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Observations on the Mono-amine Oxidase Activity of Placenta and Uterus

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It is known that under aerobic conditions the kidney can oxidize mono-amines such as tyramine or adrenaline by means of a mono-amine oxidase (Blaschko, Richter & Schlossmann, 1937), and it has also been shown that under anaerobic conditions it can decarboxylate tyrosine, dihydroxyphenyl-alanine and histidine (Werle, 1936; Werle & Hermann, 1937; Holtz, 1937; Holtz, Heise & Spreyer, 1938). More recently a number of experimental findings have suggested the possibility of the excessive production or defective inactivation of pressor amines playing a part in the production of certain types of hypertension (Blaschko, 1942).

The ideas that have been put forward in this connexion have been summarized by Schales (1947), who, in a discussion of the relation of kidney enzymes to essential hypertension, mentions the view that since mono-amine oxidase requires oxygen to inactivate amines 'the restriction of renal circulation in experimental hypertension might make it possible for some pressor amines to escape unoxidized from the kidney', and so play a part in producing peripheral vaso-constriction. Duff, Hamilton & Magner (1939) claim to have produced arteriolar lesions similar to those of malignant hypertension by repeated intravenous injection of tyramine into rabbits. Raska (1943) has reported that the mono-amine oxidase activity of kidney slices from dogs rendered hypertensive by the Goldblatt technique is decreased. His results do not suggest that this decrease is specific, since the rates of residual respiration (i.e. in the absence of added substrate) and of oxidation of DL-alanine, L-dihydroxyphenylalanine (DOPA) and histamine were also diminished. In support of this, Gerbi, Rubinstein & Goldblatt (1940) had earlier found a diminution of the residual oxygen consumption in ischaemic rabbit kidney. Mason, Robinson & Blalock (1940), on the other hand, compared the Q_{O_2} of renal cortical tissue from normal and hypertensive dogs in the presence of glucose and found no significant

difference, although they state that preliminary observations showed a striking difference in the rates of residual respiration and of ammonia formation.

Rather more indirect evidence suggesting the possibility of faulty inactivation of the pressor amine formed by decarboxylation of L-DOPA in hypertensive animals has been provided by experiments showing that perfusion of the ischaemic mammalian kidney with DOPA gives rise to the production of a pressor substance having the properties of hydroxytyramine (Bing, 1941). Moreover, the injection of DOPA into cats with experimental renal hypertension causes a marked rise in blood pressure, whereas in normal cats no effect was observed (Bing & Zucker, 1941; Oster & Sorkin, 1942).

In view of the presence in the kidney of this amine-inactivating enzyme, and the suggestions which have been put forward regarding its possible relation to experimental renal hypertension, it was decided to study the possibility of some similar enzymic mechanism being a contributory factor in the development of the hypertension which is a marked and early feature of certain of the toxæmias of pregnancy. In this connexion the finding by Bhagvat, Blaschko & Richter (1939) of a high mono-amine oxidase activity in normal uterus is of interest.

For these reasons a study was undertaken to determine whether an active mono-amine oxidase is present in placenta, since this organ is thought by many workers to play an important part in the pathogenesis of the toxæmia, and also to investigate in greater detail the activity and properties of this enzyme in uterine tissue. Before this work was completed a brief report (Luschinsky & Singher, 1948) appeared on the identification of mono-amine oxidase in human placenta. The present work confirms this observation and describes certain other properties of the enzyme in connexion with the physiology of placental tissue.

METHODS

Estimation of enzymic activity

Oxygen consumption was determined manometrically using the Warburg technique. In all cases, except where stated, the tissue was finely minced with scissors and then dispersed in 0.067M-phosphate buffer, pH 7.3, in a high-speed homogenizer of the type described by Folley & Watson (1948), so that 2 ml. of the final homogenate corresponded to 200 mg. fresh tissue; where the amount of available tissue was not sufficient to provide a large enough volume for homogenization at this concentration, it was dispersed in the smallest possible volume.

Tyramine hydrochloride (L. Light and Co. Ltd.; recrystallized from water) was used as substrate in 0.01M solution except where otherwise stated.

O₂ uptake, using 2 ml. of the tissue homogenate, was measured at 38°, and in a gas phase of O₂, over a period of 1 hr., the results being expressed as $\mu\text{l. O}_2/\text{g. fresh tissue/hr.}$ Controls for non-enzymic oxidation of the substrate, and for the O₂ consumption of the enzyme preparation in the absence of added substrate, were included in each experiment.

In experiments where it was desired to trap the aldehyde formed during the initial oxidation of the amine in order to prevent further oxidation occurring, semicarbazide hydrochloride was added to the reaction mixture to give a final concentration of 0.02M.

Ammonia was estimated using the Markham (1942) apparatus.

Materials

Rat, rabbit and guinea-pig placenta. The pregnant uterus was removed immediately after killing the animal by exsanguination. The placentas were then dissected free from the other products of conception, washed with 0.9% NaCl, blotted dry with filter paper, weighed and homogenized.

Human placentas were brought to the laboratory either immediately after delivery or after having been placed immediately in a refrigerator in which they remained for periods up to 10 hr. In most cases they were expelled *per vaginam*, although a few were obtained after Caesarian section. They were prepared by one of two methods:

(1) A small piece, approximately 2 cm. square and extending through the entire thickness of the organ, was excised, mopped free from surface blood, washed with distilled water, weighed and homogenized.

(2) The membranes and larger blood vessels were dissected off, and the remaining tissue squeezed free from as much of the contained blood as possible, after which it was washed with glass-distilled water, mopped with filter paper, weighed, and passed through a Latapie mincer. The mince was then well mixed, and a weighed sample taken for homogenization.

Rat and rabbit uterus. Immediately after killing the animals by exsanguination the uterus was removed, and, in the case of non-pregnant animals, weighed and homogenized. When taken from pregnant animals the whole uterus was removed intact and weighed; if sufficiently developed, the products of conception were then dissected away, and the uterus was mopped free from blood and amniotic fluid with filter paper, washed rapidly in 0.9% NaCl, weighed and homogenized.

RESULTS

Mono-amine oxidase activity of uterus

The presence of an active amine oxidase in dog and sheep uterus was demonstrated by Bhagvat *et al.* (1939). We have extended this finding to the non-pregnant uterus of the rat and rabbit. The results of six typical experiments are shown in Table 1.

Table 1. *Mono-amine oxidase activity of non-pregnant rat and rabbit uterus*

(Activity expressed in $\mu\text{l. O}_2/\text{g. fresh tissue/hr.}$)

Rat	Rabbit
206	505
451	489
222	692
440	500
580	628
322	621

As the object of this work was a study of the enzyme in the pregnant animal, estimations were next made on the rat uterus at various stages of pregnancy. Thirty pregnant rats were used, and

Fig. 1. Relation between mono-amine oxidase activity of pregnant rat uterus (expressed as $\mu\text{l. O}_2/\text{g. fresh tissue/hr.}$) and the weight of the uterus after removal of the products of conception.

a scatter diagram relating the enzyme activities (expressed per g. fresh tissue) to the weight of the uterus after removal of the products of conception is shown in Fig. 1. It is realized that the distribution of such weights affords only an approximate indication of the duration of pregnancy, but owing to the wide variations in the number of fetuses contained in different animals the total weight of uterus, together with products of conception, provides an even less accurate indication.

From the results given in Fig. 1 it will be seen that very high values for enzymic activity (up to 1130 $\mu\text{l. O}_2/\text{g./hr.}$) are obtained early in pregnancy,

these values tending to fall as pregnancy advances to a low level of 100–300 $\mu\text{l. O}_2/\text{g./hr.}$ shortly before delivery.

That the relatively high values obtained in the small uterus of early pregnancy are not due merely to the homogenate being necessarily more dilute, with the possibility therefore of an accompanying dilution of some inhibitory factor, is shown by the fact that equally dilute homogenates of non-pregnant uterus do not show these high values. Further, in order to control this point in the pregnant uterus, a direct comparison was made of a uterus using homogenates of three different dilutions; no significant difference between the activities of the three dilutions was observed.

From our present experimental findings it is not possible to interpret this fall in activity per unit weight as pregnancy advances, but it suggests that the enzyme may be localized predominantly in the endometrium, and that the relatively greater mass of myometrium present late in pregnancy may be responsible for the lower activity per unit weight of the whole uterus. With this possibility in view, estimations were carried out on the separated endometrium and myometrium from two human uteruses.

The first was obtained from a normal pregnancy terminated by total hysterectomy at 17 weeks on account of a rapidly growing carcinoma of the breast. The decidua (endometrium in pregnancy), which was thick and plentiful, was separated with a scalpel from the myometrium, and homogenates were made of the two portions of the tissue, the activities of which were measured simultaneously in the usual way; the decidua showed an activity of 663 $\mu\text{l. O}_2/\text{g./hr.}$, and the myometrium 97 $\mu\text{l. O}_2/\text{g./hr.}$ The second uterus, which was non-pregnant, was removed on account of menorrhagia, and gave values of 432 $\mu\text{l. O}_2/\text{g./hr.}$ for the endometrium and 171 $\mu\text{l. O}_2/\text{g./hr.}$ for the myometrium.

Two further specimens of human decidua have been obtained by Caesarian section at full term, and have given values of 1125 and 1016 $\mu\text{l. O}_2/\text{g./hr.}$

Mono-amine oxidase activity of placenta

In view of the level of enzymic activity shown by human uterine tissue, human placenta was next investigated. Using small random samples excised from the placenta (method 1, see p. 126), it was found that this tissue was also highly active, values ranging from 338 to 995 $\mu\text{l. O}_2/\text{g./hr.}$ being obtained in ten different placentas.

As mentioned above, Raska (1943) has reported a lowering of the mono-amine oxidase level in kidneys from animals rendered hypertensive by the application of the Goldblatt clamp, and since normal human placenta, as well as kidney, appears to be a rich source of the enzyme, it was decided to attempt a comparison of the activity of this enzyme

in normal placentas and in those from cases of toxæmia of pregnancy showing hypertension.

The wide variations, however, in the results quoted above obtained on isolated samples of placental tissue led us, when embarking on this comparison, to use homogenates prepared from a portion of a well-mixed mince of the whole placenta, carried out as described above under method 2. Even when using this method it was found that considerable variation exists between the activities of different placentas.

Table 2. *Mono-amine oxidase activities of normal human placentas*

$\mu\text{l. O}_2/\text{g. fresh tissue/hr.}$	mmol. $\text{O}_2/\text{whole placenta/hr.}$
665	16.5
510	9.6
660	16.5
660	12.0
925	10.2
535	8.1
406	8.2
705	11.6
825	14.3
808	14.0
555	14.9
825	10.1

The results obtained with twelve different normal placentas at full term are shown in Table 2, where the activities are given both in terms of $\mu\text{l. O}_2/\text{g. fresh tissue/hr.}$ and also as mmol. $\text{O}_2/\text{whole placenta/hr.}$ From these figures, and from the published work on other human tissues (Birkhauser, 1940; Zeller, Stern & Wenk, 1940; Blaschko, 1942; Epps, 1945), it seems that normal human placenta at term must be classed as a tissue relatively rich in mono-amine oxidase.

An active enzyme was also found in rat, rabbit and guinea-pig placenta. In the course of these experiments with animal placentas variations in the level of activity of the mono-amine oxidase were noted in placentas of different ages in different animals. A series of estimations was therefore carried out in rats at various stages of placental development. The results of these experiments are shown in Fig. 2, in which enzymic activities of the placental homogenates from different animals (expressed as $\mu\text{l. O}_2/\text{g. fresh tissue/hr.}$) are plotted against the mean placental weight for each animal.

From the results obtained it will be seen that the level of enzymic activity, expressed per g. of placental tissue, appears to increase as the placenta ages. A linear regression line has been fitted, and has a slope of 55 $\mu\text{l. O}_2/\text{g./hr.}$ for each 0.1 g. increase in mean placental weight. The correlation coefficient for the values shown in Fig. 2 is 0.76 ± 0.21 . This correlation is in contrast to the results obtained with the whole uterus mentioned above.

If, as has been postulated, any relationship exists between mono-amine oxidase activity in the kidney and experimental renal hypertension, the dependence of enzymic activity on O_2 tension and the relation of this to restriction of the renal circulation becomes important. Further, in view of the postulated association of placental ischaemia with pregnancy toxæmia, this relationship may also be of importance in connexion with the hypertension accompanying this condition. Kohn (1937) has shown that the mono-amine oxidase of pig liver is unusually sensitive to changes in O_2 tension, the rate of oxidation of tyramine being increased by 200–250% by increasing the concentration of O_2 in the gas phase from 20 to 100%. By contrast he showed that a milk xanthine oxidase preparation was almost saturated at 20% O_2 .

Fig. 2. Relation between mono-amine oxidase activity of rat placenta (expressed as $\mu\text{l. O}_2/\text{g. fresh tissue/hr.}$) and mean weight of the individual placentas from any one animal.

Using homogenates of human placenta we have compared the rates of oxidation of tyramine and of succinate at different oxygen tensions. A final concentration of 0.05M-sodium succinate was used as substrate in the succinic oxidase determinations.

Fig. 3. Effect of O_2 tension on rates of oxidation of tyramine and succinate by human placental homogenates (expressed as percentages of activity at 20% O_2).

The results of these experiments are shown in Fig. 3, in which the enzymic activities are expressed as percentages of the activity at 20% O_2 . It will be seen that whereas the rate of oxidation of succinate

is only slightly affected by increasing the concentration of oxygen above about 2.5–5%, the rate of oxidation of tyramine increases strikingly as the oxygen tension is increased, the rate in the presence of 2.5% O_2 being less than half that in 10% O_2 . In addition to the enzyme in liver, the placental mono-amine oxidase is therefore also highly sensitive to changes in oxygen tension, and any fall in the latter, such as might occur in ischaemic areas of placenta, might therefore be expected to cause a more pronounced fall in the activity of the mono-amine oxidase than in that of certain other enzymes.

Specificity of the placental mono-amine oxidase

The tyramine oxidase first described by Hare (1928) in liver was shown by Blaschko *et al.* (1937) to have a wide range of specificity, oxidizing a number of aliphatic and aromatic mono-amines. In the present work, in addition to tyramine, we have studied the oxidation of 2-phenylethylamine (L. Light and Co.), and tryptamine and DL-adrenaline (British Drug Houses Ltd.), by human placental extracts. These substrates were also used in final concentrations of 0.01 M, the estimations of enzymic activity being carried out as with tyramine. In these experiments, however, semicarbazide was added to prevent the further oxidation of the aldehyde formed by deamination, together with 0.001 M-cyanide to inhibit the oxidation of adrenaline by the cytochrome oxidase system. KCN was also added to the centre cup to prevent loss of cyanide from the main chamber.

The results of these experiments are shown in Table 3. The relative rates of oxidation of tyramine, tryptamine and 2-phenylethylamine by human placenta are of the same order as those found by Blaschko *et al.* (1937) for guinea-pig liver. The value quoted for adrenaline is somewhat higher, but although when using this substrate readings were taken every 10 min., wide variations in its rate of oxidation were found in different experiments.

Table 3. *Relative rates of oxidation of some aromatic amines by placental mono-amine oxidase*

(Extra O_2 uptake due to tyramine/g./hr. taken as 100. Reactions in the presence of 0.02M-semicarbazide hydrochloride and 0.001 M-KCN.)

Tyramine	100
Tryptamine	77
Adrenaline	128
2-Phenylethylamine	16

A number of experiments were carried out using 0.01M-histamine dihydrochloride and cadavarine dihydrochloride as substrates, but in each case oxidation by placental homogenates under our conditions was barely measurable in the course of 1 hr.

Ammonia production by placental mono-amine oxidase

The rate of ammonia production has been estimated in a few experiments in order to correlate the extra oxygen uptake with deamination. At the

end of the measurement of oxygen uptake 1 ml. of 25 % trichloroacetic acid was added to each bottle. After standing 15 min. the contents of the bottles were centrifuged, and the bottles and the precipitate washed twice with 1 ml. of 5 % trichloroacetic acid. The filtrates were then made up to 10 ml. in volumetric flasks, and samples of each taken for ammonia estimation.

In each of these experiments the oxygen consumption in the presence of semicarbazide was also estimated, in order to determine the proportion of total oxygen uptake by the placental enzyme which was due to oxidation of the aldehyde formed. The results of these experiments are shown in Table 4.

gradually increasing until the child is about 3 months old, when maximum activity appears to be reached. In a study of the DOPA-decarboxylase activity of various human and animal tissues Page (1945), using a biological method for estimating the hydroxytyramine formed, was unable to detect the presence of this enzyme in human placenta. As far as we are aware, however, the decarboxylation of other amino-acids by placental or foetal tissue has not so far been studied.

The demonstration by Kohn (1937) that the activity of the mono-amine oxidase of liver is determined by the oxygen tension has been shown to apply also to the enzyme in placenta. In the absence

Table 4. *Ratio of atoms oxygen utilized/moles ammonia produced, and ratio of oxygen utilized in the presence and absence of semicarbazide, during oxidation of tyramine by the placental enzyme*

O ₂ uptake (μ l. O ₂ /g./hr.)		NH ₃ produced (μ M/g./hr.)	Atoms O/moles NH ₃		Ratio of O ₂ uptakes (a)/(b)
Without semicarbazide (a)	With semicarbazide (b)		Without semicarbazide	With semicarbazide	
672	521	43.9	1.37	1.06	1.29
601	506	48.6	1.10	0.92	1.19
680	595	52.4	1.16	1.01	1.16
890	735	55.7	1.42	1.17	1.21

In the absence of semicarbazide a mean O:NH₃ ratio of 1.26 was obtained; when, however, the figures for the oxygen uptake in the presence of semicarbazide are used, the ratios in these four experiments become 1.06, 0.92, 1.01 and 1.17 respectively.

DISCUSSION

The findings reported in this paper establish the presence of an active mono-amine oxidase in placental extracts, confirming the recent observations of Luschinsky & Singher (1948). From a study of its action on different amines it has been shown that this enzyme, like the mono-amine oxidase present in liver, kidney and elsewhere, can oxidize a number of aromatic mono-amines, but is inactive against di-amines such as histamine or cadaverine.

In human placenta at term, this enzyme is present in relatively large amounts, and in rat placenta its activity appears to increase as the placenta matures. From the experiments which we have made it is impossible to do more than speculate on the possible significance of this enzyme in placenta, but the fact that in rat placenta its activity appears to increase as the placenta matures suggests that it may exercise some function of increasing significance as pregnancy advances, and the foetus or foetuses increase in size and differentiation. In this connexion it should be noted that Epps (1945) has shown that the kidney cortex and medulla of the newborn child are deficient in mono-amine oxidase, the activity

of any exact information as to the oxygen tensions to which the enzyme would normally be exposed under *in vivo* conditions, it is clearly impossible to interpret this property of amine oxidase in any physiological sense. It does suggest, however, that any lowering of the local oxygen tension, such as might be expected to occur in the placenta, more particularly towards the end of pregnancy, might result in an inhibition of the activity of this enzyme to a greater extent than that of the succinic oxidase system, and possibly also of other systems involving the cytochrome-cytochrome oxidase mechanism.

The high values of the mono-amine oxidase activity of the rat uterus early in pregnancy together with the apparent fall in these values, when expressed on a weight basis as pregnancy advances, suggests that the enzyme may be chiefly localized in the endometrium, and this supposition has been confirmed by examination of the separate endometrium and myometrium from human uterus. The low value found in the myometrium would indicate that this muscle is similar to the other muscular organs examined by Bhagvat *et al.* (1939), the high value of whole uterus being due mainly to an endometrial enzyme.

Experiments are now in progress in the hope of obtaining information concerning the physiological significance of this enzyme in placenta, and to ascertain whether any abnormalities of its function are detectable in the toxæmias of pregnancy.

SUMMARY

1. The presence of an active mono-amine oxidase in uterus has been confirmed. Evidence is presented indicating that in the human uterus, and possibly also in the rat uterus, it is mainly localized in the endometrium.

2. Human, rat, rabbit and guinea-pig placentas have also been found to be potent sources of the enzyme.

3. The level of enzymic activity in rat placenta (expressed per g. of tissue) appears to increase as the placenta matures *in utero*.

4. The activity of the placental enzyme, like that of the enzyme in liver, is proportional to the oxygen tension of the medium in which it acts, a fall of oxygen tension causing a marked fall in rate of enzyme action.

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Effect of Sulphydryl-combining Compounds on the Activity of the Succinic Oxidase System

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Thunberg (1916) was the first to suggest that the tissue catalyst responsible for the oxidation of succinate contained a sulphydryl group, but his evidence, viz. that colloidal sulphur inhibited the enzyme, was not very convincing. There was, in fact, better evidence available to Thunberg, since Battelli & Stern (1910) had already shown that arsenite inhibited this enzyme, and Ehrlich (1909) had suggested that the toxic action of arsenicals on tissues might be due to their combination with sulphydryl groups. This suggestion of Ehrlich has recently received convincing experimental support by the work of Peters and his colleagues (Peters, Stocken & Thompson, 1945).

Hopkins and his co-workers (Hopkins & Morgan, 1938; Hopkins, Morgan & Lutwak-Mann, 1938) were

the first to demonstrate the existence and the importance of SH groups in succinic dehydrogenase. These workers showed that succinic dehydrogenase was completely inhibited by incubation with a high concentration of oxidized glutathione (GSSG), and that the activity was completely restored by incubating the inhibited enzyme with reduced glutathione (GSH) under anaerobic conditions. They suggested that GSSG acted by oxidizing the SH groups of succinic dehydrogenase to disulphide (S.S) groups and that this reaction was reversible.

Since the publication of the papers of Hopkins and his co-workers, many studies of the action of other SH-combining compounds on succinic oxidase