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THE 5-HYDROXYTRYPTAMINE CONTENT OF THE PLACENTA AND FOETUS DURING PREGNANCY IN MICE

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5-Hydroxytryptamine (5HT) levels were measured in blood and tissues from pregnant mice. Blood levels remained constant during pregnancy and were the same as those in nonpregnant female mice. Placental levels of 5HT increased throughout pregnancy as did the foetal levels. The maternal blood volume of the placenta also increased with advancing gestation. 5HT levels were measured after treatment of the mother with 5HT, and the critical placental level of 5HT observed at about the time of death of the foetus was determined. The levels of 5HT in the placenta and foetus after treatment of the mother with several monoamine oxidase inhibitors were measured, and found to show no significant increase above the normal levels in these tissues. Treatment with cyproheptadine, a 5HT antagonist, did not delay parturition.

Previous work has shown that both 5-hydroxytryptamine (5HT) and monoamine oxidase inhibitors will interrupt pregnancy in mice if administered during the early stages (Lindsay, Poulson & Robson, 1963). This action can be prevented if progesterone or prolactin is given concurrently with these drugs, which suggests that in some way luteal secretion is inhibited and that this is responsible for the interruption of pregnancy. During the second half of pregnancy 5HT still causes death of the foetus whereas monoamine oxidase inhibitors are no longer effective. Progesterone and prolactin have little protective action in pregnancy when 5HT is administered to the mother in the later stages. It appears that the action of 5HT in the later stages of pregnancy is mainly a peripheral one, probably exerted on the placenta (Robson & Sullivan, 1963).

These findings suggest that measurements of the 5HT contents of the foetus and placenta in the second half of pregnancy in normal mice and subsequently in mice treated with 5HT would help to elucidate the mode of action of this compound. In an attempt to account for the amount of 5HT found in the untreated placenta, the maternal blood content of the placenta in the latter half of pregnancy was measured, and this was correlated with the maternal blood level of 5HT. As treatment of the mother with 5HT increased the 5HT level in the placenta, it seemed of interest to investigate the effect of monoamine oxidase inhibitors, particularly as these substances had been shown to interrupt pregnancy in the early stages.

METHODS

All experiments were performed on mature white mice of known fertility and bred in the Animal House, Guy's Hospital Medical School. The 1st day of pregnancy was counted from the finding of the vaginal plug. All parturient animals used were 19 days pregnant. Parturition was actually watched and the animals killed after the birth of two foetuses.

Extraction and assay of 5HT from the tissues

The mice were killed by dislocation of the upper cervical spine and the foetuses and placentas, together with blood and some other tissues, were removed. The tissues were weighed and stored at −10° C until required. The extraction of 5HT from the tissues was by the method recommended by Amin, Crawford & Gaddum (1954). The acetone-extracts were assayed biologically on the oestrous rat uterus or the rat stomach fundus strip preparations. The isolated uterine horn was suspended in a 5 ml. organ-bath containing aerated de Jalon solution and atropine sulphate (10^{-6}), at 30° C (Amin *et al.*, 1954). The rat isolated fundus strip was suspended in a 10 ml. organ-bath containing aerated de Jalon solution and hyoscine hydrobromide (10^{-7}), at 37° C (Vane, 1957, 1959). The contractions of both preparations produced by the acetone-extracts were abolished by bromolysergic acid diethylamide, as were those produced by 5HT itself.

Representative samples from each group of tissues were assayed biochemically (Bogdanski, Pletscher, Brodie & Udenfriend, 1956). The 5HT was extracted from a tissue homogenate with butanol and returned into an acidic aqueous phase by the addition of heptane. The fluorescence was developed by the addition of concentrated hydrochloric acid and read at *p*H 1 using an activating wavelength of 300 mμ and an emission wavelength of 540 mμ. The 5HT from the tissues was characterized by this method. In all experiments, with the exception of those involving the use of *p*-di(2-hydrazinoethoxy)benzene hydrochloride (HP 1325), the results obtained by the biological method were confirmed biochemically. The results obtained by the two methods consistently agreed.

Blood for 5HT estimations was taken from the heart using a siliconed syringe, after anaesthetizing the animal with chloroform.

Measurement of the volume of maternal blood in the placenta

Mouse erythrocytes were labelled with radio-chromium, by a modification of the method used by Veall & Vetter (1958) for the labelling of human erythrocytes. Approximately 0.7 ml. of blood was withdrawn from the mouse heart into a syringe containing 0.3 ml. of citric acid-dextrose solution, the mouse having been anaesthetized with chloroform. The erythrocytes were labelled using a sterile isotonic solution of $Na_2{}^{51}CrO_4$ and were washed and resuspended in plasma-saline to give an activity of 50 μc/ml. of blood. Labelled erythrocytes with an activity of about 20 μc (0.4 ml.) were injected intravenously into mice at various stages of pregnancy. After sufficient time (30 min) had elapsed after the injection to ensure complete mixing of erythrocytes (Robson & Sullivan, 1963), the mouse was killed and placentas and foetuses were removed together with a known volume of maternal blood and several other maternal tissues. The radioactivities of these tissues were determined using a well crystal scintillation counter, and expressed as percentages of the activity in the maternal blood. Each result represents the mean of estimations done on two or more mice.

Treatment with drugs

All mice treated with 5HT or with monoamine oxidase inhibitors were killed on the 14th day of gestation. 5HT was used as the creatinine sulphate (May & Baker), the dose administered being 40 mg/kg of the salt, subcutaneously. All assay results are given in terms of the base.

The doses of monoamine oxidase inhibitors were based on those used in previous experiments on pregnancy (Poulson & Robson, 1963). Doses of iproniazid are in terms of the phosphate, two different doses being administered. In the first experiment, 165 mg/kg of iproniazid was given subcutaneously in a single daily dose, from days 11 to 13 of gestation.

In a second experiment this dose was increased to 330 mg/kg/day, given subcutaneously on days 10 to 13. Some mice received a single dose of iproniazid on the 14th day of gestation 5 hr before they were killed.

p-Di(2-hydrazinoethoxy)benzene hydrochloride (HP 1325), another monoamine oxidase inhibitor, was used in a dose of 165 mg/kg, subcutaneously. Groups of pregnant mice were treated daily on days 9 to 13 and 11 to 13, and by a single dose 5 hr before they were killed.

Doses of tranylcypromine sulphate (8 mg/kg, subcutaneously) were administered daily on days 11 to 13 and also in a single dose 5 hr before the mice were killed. Cyproheptadine hydrochloride was used as described in the text.

RESULTS

Seasonal variation of 5HT contents of placenta and foetus

These contents were all determined on day 14 of pregnancy and all the individual results were obtained during a period of 1 year (November 1961 to November 1962). Results are shown in Fig. 1. It will be seen that there was a good deal of variability

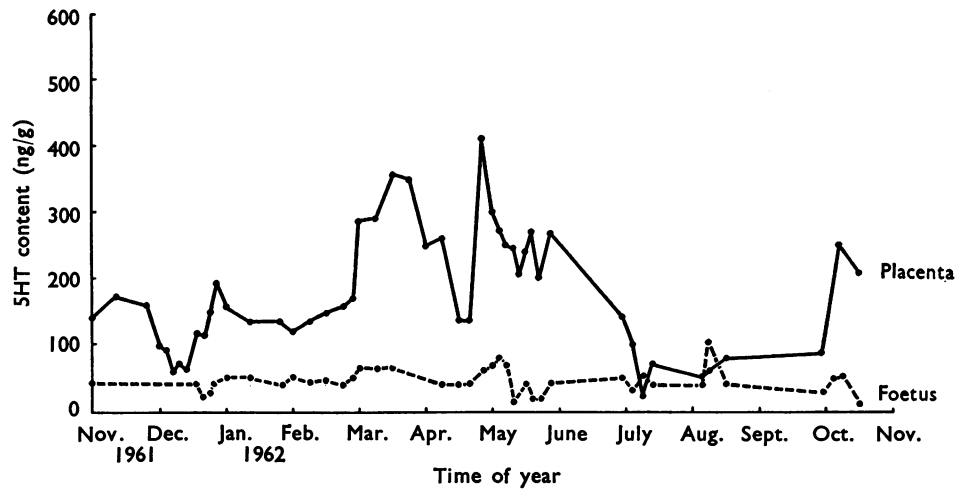

Fig. 1. Seasonal variation in 5-hydroxytryptamine (5HT) content of the placenta and foetus in 14 day pregnant mice. Each point represents a single assay.

in the placental content of 5HT at that particular stage of pregnancy, the amounts measured varying from less than 100 ng/g to about 400 ng/g. Nevertheless, there is a strong suggestion that the 5HT content of the placenta increased in the spring and summer. The seasonal variation was greater than that due to variation between individuals. The 5HT content of the foetus throughout the year also appears to show some rise at the same time as the placental content, though it must be remembered that figures obtained were at the lower limit of the sensitivity of the method. Cass (1960) has found that there is a similar seasonal variation in the 5HT content of the blood of rats.

5HT level of foetus and placenta during pregnancy

These results were all obtained over the period May to November 1962, and are shown in Fig. 2. The earliest figures are for the 12th day of pregnancy, since

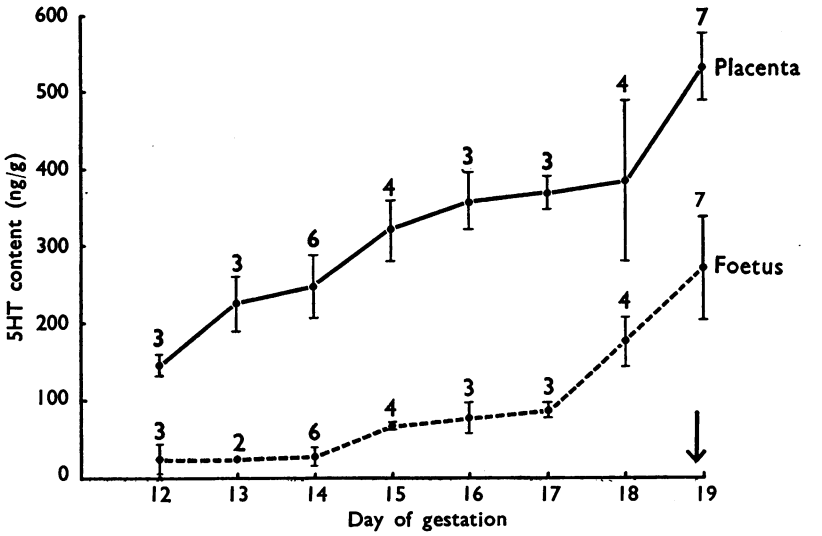

Fig. 2. Placental and foetal contents of 5-hydroxytryptamine (5HT) at various stages of pregnancy. Means and standard deviations are shown. Each result represents an assay on all the placentas (or foetuses) obtained from two mice. The number of such assays is indicated for each stage of pregnancy. The value for the foetal content at 13 days is the mean of two results. The arrow indicates time of parturition.

insufficient material to give accurate assays can be collected earlier in pregnancy. In spite of the seasonal fluctuations encountered in the 5HT content on the 14th day of pregnancy, the results nevertheless clearly show that the 5HT content of the placenta increased throughout pregnancy reaching a maximum at parturition. In the case of the foetus all figures are below 100 ng/g until the 18th day of pregnancy when there is a rise which becomes more striking at parturition, when the average value is about 250 ng/g of tissue.

Maternal blood levels of 5HT

These were measured in order to determine whether the change in placental level merely reflected changes in maternal blood level. In four nonpregnant adult female mice, levels of 320, 340, 350 and 400 ng/ml. of blood were found. In seventeen mice investigated at the 14th to 19th day of pregnancy the blood levels varied from 290 to 460 ng/ml. The mean of all the blood values was 367.5 ng/ml. (standard deviation, ± 46.7). There were no differences at the various stages of pregnancy investigated. It is thus clear that the blood content of 5HT in the mouse is not affected by pregnancy.

The 5HT content of the spleen was also determined at various stages of pregnancy, most of the assays being performed on the 14th day or at parturition. The values ranged rather widely. Results for the 14th day of pregnancy ranged from 300 to 625 ng/g (mean 480.4, standard deviation 60, twenty estimations). At parturition there were seven observations which ranged from 380 to 613 ng/g (mean 441.8, standard deviation 80). Eight assays at other stages of pregnancy came within the

same range. It is clear that the content of 5HT in the spleen, like that in the maternal blood, does not vary with the stage of pregnancy.

Increase in the blood volume of the maternal placenta

The results are illustrated in Fig. 3. It will be seen that in the earlier stages investigated, that is the 12th to 16th day, the maternal blood makes up about 10% of the whole placenta. A sudden rise then occurs and the maternal blood content progressively increases until it occupies nearly 30% of the placenta. The blood

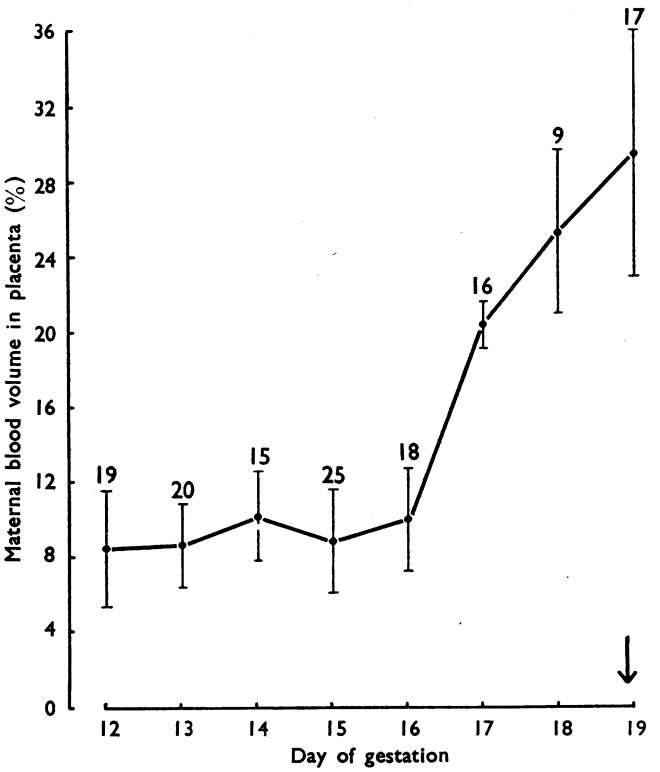

Fig. 3. Maternal blood volume of the placenta at various stages of pregnancy. Means and standard deviations are shown as well as the number of observations for each value. Each value represents an estimation on one placenta, the total number of placentas for each day of pregnancy being obtained from two or three mice. The arrow indicates time of parturition.

content of the uterus (from which the foetuses and placentas had been removed) was measured as a control and was about 5%, and showed no correlation with the stage of pregnancy.

With this knowledge and the results on the average 5HT content of the maternal blood, the results on the 5HT content of the placenta at various stages of pregnancy have been recalculated to show the 5HT content of the placenta, excluding the maternal blood (Fig. 4).

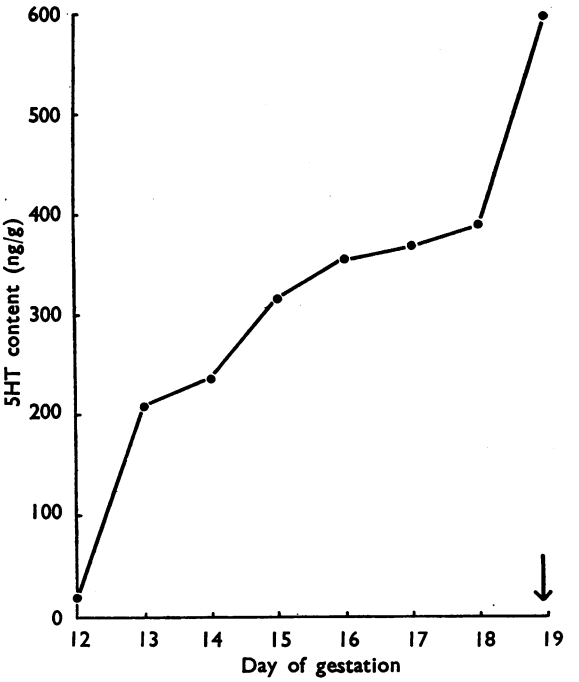

Fig. 4. Amount of 5-hydroxytryptamine (5HT) in the placenta, excluding the maternal blood, at various stages of pregnancy, calculated on the assumption that 1 ml. of blood weighs 1 g. The arrow indicates time of parturition.

Effects of injection of 5HT on placental and foetal levels

When 40 mg/kg of 5HT is injected subcutaneously into a mouse on the 14th day of gestation (as in these experiments), the foetuses usually die within 30 min of the injection. The first experiments were devised to determine how the placental and foetal levels of 5HT are affected under these circumstances and the results are summarized in Fig. 5. It will be seen that 15 min after injection the placental level was only slightly raised (by about one-third) and all the foetuses were alive. At 30 min after injection, however, there was a striking increase in the placental 5HT level, to about two- to three-times the normal value. In nine of the litters all the foetuses were dead and in the other four litters there were some dead and some living foetuses. At 60 min after injection there was a further rise in the 5HT content of the placenta and all the foetuses were dead. In the foetus there was no significant rise in 5HT content up to 1 hr after injection.

These results thus show that at the time of foetal death, that is between 15 and 30 min after injection of 5HT into the mother, there was probably a quite large rise in the placental 5HT content, but that the amount in the foetus was not affected.

Effect of 5HT injection on the maternal blood level

It seemed possible that the increase in the placental content of 5HT after the subcutaneous administration of the drug to the mother might, in part or whole, be

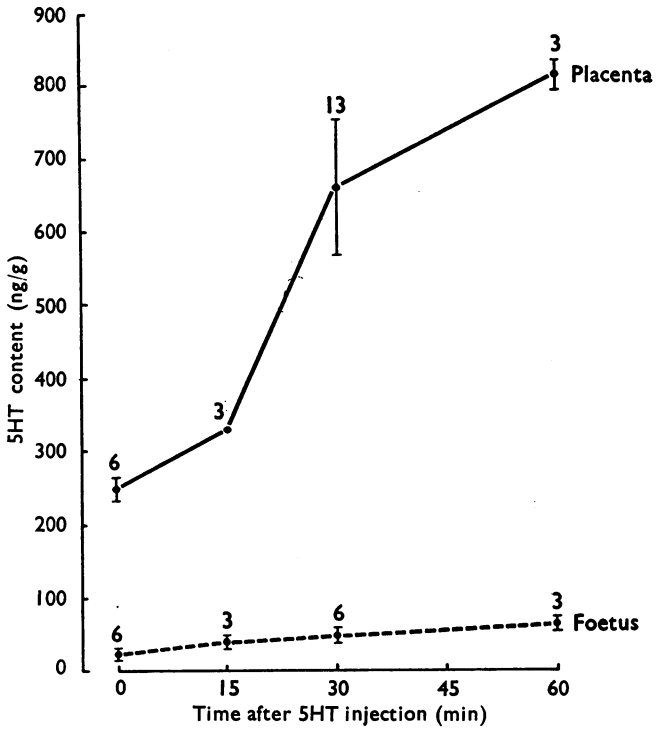

Fig. 5. Placental and foetal levels of 5-hydroxytryptamine (5HT) after subcutaneous injection of 5HT (40 mg/kg, subcutaneously) into 14 day pregnant mice. Means and standard deviations are shown. The placental mean value at 15 min shows no standard deviation as the values were all the same. Each value represents an assay on all the placentas or foetuses obtained from two mice. The total number of such assays is indicated at each point.

due to the rise in the 5HT content of the maternal blood contained in the placenta. It was thus necessary to determine the maternal blood levels which resulted from the administration of 5HT.

Four groups each of two female, nonpregnant mice were injected subcutaneously with 1.0 mg of 5HT creatinine sulphate, and were killed at intervals of 15, 30, 60 and 120 min respectively after the injections. The blood for each group of mice was pooled and the 5HT content was determined. The results (Fig. 6) show that there was a rapid rise in the 5HT content of the blood, which reached a maximum of about 8 μg/ml. 30 to 60 min after the administration of 5HT and was falling at 120 min. The results suggest that the 5HT level in the blood remains above the normal value for several hours after the administration of the salt. The significance of these results is discussed later.

Effect of amine oxidase inhibitors on placental and foetal levels of 5HT

All these experiments were planned so that the mice were killed on the 14th day of pregnancy for the estimation of the 5HT content of tissues. The assessment of these results is complicated by the fact that account has to be taken of the normal

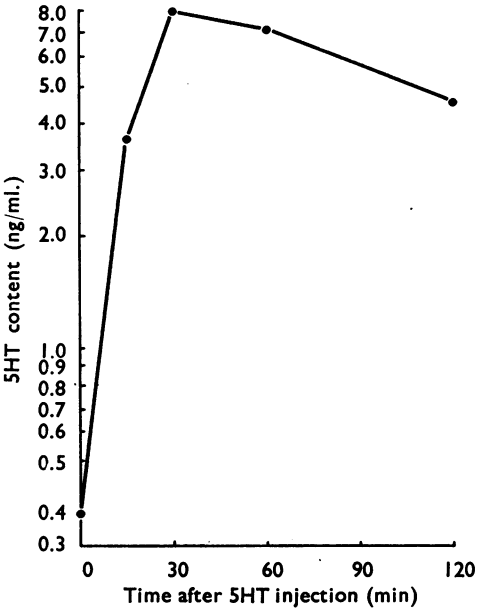

Fig. 6. Increase in 5-hydroxytryptamine (5HT) content of mouse blood (ordinate, log scale) after subcutaneous administration of 5HT (40 mg/kg).

seasonal variations in the 5HT content of the placenta. The results are therefore given together with the month of the year at which they were obtained and the various figures have to be compared with those for the nontreated animals, as shown in Fig. 1.

It is clear that iproniazid produced no significant effect on the 5HT content of the placenta and foetus, whether it was given as three daily doses or as a single dose before killing the animal. On the other hand the 5HT level of the spleen was raised (Table 1). Similarly, the potent amine oxidase inhibitor HP 1325 did not

TABLE 1

5-HYDROXYTRYPTAMINE (5HT) CONTENTS OF TISSUES FROM PREGNANT MICE TREATED WITH IPRONIAZID

All mice were killed on the 14th day of gestation. Figures in brackets indicate the number of observations in each group. All results are means ± standard deviations. * Placentas from non-treated mice at the same time as from treated animals

Dose of iproniazid (mg/kg)	Treatment	Date of observation	Contents of 5HT (ng/g) in Placenta	Normal placenta*	Foetus	Spleen
330	Daily, days 10 to 13	Jan. 1962	122·5±7·0 (4)	150	59·7±9·2 (4)	—
		March 1962	380 (1)	>300	49·0 (1)	—
330	Single dose 5 hr before death	Jan. 1962	155·5 (2)	150	40·0 (2)	—
165	Daily, days 11 to 13	March 1962	372·4±91·8 (5)	>300	68·0±17·0 (4)	1,300±50·0 (3)

TABLE 2

5-HYDROXYTRYPTAMINE (5HT) CONTENTS OF TISSUES FROM PREGNANT MICE TREATED WITH HP 1325

All mice were killed on the 14th day of gestation. All results are means ± standard deviations. * The control placental value was about 150 ng/g

Dose of HP 1325 (mg/kg)	Treatment	Number of observations	Date of observation	Contents of 5HT (ng/g) in Placenta*	Contents of 5HT (ng/g) in Foetus
165	Daily, days 9 to 13	6	Jan. 1962*	136±30·1	43·2±7·6
165	Daily, days 11 to 13	4	Jan. 1962	127·0±34·2	44·5±9·5
165	Single dose 5 hr before death	2	Jan. 1962	138·0	31·0

affect the 5HT content of the placenta and foetus (Table 2); tranylcypromine, too, was without effect and did not alter the 5HT level in the spleen (Table 3).

The effect of iproniazid on the blood 5HT was also determined. One group of six nonpregnant female mice was injected with iproniazid (330 mg/kg, subcutaneously) 5 hr before they were killed. Another similar group served as controls. The blood from each group was pooled and assayed biologically. The 5HT contents of the control and treated groups were respectively 292 and 274 ng/ml. The treatment with this amine oxidase inhibitor thus had no effect on the 5HT blood level.

TABLE 3

5-HYDROXYTRYPTAMINE (5HT) CONTENTS OF TISSUES FROM PREGNANT MICE TREATED WITH TRANYLCYPROMINE

All mice were killed on the 14th day of gestation. Figures in brackets indicate the number of observations in each group. All results are means ± standard deviations. * Placentas from non-treated mice at the same time as from treated animals

Dose of tranyl-cypromine (mg/kg)	Treatment	Date of observation	5-Hydroxytryptamine content (ng/g) in Placenta	Normal placenta*	Foetus	Spleen
8	Daily, days 11 to 13	Oct. 1962	256 (2)	250	23 (2)	397 (2)
8	Single dose 5 hr before death	Aug. 1962 Oct. 1962	71±5·6 (3) 300 (1)	50–100 250	37±7·9 (3) 21 (1)	375±75 (3) 350 (1)

Relation of 5HT to parturition

The rising level during pregnancy of 5HT in the placenta and foetus suggested that the high level of the drug at parturition might play some part in its initiation. This was tested by giving cyproheptadine, a potent 5HT antagonist, to mice for some 2 days preceding parturition. The expectation that this would delay parturition was not fulfilled.

Seven pregnant mice received subcutaneously 0.2 mg of cyproheptadine in the morning and 0.4 mg in the evening of days 18 and 19 of pregnancy. Six of the

mice had litters by the morning of the 20th day. The remaining mouse was similarly treated on the 20th day and had had a litter by the morning of the 21st day. Three mice received an even more intensive treatment starting on the 18th day. They were injected with 0.4 mg of cyproheptadine morning and evening and were deprived of food during the daytime. They were given 0.8 mg of cyproheptadine in 5 g of diet at night. One mouse had a litter on the 19th day; the other two had had litters by the morning of the 20th day. There was thus no evidence whatever that the treatment delayed parturition.

DISCUSSION

These experiments have shown quite clearly that the 5HT content of the placenta and foetus in the mouse increases from the 12th day of gestation, that is the earliest time at which such measurements could be made, until parturition. The 5HT content of the maternal blood showed no such change, so that presumably the foetus or placenta, or both, were responsible for the increase. The most rapid rise in both was observed just before parturition; in the foetus this started at about the 18th day and in the placental tissues, that is the placenta without the maternal blood, 1 day later, on the 19th day (Fig. 5). This result is perhaps suggestive evidence that the foetus is the primary site accounting for the rise in 5HT in the intra-uterine tissues though it does not exclude the possibility that the placenta too is involved in the process. The cause of the rise in the foetal 5HT content is not known, but it may coincide with the appearance of enterochromaffin cells in the foetus, though there is no evidence on this question in the literature. Faustini (1955), however, found that in the embryonic calf there was a parallel relationship between the development of the enterochromaffin cells and the 5HT content of the intestinal tract, and the same may apply to the mouse foetus. This requires investigation.

The significance of the rise in 5HT content of the foetus and placenta during pregnancy is quite unknown. It may indeed be of no physiological importance and merely secondary to the development of certain 5HT-producing tissues, and to the storage of the 5HT. It was tempting to speculate whether the 5HT played any part in the initiation of parturition, since it is well known that the uterine muscle of the mouse is quite sensitive to the drug. If this was so the 5HT would have to act locally and not after absorption into the maternal circulation, since the maternal blood level does not increase during pregnancy. This is not impossible, since progesterone, acting locally, can play a part in the control of uterine activity during pregnancy (Csapo, 1961; Forbes, 1961). No evidence in support of the hypothesis was, however, obtained in experiments involving the use of cyproheptadine. This drug, a powerful antagonist of 5HT, did not delay parturition, as would have been expected if 5HT were concerned in the initiation of parturition. It should be added that this evidence does not completely eliminate the possibility that 5HT plays some part in the initiation of parturition since, as is well known, it is sometimes difficult to antagonize, by the systemic administration of drugs, the effect of substances produced locally near their site of action.

The changes in the 5HT content of the foetus and placenta following the administration of 5HT have now to be considered, in relation to the mechanism responsible

for the rapid death of the foetus which occurs after the administration of the drug. Since there is no significant increase in foetal content of 5HT at the time of foetal death, this result is in all probability not due to a direct toxic action of 5HT on the foetus. This conclusion agrees with that reached by Robson & Sullivan (1963), who found that the direct injection of comparatively large doses of 5HT into the foetus, producing concentrations in the foetus much higher than those seen in the present experiments following the administration of 5HT to the mother, did not produce any deleterious effects on the foetus. We are thus left with the likelihood that the toxic action of 5HT on the foetus is secondary to its effect on the placenta. This conclusion was also reached by Robson & Sullivan (1963) on the basis of the results on the action of 5HT on placental blood flow and function. It is noteworthy that occasionally (though not in the present experiments) foetal death may occur some 15 min after administration of the drug, when the placental content has increased to only a small extent. Further deductions can be made from a consideration of maternal blood and placental contents of 5HT at various stages after the injection of 5HT, and the amount of maternal blood present in the placenta at the time when these studies were performed. Calculations have been made which are presented in Table 4. The theoretical values are based on the assumption that

TABLE 4

ACTUAL AND THEORETICAL VALUES OF 5-HYDROXYTRYPTAMINE (5HT) CONTENT OF THE PLACENTA AFTER INJECTION OF 5HT

5HT (40 mg/kg) was injected subcutaneously on the 14th day of gestation. For method of calculating theoretical values, see text

Time after injection (min)	5HT in placental tissue (ng/g)	
	Actual values	Theoretical values
15	334	533
30	663	885
60	818	816

the amount of maternal blood in the placenta is about 8% of the total placental weight and that this maternal blood contains the same concentration of 5HT as does the systemic maternal blood ; it is furthermore assumed that the 5HT content of the remainder of the placenta has not altered as a result of the 5HT administration. The results are interesting. At 15 and 30 min the actual amount of 5HT in the placenta was lower than the calculated value. This result is not unexpected since 5HT interferes with blood supply to the placenta so that complete mixing of the systemic maternal blood is delayed (Robson & Sullivan, 1963). Later still (at 60 min) the placental content of 5HT was essentially the same as the calculated value, as would be expected if full mixing had occurred by this time.

The failure of the amine oxidase inhibitors to increase the placental 5HT content is puzzling. Both iproniazid and HP 1325 are known to increase the 5HT content of various tissues *in vivo*, for example the brain and the liver, and such an effect was actually demonstrated with iproniazid on the spleen in the present experiments. Moreover Ibrahim (1961) has shown that the administration of iproniazid to mice for 2 days decreases the amine oxidase activity of the placenta. This was found

at the same stage of pregnancy as in the present experiments. This discrepancy could be explained if it were assumed that in the mouse placenta the rate at which 5HT is metabolized is not appreciably affected when the action of amine oxidase is partially blocked. Moreover, the fact that the three amine oxidase inhibitors investigated did not raise the placental level of 5HT agrees with the finding that these drugs do not produce any deleterious effect on the gestation at this stage. Any appreciable increase in the 5HT content of the placenta (such as was, for example, found in the spleen) would have been expected to be rapidly toxic to the uterine contents.

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