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ANALYSIS OF ACTIONS OF 5-HYDROXYTRYPTAMINE IN PREGNANCY

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SUMMARY

1. When 5-hydroxytryptamine creatinine sulphate is injected subcutaneously in a dose of 2 mg into mice during the second half of pregnancy, the foetuses die within 1 hr. The mode of action of 5-hydroxytryptamine (5-HT) in producing this effect has been studied.

2. It has been demonstrated that this is not a direct toxic effect of 5-HT. Very little passes through the placenta into the foetus, and after the injection of much larger amounts directly into the foetus no lethal effect was observed.

3. It was shown that 5-HT had little effect on uterine motility *in vivo*, and that the much larger contractions produced by oxytocin did not produce any effect on the foetus.

4. No constriction of the umbilical vessels was produced by 5-HT injected into the mother, nor did the local application of 5-HT to the cord produce any effect.

5. The administration of 5-HT to the mother markedly reduced the passage of ^{22}Na from the maternal circulation into the placenta and foetus. This was accompanied by a reduction in the blood supply to the placenta from a normal value of 87 μl./min/g to 4 μg/min/g.

6. It is suggested that the effect of 5-HT in producing foetal death is a consequence of the reduction in the transfer function of the placenta.

INTRODUCTION

It has been shown previously that 5-HT will interfere with pregnancy in mice at all stages. In the first half of pregnancy the effect occurs when the drug is given daily for several days, e.g. from the first to sixth day, and appears to be due to an interference with the hormonal mechanism responsible for the maintenance of gestation (Lindsay, Poulson & Robson, 1963).

In the second half of pregnancy the effect of 5-HT comes on very rapidly

and usually causes death of the foetuses within 0·5 hr following a single injection (Poulson, Botros & Robson, 1960). It seemed likely at first that this was due to a direct toxic effect of the drug on the foetus, but this was found not to be so. The lethal action of 5-HT was thus investigated systematically and the experiments described in this paper illustrate our approach to the problem. They can be subdivided under these headings:

(*a*) Direct effect of 5-HT on the foetus.

(*b*) Effect on uterine activity *in vivo*.

(*c*) Action on vessels of the umbilical cord.

(*d*) Effect on the passage of radio-sodium from the mother into the placenta and foetus.

(*e*) Effect on the maternal blood supply to the placenta.

METHODS

Experiments were performed on white mice bred in the Animal House of Guy's Hospital Medical School. The mice were kept in groups of five females and one male and vaginal plugs looked for daily. The day of finding the vaginal plug is called day 1 of pregnancy.

5-Hydroxytryptamine creatinine sulphate. 5-Hydroxytryptamine creatinine sulphate (May & Baker) was used throughout and the doses given are expressed in terms of the salt. To produce the lethal effect on the foetus a standard dose of 2 mg of the salt (equivalent to 0·87 mg of base) was given subcutaneously to the mother.

Anaethesia. Ether was used in the early experiments, but was unsatisfactory, since it reduced markedly the lethal effect of the 5-HT. Subsequently Numal (di-ethylaminoallyl isopropyl barbiturate acid) (Hoffmann-La Roche) was used subcutaneously in a dose of 130–150 mg/kg. This is a veterinary anaesthetic and has been found to be very satisfactory for mice and rats, a single dose subcutaneously producing anaesthesia lasting for some hours.

Observations on the foetus. It was necessary to determine accurately the condition and viability of the foetus. To do this, and to avoid artifacts, it was important that the anaesthetized mother be kept under carefully controlled conditions. After induction of anaesthesia, the mother was placed on a warmed plate with a variable heat control. Using thermistors, the rectal temperature of the mother was recorded and also the temperature between the mother and warm plate; this latter is important since if the temperature goes much above 40° C the mother may start wriggling. The rectal temperature was maintained at 36–38° C. The abdomen was opened and one horn of the uterus mobilized and carefully packed around with moist swabs. The uterus was then incised, the membranes ruptured, and the foetus gently exposed leaving the placenta still attached to the uterine wall. The whole area was irrigated with a slow drip of Tyrode solution with 5 % gelatine at 37° C. The foetus was supported on the turned up end of a Perspex rod 6 mm in diameter, and about 30 cm in length. A microscope lamp was focused to shine down this rod and so served to transilluminate the foetus without altering its temperature. The foetus and, if necessary, the umbilical vessels could then be examined using a dissecting microscope at magnifications up to × 35. In this way it is always possible to observe the heart action in detail when the foetus itself is transilluminated; sometimes these observations can be made even by transillumination through the intact uterine wall (Fig. 1).

Two criteria were used to assess the condition of the foetus, (1) its mobility, which only occurs spontaneously at a foetal age of 16 days or more, and (2) the cardiac activity which included the auricular and ventricular rates, degree of dissociation between these, and emptying of the chambers, all of which could be quite clearly seen.

Assay of 5-HT in tissues. The tissues were extracted and assayed both biologically and fluorometrically as described by Robson & Senior (1964). All the results of assays are expressed in terms of 5-HT base.

Measurement of uterine activity. Uterine activity was measured on mice in the 15 or 16th day of pregnancy anaesthetized with Numal and kept warm as described. The uterus was exposed and its contractions measured using a Cushny myocardiograph (see Robson & Schild, 1938). The levers of the myograph were stitched to the uterus to record the contractions of about 1·5 cm length of muscle. This method was used to measure the contraction of both the longitudinal and circular muscle at a site containing a foetus.

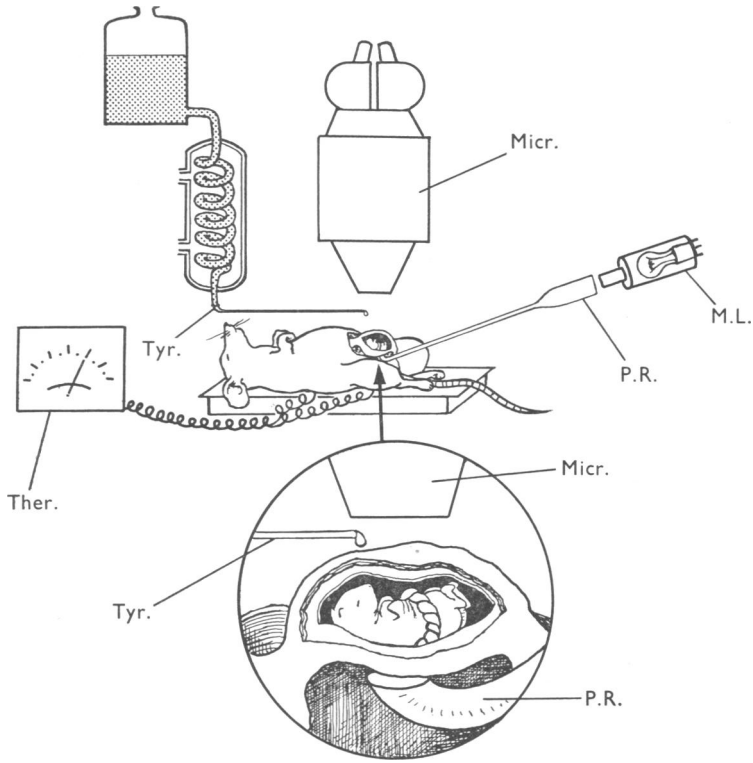

Fig. 1. Diagram of set-up used for the examination of the foetus and umbilical vessels in the anaesthetized mouse. By transillumination using a Perspex rod it is possible to count the foetal heart rate through the intact uterine wall. Micr., dissecting microscope, P.R., Perspex rod, M.L., microscope lamp, Ther. thermistor thermometer, Tyr., Tyrode/gelatine drip.

The heart rate of the foetus in that part of uterus where the contractions were being recorded was counted by transillumination through the intact uterus.

Observation of umbilical cord vessels. The mother was anaesthetized and the foetus exposed as described above. In some experiments the yolk sac splanchnopleure was left intact and the umbilical cord observed by transillumination. In other experiments, including those in which drugs were applied directly to the cord, the amniotic sac was opened and the cord placed across the end of the Perspex rod. In both cases the umbilical vessels could be observed with a binocular dissecting microscope at a magnification of $\times 35$ using an eyepiece micrometer to measure the diameter of the vessels. At this magnification the blood flow within the

vessels is just visible. Special care was taken to apply drugs at body temperature as the vessels are very temperature-sensitive.

Experiments with radio-sodium. When the experiments involved the use of 5-HT, 2 mg of the creatinine sulphate were given subcutaneously. Five minutes later, radio-sodium was injected intravenously as isotonic NaCl into the tail vein of the mother. Control animals received the radio-sodium without previous 5-HT treatment. The isotope used, ^{22}Na (in a dose of 3 μ C) was obtained from the Radiochemical Centre, Amersham, as isotonic sodium chloride and was diluted in non-radioactive isotonic NaCl to give the required amount in a volume of 0·2–0·3 ml.

At various intervals foetuses, placentae, maternal blood (and usually some other tissues as well) were removed and put into test-tubes. In early experiments it was found that during the first 5 min or so after injection, the radio-sodium rapidly diffused extravascularly and the maternal blood level thereafter remained reasonably constant for the next 0·5 hr, i.e. the duration of the experiment. Subsequently only a single blood sample was taken at the end of the experiment and used as a standard with which the radio-sodium content of the various tissues examined was compared. Thus in all experiments the radio-sodium content of the tissues was expressed as a percentage of the maternal blood content.

The levels of radio-sodium found in the blood of the control animals and those treated with 5-HT were substantially the same. Hence, differences in the radio-sodium content of specific tissues in the two groups could not be attributed to differences in the maternal blood level.

Samples were usually stored overnight at $-20°$ C, and the radioactivity then determined, the specimens weighed and the results for each tissue expressed after correcting for background as:

$$\frac{\text{count/g of tissue (wet weight)/sec}}{\text{count/ml. of maternal blood/sec}} \times 100.$$

These experiments were done in two different ways. In the first (four experiments), the mice were anaesthetized with Numal (130 mg/kg subcutaneously) plus a little occasional ether if necessary. They were placed on a warmed table and the body temperature controlled as described. The abdomen was opened and the uterus carefully exposed to ensure that the foetuses were in good condition. The mice were then injected with 5-HT and 5 min later with radio-sodium. At various intervals up to 30 min after the sodium injection, a single foetus with its corresponding placenta was removed after its condition (heart rate, movements and colour) had been noted, and the radioactivity determined. At any site in which the placenta showed any sign of detachment due to previous manipulation of the uterus the placenta and foetus were discarded.

In the second method (three experiments) the mice were left intact and no anaesthetic was used. The mice were injected with the 5-HT and with radio-sodium, and at intervals up to 30 min were rapidly killed with chloroform. The thorax was opened, the heart cut open while still beating and 0·1 ml. of blood taken for counting. The whole uterus was then quickly removed and blotted; the foetuses and placentae were taken for counting.

The results obtained by these two methods were in good agreement and are not discussed separately.

Experiments to measure blood flow using labelled red blood cells. The placenta in the mouse is small (weighing about 100 mg) and is in intimate contact with the uterus, from which it obtains its blood supply through a complex system of vessels. Conventional methods of measuring blood flow are therefore unsuitable for the placenta and it was decided to use a method which involved the administration of a radioactive isotope. Since it had already been shown that 5-HT affected the passage of sodium into the placenta and foetus, the usual method which has been used clinically and which involves the measurement of the rate of disappearance of sodium injected into the chorio-decidual space (Veall & Vetter, 1958) was obviously unsatisfactory. A method was therefore used which would measure the rate of entry of the maternal blood into the placenta.

If labelled erythrocytes are injected intravenously into the mother they will mix with the maternal blood, and the labelled cells will appear in a given organ at a rate depending largely on the blood flow to that organ. Ultimately full mixing with all of the maternal blood occurs and the radioactivity of a particular organ will then remain constant and when compared with that of the mixed venous (maternal) blood will serve as an indication of the blood volume of that organ. Thus in the placenta the rate of increase of radioactivity during the first few minutes after the injection of labelled cells will give a measure of the blood flow to the placenta, and from the final value obtained it will be possible to calculate the fraction of the placenta occupied by maternal blood.

If a drug reduces the blood flow to the placenta, for example, by constricting the uterine blood vessels, this will be mirrored in a decreased rate of entry of radioactivity into the placenta. The final amount of activity obtained, however, will be the same, unless there has also been a change in the maternal blood volume of the placenta. Such a change can also be investigated by allowing full mixing of the labelled cells to take place before administering the drug under investigation. If the drug then produces no change in the radioactivity of the placenta it will indicate that it has no effect on the volume of the placenta occupied by maternal blood.

In early experiments radio-iodinated serum albumen was used instead of labelled erythrocytes, but this was found to be unsatisfactory since the material always contains traces of free iodide which is concentrated by the placenta, thus giving rise to erroneous results. Mouse erythrocytes labelled with chromium were therefore used and this has proved very satisfactory. The method of labelling the cells has been described by Robson & Senior (1964); a volume of 0·2–0·3 ml. of a suspension with an activity of 100μC/ml. was used.

As with the sodium studies the experiments were performed in two ways:

(*a*) The labelled erythrocytes were injected intravenously into the tail vein of anaesthetized mothers, and the placentae (and foetuses) were removed at intervals of up to 30 min.

(*b*) Unanaesthetized mothers were injected intravenously with labelled cells and killed at various times after the injection. When used, the 5-HT (2 mg) was injected subcutaneously 5 min before the administration of the labelled cells. As in the experiments with radio-sodium, 0·1 ml. of the maternal blood was collected from the heart, after opening the thorax.

The results obtained with the two methods were very similar and are not discussed separately.

Counting methods. The activity of the tissues containing radio-sodium or chromium was measured using a Dynatron/Ekco well crystal scintillation counter.

Adrenalectomy and ovariectomy were performed under Numal anaesthesia using dorso-lateral incisions.

RESULTS

Investigation of direct toxic action of 5-HT on the foetus. The first possibility to be considered was that the 5-HT after injection into the mother crossed the placental barrier in amounts which were either directly toxic to the foetus, or which produced effects on its cardio-vascular system which resulted in foetal death. Two types of experiment were therefore performed. In the first, 5-HT was injected subcutaneously into the mother, and the dead or dying foetuses removed and their 5-HT content assayed. In the second type of experiment, the foetuses were exposed in untreated mothers and injected directly, either subcutaneously or intraperitoneally, with 5-HT in amounts equal to or greater than those which had been found following injection into the mother, to see if this resulted in foetal death.

In order to keep the conditions constant, both types of experiment were performed in the same way, i.e. the mother was anaesthetized, and the foetuses exposed. The 5-HT was injected into either the mother or foetuses; the foetuses were examined and the heart rate was counted for the next 60–70 min, or until they were dead. The tissues were then removed and kept at $-20°$ C until assayed for 5-HT content. In addition, the first type of experiment was also performed in intact unanaesthetized mice killed at various intervals after injection. Again foetuses were chosen for assay just after death. Since the results obtained in anaesthetized and unanaesthetized animals were not different, the two groups were combined.

TABLE 1. 5-HT content of foetus and placenta after injection of 5-HT into the mother

Day of gestation	5-HT creatinine sulphate administered (mg)	Min after injection	5-HT content (μg/g)	
			Foetus	Placenta
14	0	—	0·025 ± 0·005*	0·248 ± 0·016
14	2·0	60	0·171 (3)†	1·620 (3)
14	2·0	52	0·073 (6)	1·180 (6)
15	0	—	0·066 ± 0·003*	0·321 ± 0·020
15	2·0	42	0·054 (5)	0·369 (5)
15	2·0	41	0·093 (4)	0·350 (4)
15	2·0	54	0·041 (6)	0·800 (6)
16	0	—	0·073 ± 0·012*	0·358 ± 0·021
16	3·0	84	0·186 (6)	2·100 (6)
16	2·0	12	0·093 (7)	0·547 (7)

* Mean ± S.E.

† Figures in brackets denote number of foetuses or placentae pooled for assay. All taken from one mother in every case.

All foetuses were dead when taken for assay.

The 5-HT content refers to the base.

(*a*) *Values of 5-HT found after injection into the mother.* The results are shown in Table 1.

It can be seen that the foetuses of untreated animals contain only small amounts of 5-HT, less than 0·1 μg/g of tissue. The values in foetuses from mothers treated with 5-HT were either similar to the control values or only slightly elevated. In the placenta, some of the figures were several times the control values.

It can thus be concluded that at the time when the foetuses were all dead as a result of the injection into the mother, the amounts of 5-HT found in the foetus were only moderately or not at all raised above those normally present in the untreated foetus.

(*b*) *Effect of 5-HT injected into the foetus.* It was decided to inject the foetuses (weight about 100–350 mg) with 1–5 μg of 5-HT creatinine sulphate (i.e. 0·43–2·17 μg 5-HT base) which is about 6–50 times the amount found at foetal death after administration of the substance to the mother.

The foetuses were observed for at least 1 hr after the direct injection of 5-HT. In no instance was the foetus killed by this amount of 5-HT, and usually heart rates were well maintained, with active movements in the older foetuses. After the period of observation the injected foetuses with their placentae were removed and the 5-HT contents determined. The amounts of 5-HT found were considerably higher (about 1·5–8·5 times) than those in foetuses which died after the injection of the 5-HT into the mother.

It can be concluded from these experiments that:

(*a*) following the injection of 5-HT into the mother, very little amine passes through into the foetus;

(*b*) the direct injection of much larger amounts into the foetus produces no obvious toxic effects on the foetus.

Effect of 5-HT on uterine contractions. A well known effect of 5-HT is that it causes contractions of the uterus *in vitro*. It seemed possible that such an effect, occurring *in vivo*, might account for the lethal action of the drug on the foetuses, and this was investigated.

Experiments were performed in five mice, 15 or 16 days pregnant. In four of these the contractions of the longitudinal muscle, and in one those of the circular muscle, were recorded. In four of these experiments including that in which the circular contractions were recorded, the injection of 2 mg 5-HT subcutaneously into the mother had little or no effect on uterine activity. Nevertheless, within 15–30 min of the injection all the foetuses were dead.

In the 5th animal the effect of 5-HT was compared with that of oxytocin with the result shown in Fig. 2. One unit of oxytocin injected subcutaneously into the mother caused a marked increase in tone and in the amplitude of the uterine contractions. The viability of the foetus was not affected and 32 min later the foetal heart rate was unimpaired at 196 beats/min. The mother was then injected subcutaneously with 2 mg 5-HT which caused some increase in uterine activity though the effect was less marked than that of oxytocin. Within 10 min the foetal heart rate had fallen to 9 beats/min, and 15 min later the foetal heart had stopped beating.

It thus seems that foetal death produced by the administration of 5-HT is not a result of excessive uterine activity since oxytocin, which had a more marked effect on uterine activity, did not produce any deleterious effect on the foetus.

Observations on the umbilical vessels

Injection of 5-HT into the mother. In two mice, 15 days pregnant, and in two, 16 days pregnant, the amniotic sacs were opened, the foetuses left attached to the placentae *in situ*, the cord vessel diameter measured, and

the foetal heart rate counted. After the umbilical vessels had recovered from the effects of the manipulation, the mothers were injected subcutaneously with 2·0 mg of 5-HT.

In a 5th animal, 15 days pregnant, the foetuses were left in the amniotic sacs, and the umbilical vessels measured through the sac walls. The mother was then injected intravenously with 1·3 mg 5-HT.

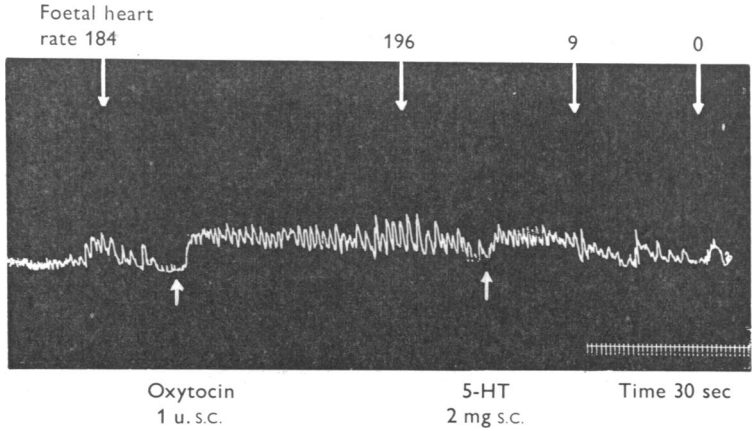

Fig. 2. The effect of oxytocin and of 5-HT on uterine contractions and foetal heart rate in the anaesthetized mouse is shown. The contractions of the longitudinal muscle are recorded at a part of the uterus containing a foetus. Top arrows show foetal heart rates counted by transillumination. Lower arrows show firstly the injection of 1 unit of oxytocin subcutaneously (s.c.) into the mother, and secondly of 2 mg 5-HT creatinine sulphate. Time intervals = 30 sec.

In all animals the foetuses were observed until they died (usually in 30–40 min). No constriction of the vessels was seen until the foetuses were either dead, or the heart had almost stopped beating (rate less than 20/min and very irregular). In one case the vessels dilated, the artery from 310 to 390 μ and the vein from 285 to 310 μ about 10 min before the heart started to fail. In two of the animals marked constriction of the umbilical vessels was seen *after* the death of the foetuses.

Application of drugs directly to the cord. Experiments were done on four foetuses in two mice, at the 15th day of pregnancy. The cord was exposed and kept moist in warm gelatine/Tyrode solution. The drugs were dissolved in Tyrode and warmed to 37° C before application to the surface of the cord. The test solution (1 ml.) was then applied to the cord over a period of 2–3 min. To be certain that there was no difficulty of penetration of drugs, the membranes surrounding the cord vessels were dissected off in two of the foetuses.

5-Hydroxytryptamine. Solutions of 5-HT creatinine sulphate in concentrations ranging from 10 μg/ml. to 1·0 mg/ml. had no effect on the

diameters of the umbilical vessels. A concentration of 10 mg/ml. did produce a constriction on the artery of one foetus, from 400 to 245 μ, which lasted for only 3 min but on two other foetuses (one in a different mother) the same concentration produced no effect.

Adrenaline, noradrenaline, acetylcholine and histamine. Adrenaline, noradrenaline or acetylcholine in concentrations ranging from 10 μg/ml. to 1 mg/ml., and histamine 10 μg/ml. had no effect on the diameters of the vessels.

Temperature sensitivity. The umbilical vessels constricted if solutions of any of the above drugs, or even Tyrode solution, were applied at room temperature.

This constriction of the vessels confirmed that the technique of observation was satisfactory and that constriction could be measured if produced.

Effects on placental function

Transfer of radio-sodium from the maternal blood into the placenta and foetus. Experiments were done in order to determine whether 5-HT interfered with the passage of nutrients from mother to foetus. Sodium is a good indicator of placental function with regard to transfer. Radio-sodium was injected intravenously into the mother and the content in the placenta and foetus measured after various intervals as described in Methods.

Seven experiments were done in mice on the 15th or 16th day of pregnancy but as the results were not significantly different from each other they are all presented together.

It was found that the passage of radio-sodium into the placenta was greatly decreased by the 5-HT treatment, and that radio-sodium transfer to the foetus was almost completely prevented. Figure 3 shows the average percentage of radio-sodium present in the placenta and foetus during the first half hour following injection.

The results show that sodium passes rapidly into the placenta in untreated animals during the first 10–15 min by which time the radio-sodium content of the placenta is about 70% of that found in the maternal blood. In the foetus, equilibrium occurs much more slowly and is not complete at 30 min, though at that time the level in the foetus is already about 35% of that present in the maternal blood. In the animals treated with 5-HT the passage of radio-sodium into the placenta is much slower so that by 30 min the placenta contains less than 25% of that present in the maternal blood. The passage into the foetus is very slow indeed, and even at 30 min the foetal content is only about 2% compared with 35% in the controls.

The impaired transfer of sodium to the foetus in the treated animals was not a result of foetal death since at 10 min all the foetuses were alive

although the heart rates were slower in some of them; at that time the radio-sodium content was less than 1/100th of that in the control foetuses. By 20 min the majority of the foetuses were alive though the heart beat was usually slow and irregular. At 30 min most of the foetuses were dead.

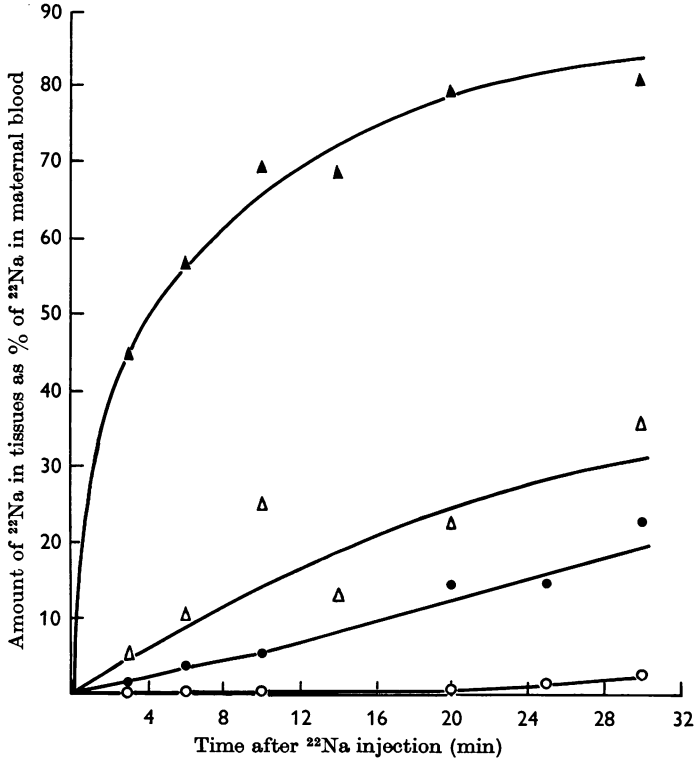

Fig. 3. Showing the effect of 5-HT on the rate of entry of ^{22}Na into the placenta and foetus. The sodium was injected intravenously at zero time. In one group 2 mg 5-HT creatinine sulphate was injected subcutaneously into the mothers 5 min previously. ▲—▲ Placentae in untreated mice. △—△ Foetuses in untreated mice. ●—● Placentae in 5-HT treated mice. ○—○ Foetuses in 5-HT treated mice.

Effect of 5-HT on blood flow to the placenta. It seemed possible that the reduced transfer of sodium produced by 5-HT might simply be due to a reduction in the blood supply and vascularity of the placenta. The blood flow to the placenta and the maternal blood volume of the placenta was therefore measured. As in the experiments with radio-sodium, the radio-chromium content of the placenta and other tissues is expressed as a percentage of that in the maternal heart blood.

Altogether nine mice, 16 or 17 days pregnant, were used, five controls and four treated with 5-HT, three in each group involving the use of anaesthetized mice.

The results are shown in Fig. 4. In the control animals the maternal systemic blood mixes rapidly with that in the placenta so that in less than 5 min the amount of radioactivity in the placenta had practically reached a maximum and thereafter the value remains constant at around 17%. In the treated animals the rate of entry of labelled cells into the placenta is markedly reduced, so that it took about 20 min for the value to reach the maximum. This maximum was not significantly different from that found in the controls.

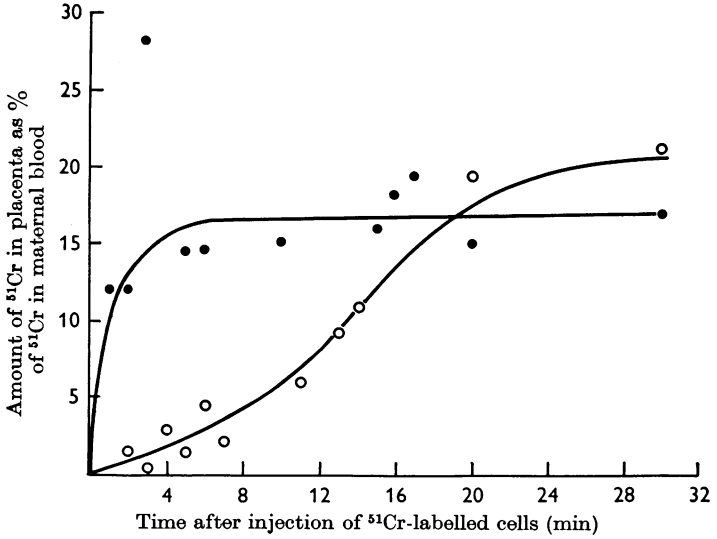

Fig. 4. Showing the effect of 5-HT on the rate of entry of ^{51}Cr-labelled mouse erythrocytes into the mouse placenta. The labelled cells were injected intravenously into the mother at zero time. In one group 2 mg 5-HT creatinine sulphate was injected subcutaneously 5 min previously. ●—● Control animals not treated with 5-HT. ○—○ 5-HT treated animals.

This maximum value of 17% found both in the control and treated animals represents the percentage of the placenta occupied by maternal blood. As a rule no radioactivity was found in any of the foetuses.

Effect of 5-HT on the maternal blood volume of the placenta. In the experiments described the amount of labelled blood present in the placenta of animals treated with 5-HT for about the first 20 min was less than in the untreated animals. This could be due either to: (*a*) constriction of the blood vessels supplying or draining the placenta, causing reduced rate of entry of blood into the placenta, (*b*) constriction of the maternal blood space of the placenta lasting for about 20 min, or (*c*) a combination of these two effects.

For anatomical reasons the second explanation seemed unlikely but was, nevertheless, investigated in the following way.

Two unanaesthetized mice were injected intravenously with labelled red cells. Twenty-five minutes later, i.e. after full mixing had occurred, one animal received 2 mg of 5-HT subcutaneously. Five minutes later both mice were killed and the placentae removed and counted.

In the control mouse the average maternal blood content of the placentae was $17{\cdot}1 \pm 2{\cdot}75\%$, and in the treated animal the value was $15{\cdot}9 \pm 1{\cdot}07\%$, not significantly different. It would thus appear that 5-HT does not cause constriction of the maternal blood spaces within the placenta. The reduction in the amount of radioactivity observed in the placentae for 20 min after the administration of 5-HT (as seen in the experiments described above), must therefore be due to constriction of the vessels supplying or draining the placenta.

Foetal survival following death of the mother. The results described strongly suggest that 5-HT produces death of the foetuses by interfering with their nutrition through an action on the maternal blood supply to the placenta. It is known, however, that foetuses are very resistant to anoxia and this raised the question whether anoxia could result in foetal death in 0·5 hr, i.e. the period that elapses before 5-HT produces its fatal effect.

To investigate this six mice (16 days pregnant) were decapitated and exsanguinated. They were then immersed in a water-bath maintained at 37° C. At various intervals one mouse was removed from the water-bath, and the foetuses examined. The sequence of events was as follows: after 15 *min* occasional foetuses still showed movements of the limbs. All showed abnormalities in cardiac action consisting of slowing, irregularities, and auricular-ventricular dissociation. After 20 *min* movements were no longer seen and in the majority of foetuses the heart had stopped beating. In the remainder (six out of fifteen) the ventricular rate was down to 10–20 beats/min and was irregular. Auricular contractions were only seen in one foetus. After 30 *min* sixteen out of eighteen foetuses were dead and the hearts had stopped beating. The remaining two had an irregular ventricular rate of about 10 beats/min.

To compare the sequence of events described above with that following the administration of 5-HT, three mice (16 days pregnant) were injected with 2 mg 5-HT subcutaneously and left intact until killed, when the foetuses were similarly examined. After 30 *min* no movements were observed in any of seven foetuses. In six of them, however, the ventricle (but not auricle) was still beating irregularly at about 10 beats/min. After 40 *min.* no foetal movements were seen, and in nine out of fifteen the heart had stopped completely. In four others the ventricular rate was very slow (10–20 beats/min) with no auricular beats.

It would thus seem that anoxia caused either by death of the mother, or

by administration of 5-HT, produces a similar sequence of effects on the foetus.

Effect of adrenalectomy and ovariectomy. It seemed possible, though unlikely, that the effect of the 5-HT might be mediated through the adrenals or ovaries. To exclude this possibility seven mice (16 days pregnant) were adrenalectomized and in two of these the ovaries were also removed. As expected, this itself had no effect on the foetuses when examined 3–4 hr later. Next 2 mg 5-HT was injected subcutaneously into the mothers and produced death of the foetuses within the usual time.

It is therefore concluded that 5-HT can exert its lethal effect on the foetus in the absence of the adrenals and ovaries.

DISCUSSION

These experiments were undertaken in order to elucidate the mechanism by which 5-HT so rapidly produces its lethal effect on the foetus if injected into mice during the second half of pregnancy. Originally the most likely hypothesis seemed to be that this was merely a direct toxic effect on the foetus, which was more susceptible to the drug than the mother. This is known to be true for a number of nucleotoxic drugs such as 6-diazo-5-oxo-L-norleucine (DON) which will produce death of the foetus in a dose less than 1/100 of that required to kill the mother (Jackson, Robson & Wander, 1959). Such a finding, however, was not obtained with 5-HT since, following the injection of this substance into the mother, foetal death may occur at a time when the 5-HT content of the foetus has not appreciably increased. Furthermore, the injection of much larger amounts directly into the foetus produced no deleterious effects.

One of the well known effects of 5-HT in rodents is to contract the uterus *in vitro*; it seemed possible that such an effect might in some way cause the death of the foetus *in vivo*. This possibility was definitely ruled out since 5-HT produced little increase in uterine tone in the intact animal, while oxytocin, though it markedly increased uterine contractions, had no effect on the viability of the foetus.

Pepeu & Giarman (1962) found that 5-HT rapidly produced death of the foetus in rabbits and they ascribed this in part at least to a constriction of the vessels in the umbilical cord. Panigel (1959) has also reported that the human umbilical vessels *in vitro* are very sensitive to 5-HT. In the present experiments on mice no such action was observed on the cord vessels *in situ*.

All these negative results suggested that 5-HT produced its action indirectly, through the mother, probably through an acute impairment of foetal nutrition. It is well known that foetuses can survive prolonged periods of anoxia and even for some time following the death of the mother.

Our results have shown that if precautions are taken to maintain the mother and foetuses at about 37° C, then the death of the mother is rapidly followed by depression of foetal cardiac activity and foetal death may occur within 20 min. Thus if 5-HT can markedly impair foetal nutrition this could lead to foetal death within the period observed.

Our results show that 5-HT greatly depresses the transfer of radio-sodium from the mother to the foetus. If it is accepted that sodium is a good indicator of placental transfer function, then these results suggest that 5-HT could produce its lethal effect on the foetus by markedly reducing the transfer function of the placenta. This is further supported by the fact that the transfer of radio-sodium to the foetus is almost completely inhibited during the 30 min period of observation following the 5-HT injection, and that during this time the sequence of events is remarkably like that seen in the foetus following the death of the mother. In both cases, there is an early deterioration of cardiac function and cessation of spontaneous movements with slowing of the heart rate, dissociation between the auricle and ventricle, incomplete emptying with finally only occasional ventricular beats preceding death. Attempts to measure the oxygen tension within the foetus have so far been unsuccessful since no sufficiently small and yet stable oxygen electrode is available.

The experiments with labelled erythrocytes show that, if injected intravenously into pregnant mice, they mix with the maternal blood in the placenta and that this mixing is nearly complete within 5 min. Once mixing has been completed no further increase in the radioactivity of the placenta occurs so that it can be concluded that the final volume reached (17 %) represents the maternal blood volume of the placenta. This assumes that there is no change in the haematocrit value within the placenta.

In the 5-HT treated animals the labelled blood mixes more slowly with that in the placenta so that full mixing takes about 15–20 min, but reaches the same final volume as in the control animals. Since we have also shown that no vaso-constriction occurs within the placenta, this slower entry of maternal blood indicates reduced blood flow through the placenta. Such an effect can be explained by constriction of the vessels supplying and or draining the placenta.

From the data given it is possible to calculate the actual blood flow through the placenta. If it is assumed that the inflowing blood mixes completely with that already in the placenta, i.e. that there is a good degree of turbulence within the placenta, then the flow rate is given by the formula:

$$\ln \frac{c}{c-a} = \frac{Qt}{V}$$

where Q = flow in ml./min,
t = time for fractional filling,
V = volume of placenta,
c = concentration of labelled cells in the inflowing blood,
a = concentration of labelled cells in the placenta at time t.

From the data obtained it can be calculated that the blood flow in the normal mouse placenta on the 16th or 17th day of pregnancy is 87 μl./min/g wet wt. Upon injection of 2 mg 5-HT creatinine sulphate subcutaneously, the flow is reduced to 4 μl./min/g wet wt. If the flow follows Poiseuille's equation, this reduction would be produced by a constriction of the vessels only to 46% of their original diameter. These figures are calculated from an average placental weight of 90 mg, a total blood volume in the placenta of 17·2% and a 5 min mixing volume taken from Fig. 4 of 15·8% in the controls and 1·8% in the 5-HT treated animals.

Thus the lethal action of 5-HT on the foetus seems dependent on an interference with foetal nutrition. Such an effect could conceivably be due to two types of action:

(1) the reduction of the blood supply to the placenta in some way interferes with the passage of sodium through the placenta from the mother to the foetus. This could result merely from changes in the haemodynamics leading to a decrease in the passive transfer of sodium to the foetus though it seems unlikely that this could produce such a marked effect (Davson, 1959). Alternatively, since the oxygen consumption of the placenta is known to be high (Campbell, Dawes, Fishman, Hyman & James, 1965), there could be an interference with placental nutrition or oxygenation which would inhibit the mechanisms responsible for the active transport of sodium.

(2) the action of 5-HT is exerted directly on the membranes separating the maternal and foetal circulations, reducing its permeability.

That 5-HT can produce a 'selective alteration in cell membrane permeability' has been shown by Pickles (1956). At present it is impossible to differentiate between these two effects but it is hoped to discuss this further in the light of additional experiments.

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