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THE ORIGIN OF HUMAN PLACENTAL OXYTOCINASE

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SUMMARY

1. Placentas from normal women and from those with pre-eclamptic toxaemia, taken immediately after labour, were examined for oxytocinase activity and analysed morphometrically.

2. Both enzyme activity and volume of syncytiotrophoblast were significantly reduced in placentas from patients with toxaemia.

3. There was a positive correlation between the enzyme activity and the proportion of trophoblast in the placenta. When the activity was expressed per unit volume trophoblast, there was very little difference between placentas, whether from normal subjects or from patients with toxaemia.

4. It is concluded that human placental oxytocinase is located in the syncytiotrophoblast.

INTRODUCTION

Homogenates of human placenta contain an enzyme, oxytocinase, which inactivates oxytocin. It has been suggested that this enzyme is located in the placental syncytiotrophoblast, but there is no direct evidence that this is so. We have therefore taken portions of human placenta, from normal subjects and from patients with pre-eclamptic toxaemia, and compared their oxytocinase content with the volume of syncytiotrophoblast present, to see whether there is a correlation between them.

This work has already been briefly reported (Mathur & Walker, 1969).

METHODS

The placentas were collected in ice immediately after delivery. The membranes were trimmed, and the cord was cut near its insertion. The placenta was then washed under running tap water to remove blood clots. The volume was determined by water displacement. Ten to fifteen pieces, each about 0·3 g, were removed from different cotyledons for estimation of enzyme.

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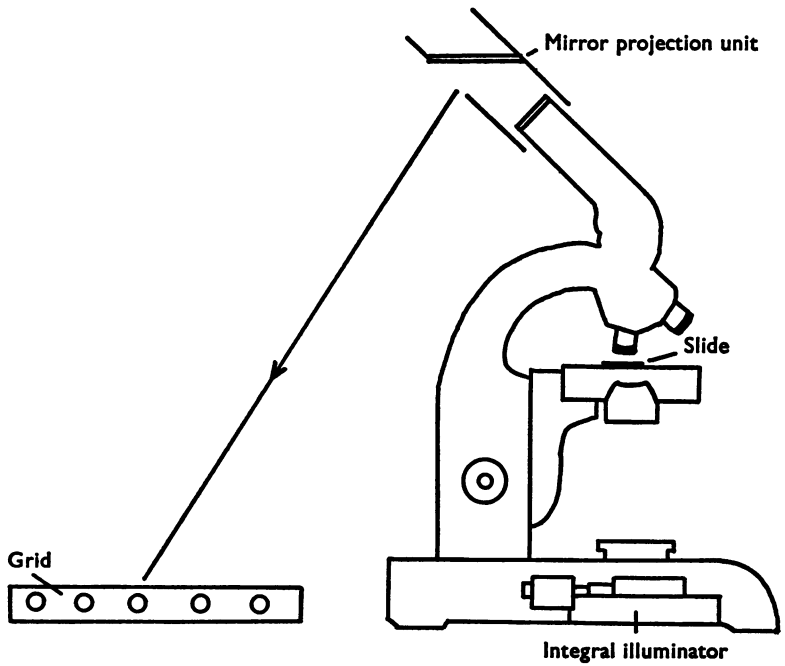
Estimation of oxytocinase. Pieces of placenta were homogenized in twice their volume of 0·066 M phosphate buffer at pH 7·4. The homogenates were centrifuged at 2000 rev/min for 30 min, and the supernatants were incubated at 37° C for various periods with synthetic oxytocin (Syntocinon, Sandoz) in order to measure the rate of inactivation (see Mathur & Walker, 1968). The initial substrate concentration was 0·5 u./ml. and the reaction was studied below 65 % inactivation of substrate, since under these conditions it behaves as a first-order reaction (Melander, 1965). Enzyme activity was expressed as k_1, the first-order velocity constant, where

$$k_1 = \frac{2{\cdot}3}{t} \times \log \frac{a}{a-x},$$

a is the initial oxytocic activity, $(a-x)$ is the activity remaining after time t, and t is the time in min.



Text-fig. 1. Apparatus used for microscopic analysis of sections of placenta.

Oxytocic activity was assayed on the superfused uterus of the rat. The method was essentially that of Gaddum (1953) with the following modifications: (*a*) only the lower two thirds of each uterine horn was used, (*b*) Munsick's solution (Munsick, 1960) was allowed to flow at a rate of about 1 ml./min and (*c*) samples for assay were applied at intervals of 5 min. In seventy-eight assays the index of precision (λ) was 0·056, and the limits of the error ($P = 0{\cdot}05$) were 85 and 115 %. In twenty-four assays, the mean difference between duplicates was less than 6 %. A paper discussing this method is in preparation (V. S. Mathur, unpublished).

Measurement of volume of syncytiotrophoblast. The rest of the placenta was immersed in 4 % formaldehyde (v/v) in saline for at least 10 days, and then cut into strips 1 cm thick. The volume proportions of the various tissues of the placenta were determined

TABLE 1. Morphometric analysis of placentas

			Per cent volume proportions				
			Macroscopic		Microscopic		
Normal		Vol. (ml.)	Parenchyma	Non-parenchyma	Villous tissue (per cent parenchyma)	Intervillous space	Trophoblast (per cent villous tissue)
1		250	79·3	20·7	58·2	41·8	24·0
2		575	80·5	19·5	58·0	42·0	25·5
3		600	81·9	18·1	59·6	40·4	22·3
4		500	82·6	17·4	57·0	43·0	28·0
5		700	76·8	23·2	62·2	37·8	24·0
Mean ± S.E. of mean		525 ± 76	80·2 ± 1·0	19·8 ± 1·0	59·0 ± 0·9	41·0 ± 0·9	24·8 ± 0·9
Toxaemic	B.P. (mm Hg)						
6	160/110	500	79·8	20·2	58·4	41·6	24·8
7	180/120	400	77·4	22·6	60·8	39·2	16·1
8	180/130	450	81·5	18·5	56·4	43·6	14·0
9	160/100	400	85·2	14·8	59·8	40·2	23·1
10	200/140	440	81·8	18·2	57·4	42·6	13·1
11	120/90	500	83·0	17·0	60·8	39·2	22·2
12	160/110	375	79·2	20·8	60·2	39·8	17·0
Mean ± S.E. of mean		438 ± 19	81·1 ± 1·0	18·9 ± 1·0	59·1 ± 0·7	40·9 ± 0·7	18·6 ± 1·8*

* Significantly different from normal ($P = < 0·03$).

morphometrically by the method of Aherne & Dunnill (1966*a*) by which the tissue is viewed against a grid and the proportion of points on the grid falling on a component of the tissue gives the proportion by volume of that component. For macroscopic analysis ten to twelve strips were examined, and 1000 points were counted. For microscopic analysis eight to ten blocks were prepared, and sections 5 μm thick were cut from each block and stained with haematoxylin and eosin. Twenty different fields were examined in each section, and in all 3000 points were counted. Instead of viewing the sections through an integrating eyepiece fitted with a graticule, as described by the original authors, we projected the image of the slide onto a white sheet marked with a grid (Text-fig. 1). A mirror was attached to the monocular eyepiece of the microscope, and with maximal light from the integral illuminator in a dark room it was possible to obtain a clear image of the section on the grid. The proportion of points on the grid falling on the image of a component of the placenta gave the proportion by volume of that component (for a discussion of the method see Aherne & Dunnill, 1966*a*).

TABLE 2. Volume (ml.) of components of placentas

Normal	Parenchyma	Non-parenchyma	Villous tissue	Intervillous space	Trophoblast
1	200	50	116	84	28
2	463	112	268	195	68
3	492	108	295	197	65
4	413	87	235	178	66
5	539	161	334	205	80
Mean ± S.E. of mean	421 ± 59	104 ± 18	250 ± 37	172 ± 22	61 ± 8·7
Toxaemic					
6	400	100	233	167	58
7	310	90	188	122	30
8	366	84	205	161	29
9	341	59	204	137	46
10	360	80	205	155	27
11	415	85	253	162	56
12	296	79	177	119	30
Mean ± S.E. of mean	355 ± 17	82 ± 4·7	209 ± 10	146 ± 8	39 ± 5·1*

* Significantly different from normal ($P = < 0{\cdot}05$).

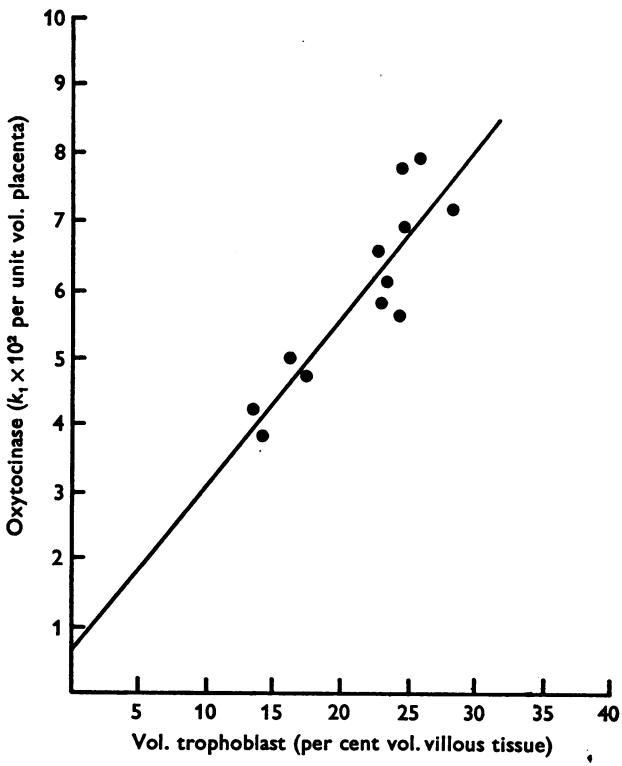
RESULTS

The detailed analysis of twelve placentas, five from normal subjects and seven from patients with toxaemia of pregnancy, is given in Tables 1 and 2. There was no significant difference between the mean placental volumes in the two groups, nor between the mean volume proportions of parenchyma and of villous tissue. The only differences seen were reductions in the mean proportion and volume of syncytiotrophoblast in placentas from patients with toxaemia. Sections from these placentas showed that the villi were congested and smaller than normal, and that the layer of

TABLE 3. Oxytocinase activity of placentas ($k_1 \times 10^2$ per unit volume)

Normal	Whole placenta	Trophoblast
1	7·8	69·4
2	7·9	66·8
3	6·5	59·8
4	7·2	54·7
5	5·6	49·3
Mean ± S.E. of mean	7·0 ± 0·43	60·0 ± 3·8
Toxaemic		
6	6·9	59·3
7	5·0	66·5
8	3·9	60·5
9	6·1	53·0
10	4·2	68·5
11	5·8	51·6
12	4·7	58·8
Mean ± S.E. of mean	5·2 ± 0·41*	59·7 ± 2·3

* Significantly different from normal ($P = < 0{\cdot}02$).



Text-fig. 2. Correlation between oxytocinase activity and volume of trophoblast.

syncytium was thin and contained few nuclei. The foetal capillaries were in close proximity to the syncytium (Pl. 1).

Table 3 gives the oxytocinase activity. There was a highly significant positive correlation between the activity per unit volume placenta and the percent villous tissue occupied by trophoblast given in Table 1 (Text-fig. 2; $r = 0{\cdot}89$, $n = 12$, $P = < 0{\cdot}001$). It will be seen that the oxytocinase activity per unit volume placenta was significantly less in the abnormal group, but that when the enzyme activity was calculated per unit volume trophoblast (Table 3, last column), there was very little difference between one placenta and another.

DISCUSSION

Various lines of evidence point to the syncytiotrophoblast as the site of placental oxytocinase. Semm & Waidl (1962) and James (1966) incubated sections of human placenta with L-cystine-di-β-naphthylamide, and found that the colour which marked the release of β-naphthylamine was confined to the syncytiotrophoblast. However, the substrate used by these workers is not specific to oxytocinase; it is broken down, for example, by the plasma of men and non-pregnant women, neither of which inactivates oxytocin (Rydén, 1966). The colour observed may therefore have been due to the action of other peptidases. Other evidence is mostly indirect. The plasma of pregnant women contains oxytocinase, identical with the placental enzyme, which is thought to be derived from the placenta. If this is so, the fact that the plasma enzyme activity increases during the course of pregnancy parallel to the growth of the syncytiotrophoblast suggests that the oxytocinase originates in this tissue.

The results reported here provide more direct evidence for this hypothesis. Although the percentage of villous tissue occupied by trophoblast (Table 1) and the oxytocinase activity per unit volume placenta (Table 3) varied widely, there was a highly significant correlation between them (Text-fig. 2). Moreover, when the enzyme activity was expressed per unit volume trophoblast, a remarkably constant figure was obtained (Table 3). These findings applied both to normal placentas and to those from patients with toxaemia, in some of which the volume of trophoblast was much reduced (Table 2).

The results of the analysis of placental components in the normal group shown in Tables 1 and 2 are similar to those of Aherne & Dunnill (1966*a*) and Wong & Latour (1966). Aherne & Dunnill (1966*b*) found that in patients with toxaemia there was a reduced volume proportion of trophoblast, and we also have found that the mean volume of trophoblast in the toxaemic series was significantly lower than that in the normal series (Table 2). In our series of seven abnormal placentas, the four with the

lowest volumes of trophoblast were from patients with the highest levels of blood pressure (Tables 1 and 2, cases 7, 8, 10 and 12). This agrees with the work of Maqueo, Chavez Azuela & Dosal de la Vega (1964), who found a correlation between the degree of syncytial degeneration and the severity of the disease.

It is not known how placental oxytocinase reaches the maternal blood. The enzyme activity of uterine venous blood is no greater than that of systemic blood (Riad, 1962), and it is therefore unlikely that the enzyme diffuses out of the placenta into the retro-placental blood space. After measuring the oxytocinase activities of plasma and placenta in women with prolonged labour, Mathur & Walker (1968) put forward the hypothesis that the enzyme reaches the mother's blood-stream in fragments of syncytial tissue which become detached from the placenta during pregnancy (Hamilton & Boyd, 1966) and are later broken down by proteolytic enzymes (Carr, 1964). One of the assumptions on which this hypothesis depends is that oxytocinase is located in the syncytiotrophoblast; this assumption is justified by the findings reported in this paper.

By combining morphometry with enzyme estimation, it has been possible to find the location of oxytocinase in the placenta. It is suggested that this method might be applied to other organs and other enzymes.

We are grateful to Mr Arthur Williams for supplying us with material from patients under his care in the Nuffield Department of Obstetrics and Gynaecology, Oxford, and for the advice on histology given to us by the late Dr D. S. R. Kirby.

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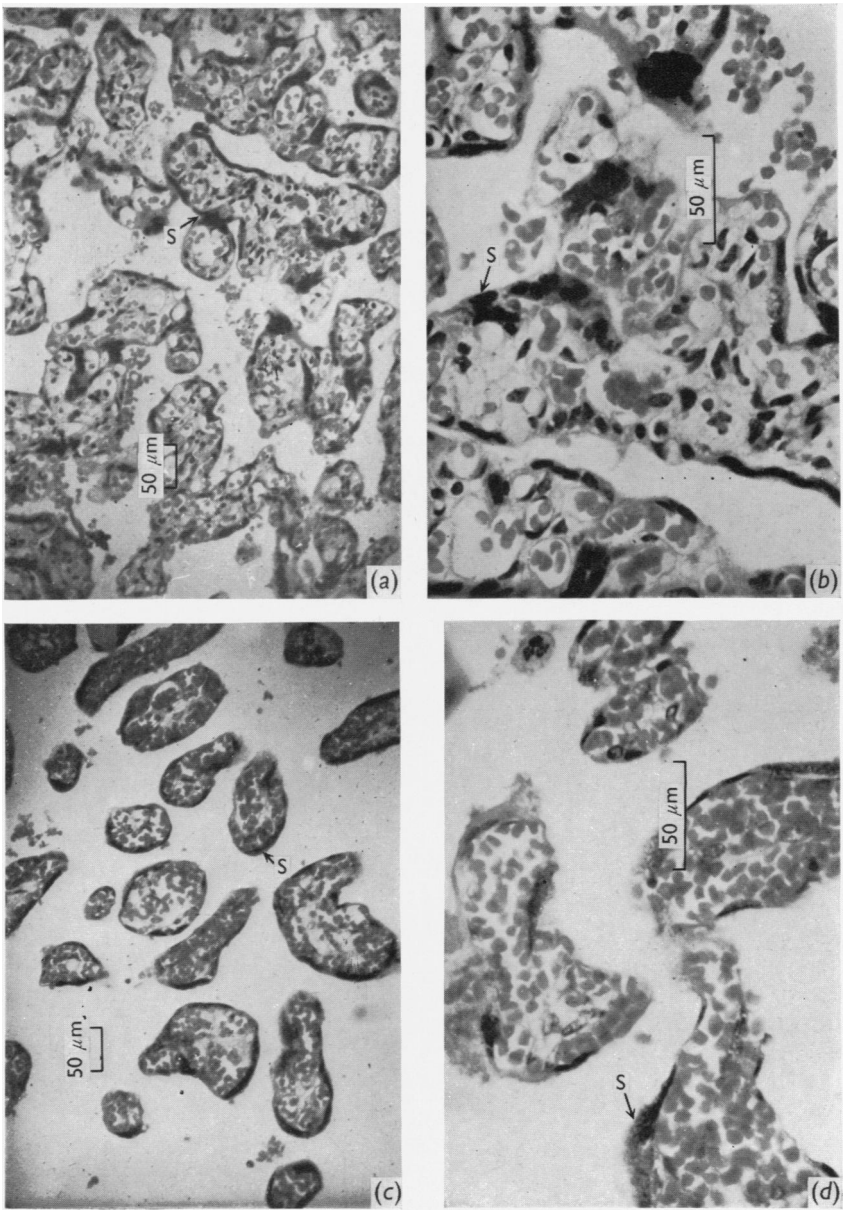
EXPLANATION OF PLATE

PLATE 1

Photomicrographs of placentas from a normal subject (*a* and *b*) and a patient suffering from toxaemia (*c* and *d*). In the abnormal placenta there is degeneration of the syncytiotrophoblast (S) and congestion of the villi.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