of the literature, however, has not revealed any such work on the placenta. Krupp and Krupp (1960) working on serum 5-HT levels have also reported similar findings. We are further in agreement with these workers that we could not establish any correlation of the serotonin concentrations with the clinical severity of the disease individually. We, however, differ from Krupp and Krupp (1960) that there is no cause and effect relationship with serotonin levels and pre-eclampsia. The presence of increased concentrations of serotonin in the placenta, especially in acute toxaemia, may be due either to storage or increased production of the hormone in the placenta. Liver damage may also be a factor in the above process by its inability to metabolise or detoxicate serotonin thus raising the blood and tissue levels. Reduced excretion due to kidney disease may also confuse the picture. The persistent raised levels of placental serotonin in pre-eclampsia and eclampsia did not appear to have any correlation with the administration of any particular drug like reserpine. In group D the high concentrations found in the multiparous group is also significant though no correlation could be obtained on studying the age of the patients. From the above it is very interesting to speculate that serotonin appears to have some aetiologic pressor effect in pre-eclamptic toxaemias and eclampsia. The exact cause and effect relationship can

however only be elucidated with further research.

Summary

(1) The placental serotonin in normal cases and in patients suffering from essential hypertension, pre-eclamptic toxaemias, accidental haemorrhage and eclampsia has been estimated by bioassay.

(2) Method (modified Erspamer's method), materials and technique are given in detail. The similarity between the properties of serotonin and the symptom-complex of toxaemias of pregnancy is discussed.

(3) Significant rise in placental serotonin has been obtained, particularly in eclampsia and severe pre-eclamptic toxaemia of pregnancy. The raised levels are higher in multiparas. No individual correlation between the raised levels and clinical severity of the disease could be established.

(4) The significance of raised placental serotonin has been discussed in eclampsia and pre-eclamptic toxaemia.

(5) There is a probability that serotonin may have some aetiologic pressor effect in toxaemias of pregnancy.

(6) Levels of placental serotonin in accidental haemorrhage were lower than those found even in cases of normal labour. This is another significant factor showing that the pathology in accidental haemorrhage may be quite different to that found in toxaemias of pregnancy, contrary to the popular belief.

Acknowledgment

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Last but not least we would like to thank many others too numerous to mention, who with their help have made this study possible.

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