# THE SITE OF INCREASED FORMATION OF HISTAMINE IN THE PREGNANT RAT

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During the last third of pregnancy the rat produces large amounts of histamine, as is indicated by its excretion in the urine (Kahlson, Rosengren & Westling, 1958). In undisturbed pregnancy the urinary excretion of histamine increases from about the 15th day onwards. This increase reaches a peak 1–2 days before parturition and falls steeply to the non-pregnant level at about 1 day before parturition. The amount excreted in 24 hr in some instances was of the same magnitude as the calculated total amount of histamine present in the whole rat. None of the tissues examined in pregnant rats were conspicuously richer in histamine than corresponding tissues in non-pregnant rats. The uterus, placentas and foetuses had a rather low histamine content. In the attempt to identify the tissue responsible for the excessive formation of the amine we were guided by the observation that the urinary excretion of histamine was greater the larger the number of young in the litter.

Three types of experiments are included in the present report. First, the formation will be recorded of $^{14}C$-histamine from injected $^{14}C$-L-histidine in pregnant and non-pregnant rats. Secondly, the effect of removal of the foetuses on the urinary excretion of histamine will be described. Thirdly, a record is presented of the rate of histamine formation *in vitro* in foetuses and new-born young. A brief report of some of the latter two types of experiments has been given to the Physiological Society (Kahlson, Rosengren, Westling & White, 1958).

## METHODS

*Animals and drugs.* The rats were of the same stock as those used in the experiments already reported, in which the care of the animals, the collection of urine samples and the synthetic histamine-free diet is described (Kahlson, Rosengren & Westling, 1958). In female rats fed on this diet almost all the urinary histamine is in the free form. In some experiments aminoguanidine sulphate (Eastman Kodak) in a dose of 20 mg/kg was injected daily under the skin to diminish the destruction of histamine by histaminase. The duration of pregnancy in the stock of rats used was 22–24 days.

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*Assay of urinary histamine.* Urine was collected in 24 hr samples and free histamine estimated on the guinea-pig's gut as already described (Kahlson, Rosengren & Westling, 1958). The histamine values given in the present paper refer to free histamine expressed as micrograms of the base.

*Removal of the foetuses* was done under ether anaesthesia. A fairly large mid-line incision was made through the skin of the back and then, on both sides, parallel but shorter incisions were made laterally through the muscles. The left ovary and uterine horn were gently withdrawn and examined, and each foetus was removed through a small hole made opposite to the attachment of its placenta. After replacement of the left ovary and uterine horn the same procedure was performed on the right side. Within 1 hr after the closure of the muscle and skin incisions the rats moved around normally. To see whether the procedures employed in removing the foetuses influenced the urinary excretion of histamine, a sham operation was performed in two pregnant rats: this involved ether anaesthesia and all the procedures mentioned except that no incisions were made in the uterine horns and the foetuses were left in place.

*Measurement of histamine formation in vitro.* This was done mainly by methods developed in Schayer's laboratory (Schayer, Davis & Smiley, 1955; Schayer, 1956). During a visit of Dr Schayer to our laboratory he modified the original method. The standard technique employed in our laboratory is as follows: Tissues minced with scissors and suspended in the same volume (0·5–2·0 ml.) 0·1 M sodium phosphate buffer of pH 7·4 containing 0·2% glucose were incubated with 40 $\mu$g $^{14}$C-L-histidine monohydrochloride for 3 hr at 37° C under pure nitrogen (to prevent the action of oxidizing enzymes). This histidine labelled in the 2-position in the imidazole ring has an activity of $9\cdot8 \times 10^6$ counts/min/mg base, measured at zero thickness by means of a flow counter. (The radioactive histidine used was synthesized by Dr R. W. Schayer). 1 $\mu$g of the labelled histidine quantitatively converted to $^{14}$C-histidine dipicrate should give 650 counts/min measured at infinite thickness in a flow counter.

At the end of the incubation 66·4 mg histamine dihydrochloride as carrier and 50 mg L-histidine monohydrochloride as a diluent were added. (Non-isotopic L-histidine was added to dilute the $^{14}$C-L-histidine to approximately the same specific activity as the carrier histamine; this reduced the hazard of contamination of the carrier by a minute quantity of histidine of extremely high specific activity). The amount of carrier (66·4 mg) is equivalent to 40 mg of base and 206 mg of the dipicrate. In order to ensure complete mixing the incubation mixture was stirred repeatedly for 15 min. Trichloroacetic acid (TCA) was added to give a final concentration of about 5%. After at least 1 hr the mixture was filtered through paper and the precipitate washed with 5% TCA, after which the TCA was extracted from the filtrate with ether.

*To extract the histamine* the filtrate was diluted with water to 30 ml. and 3 ml. 11 N-NaOH and 7·5 g anhydrous Na₂SO₄ were added; after this extraction with *n*-butanol was carried out three times with 15 ml. each time for 15 min. All extractions were done using a mechanically driven shaker unless otherwise stated. The combined butanol fractions (45 ml.) were extracted twice with 20 ml. portions of an alkaline solution of L-histidine monohydrochloride (1 mg/ml.). This histidine solution (40 ml.) was extracted twice with 20 ml. portions of butanol for 10 min. All butanol fractions were then combined (45 + 40 = 85 ml.) and extracted by hand-shaking for a few minutes with successive samples of 20 ml. 0·5 N-HCl, 15 ml. 0·1 N-HCl and 15 ml. 0·1 N-HCl. The combined HCl extracts were evaporated to dryness.

*Purification* of the extracted histamine was carried out by dissolving the residue, consisting mainly of histamine dihydrochloride, in 2 ml. water and adding 180 mg picric acid in 2 ml. absolute ethyl alcohol. The crystals of histamine dipicrate were isolated by filtration (glass-sintered Buchner Funnel, 'Pyrex', capacity 2 ml.), rinsed with cold absolute ethyl alcohol and ether and transferred to a counting plate and counted.

For final purification the histamine was converted into pipsyl histamine. To this end the picric acid was removed by passing the dissolved histamine dipicrate through a small anion exchange column (Dowex-1 in the chloride form). The dried eluate (consisting of histamine dihydrochloride) was dissolved in 2–3 ml. water. To this solution were added 280 mg pipsyl chloride (para-iodobenzenesulphonyl chloride), dissolved in 3 ml. dioxane, and 250 mg sodium bicarbonate.

This mixture was left for 30 min in a mechanical vibrator. Water was gradually added until crystallization was complete. The crystals were isolated by filtration and rinsed with cold alcohol. The crystals of pipsyl histamine were dissolved in hot acetone, active carbon added and the carbon then removed by filtration. The purified pipsyl histamine crystallized on adding water gradually. After filtering, the crystals were rinsed with 25 % (w/v) cold ethyl alcohol, transferred to a counting plate and counted. The sample was recrystallized (including treatment with active carbon) until it showed constant radioactivity.

*Measurement of radioactivity* was made at infinite thickness in a flow counter (98·7 % helium and 1·3 % butane) on crystals of histamine dipicrate and pipsyl histamine respectively. The sample was mounted on a plate of about 2·5 cm diameter. At least 1000 counts were taken for each sample on a background of 21–24 counts/min. The values given for $^{14}$C-histamine, in the form of histamine dipicrate, are counts/min above background.

*Determination of urinary $^{14}$C-histamine.* To each 24 hr sample of urine 66·4 mg histamine dihydrochloride (carrier) and 50 mg L-histidine hydrochloride (diluent) were added. After thorough mixing the sample was subjected to the procedures described above as applied to solutions after TCA had been removed.

### RESULTS

### *Urinary excretion of $^{14}$C-histamine after injection of $^{14}$C-histidine*

After injection of $^{14}$C-L-histidine under the skin in rats, $^{14}$C-histamine appears in the urine (Schayer, Wu & Smiley, 1954). To see whether the high level of urinary histamine during the last third of pregnancy, as noted by bioassay, is paralleled by increased histidine decarboxylase activity, the urinary excretion of $^{14}$C-histamine subsequent to a subcutaneous injection of $^{14}$C-L-histidine was determined.

The results are given in Table 1. To reduce the destruction of the formed $^{14}$C-histamine, the rats were given the histaminase-inhibitor aminoguanidine, 20 mg/kg once daily, during the whole course of the experiment. 2 mg/kg $^{14}$C-L-histidine hydrochloride was injected under the skin at about 9 a.m. and urine was collected for the following three 24 hr periods. In Table 1 the figures for $^{14}$C-histamine excreted are given as a fraction of the total amount

TABLE 1. Urinary excretion of $^{14}$C-histamine after subcutaneous injection of $^{14}$C-histidine 2 mg/kg in pregnant and non-pregnant female rats. The figures within parentheses indicate the day of pregnancy

| Rat no. | Weight (g) | Sexual state | $^{14}$C-histamine excretion (molecules/$10^6$ molecules histidine injected) | | |
| --- | --- | --- | --- | --- | --- |
| | | | 1st day | 2nd day | 3rd day |
| 5 | 200 | Non-pregnant | 440 | 100 | 70 |
| 11 | 155 | 5 days after parturition | 530 | 140 | 90 |
| 12 | 200 | Pseudopregnant | 640 | 90 | — |
| 15 | 320 | Assumed pregnant, no young born | 480 | 100 | 50 |
| 16 | 275 | Non-pregnant | 570 | 100 | 60 |
| 6 | 255 | Pregnant, 9 young | 1720 (19) | — | — |
| 11 | 165 | Pregnant, 8 young | 1950 (18) | 850 (19) | 480 (20) |
| 13 | 315 | Pregnant, 9 young | 1370 (18) | 280 (19) | 230 (20) |
| 14 | 290 | Pregnant, 10 young | 1380 (16) | 450 (17) | 260 (18) |

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theoretically obtainable from the injected ^{14}C-histidine. To avoid decimals this fraction was multiplied by $10^6$. The figures can therefore be said to indicate the number of histamine molecules excreted per $10^6$ molecules of injected histidine.

From Table 1 it will be seen that the rats which received injections of histidine during the last week of pregnancy excreted more ^{14}C-histamine than the non-pregnant or pseudopregnant rats. Special mention should be made of rats nos. 14, 15 and 16 which were studied simultaneously. Nos. 14 and 15 had been mated at about the same time and were assumed to be pregnant on
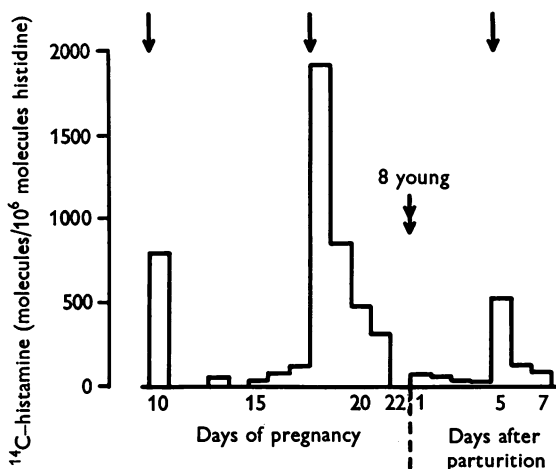


Fig. 1. Urinary excretion of ^{14}C-histamine in rat no. 11, wt. about 160 g. The ordinate represents ^{14}C-histamine expressed as number of histamine molecules excreted per $10^6$ molecules of injected histidine. Each arrow at the top of the diagram indicates subcutaneous injection of 330 $\mu$g ^{14}C-histidine.

the evidence of vaginal smears. In rat no. 14 the urinary histamine, as determined on the guinea-pig's gut, was high (370–670 $\mu$g/24 hr) as was the excretion of ^{14}C-histamine. This rat subsequently bore ten young. Rat no. 15, however, behaved differently. The urinary histamine from the 15th day onwards was not increased and the excretion of ^{14}C-histamine was not elevated as compared with the non-pregnant rat no. 16. Eventually rat no. 15 bore no young, indicating that this rat had been either pseudopregnant or that the foetuses had been absorbed in the uterus.

Fig. 1 shows the excretion of ^{14}C-histamine in a female rat (no. 11). Three subsequent injections of the same amount of ^{14}C-histidine were given, the first at the 10th day of pregnancy, the second at the 18th day and the third at the 5th day after parturition (8 young). The second injection of ^{14}C-histidine evoked the largest excretion of ^{14}C-histamine.

The experiments recorded here confirm, by a more direct method, our previous observations of an increased production of histamine during the last week of pregnancy (Kahlson, Rosengren & Westling, 1958).

## *Urinary histamine after removal of the foetuses*

It is known from various reports that if the foetuses are removed deliberately without dislodging the placentas, the rat remains physiologically pregnant by a series of criteria easily recognizable at autopsy (for literature see Newton, 1949). This technique of foetal removal with placental retention appeared useful for the investigation of the part played by the foetuses and the other products of conception in the increased formation of histamine.
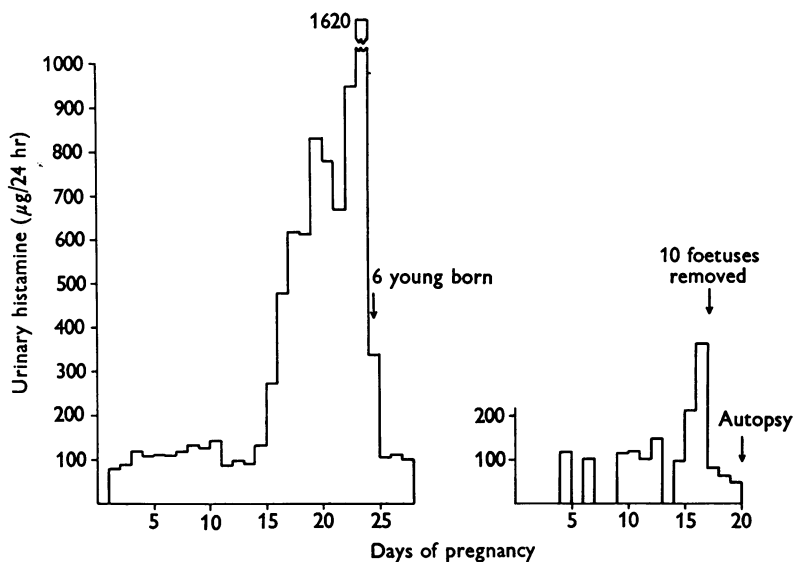


Fig. 2. Urinary excretion of histamine in undisturbed pregnancy (left side of the figure) and in a rat where the foetuses were removed at the 17th day of pregnancy (right side). Throughout the whole course of the observations the rats were under the influence of the histaminase-inhibitor aminoguanidine.

In three rats the foetuses were removed at about the 17th–19th day of pregnancy when increased formation of histamine was indicated by high values for urinary histamine. At autopsy 3 days after removal of the foetuses it was noted that the attributes of pregnancy persisted except for the absence of foetuses: the foetal and maternal placentas were retained, the endometrium was proliferated and the ovaries contained large corpora lutea. In two other rats a sham operation was performed. A typical experiment is shown in Fig. 2. Both rats were given aminoguanidine to reduce the rate of deamination of formed histamine. It will be seen that in undisturbed pregnancy, ni the

rat represented in Fig. 2 (left side), the urinary excretion of histamine increases from the 15th day onwards. This increase reaches a peak the day before parturition. On the day of parturition the urinary histamine falls steeply. In the other rat the foetuses were removed at the 17th day of pregnancy, that is, on the second day of increased excretion of histamine and before it had reached its peak value. After removal of the foetuses the urinary histamine rapidly reverted to the pre-pregnant level or even lower.

TABLE 2. Urinary histamine in three rats from which the foetuses were removed and in two rats subjected to sham operation. The figures for histamine excretion followed by parentheses are mean values. The number of observations are given in parentheses. Rat no. 12 was under the influence of aminoguanidine

| | Removal of foetuses | | | Sham operation | |
|---|---|---|---|---|---|
| Rat no. ... | 11 | 12 | 20 | 25 | 26 |
| Day of pregnancy at operation | 19 | 17 | 17 | 19 | 18 |
| No. of young in litter | 4 | 10 | 7 | 7 | 7 |
| Histamine excretion during pregnancy ($\mu$g/24 hr) | | | | | |
| Days 1–7 | 68 (2) | 113 (2) | 57 | 45 (5) | 78 (6) |
| Days 8–14 | 70 (2) | 117 (5) | 49 (6) | 40 (6) | 60 (7) |
| 1 day before operation | 96 | 365 | 114 | 52 | 177 |
| 1 day after operation | 22 | 84 | 42 | 78 | 186 |
| 2 days after operation | — | 65 | 24 | 82 | 302 |
| 3 days after operation | — | 51 | 24 | 68 | 216 |

Observations on five rats are summarized in Table 2. In all three rats from which the foetuses were removed the increased formation of histamine, as reflected by its urinary excretion, ceases promptly. The values for urinary histamine are even lower than the values before pregnancy and before the onset of the elevation. This may be due to increased inactivation of histamine in the remaining products of conception. The pregnant rat uterus and placenta are reported to exert high histamine-inactivating activity (Roberts & Robson, 1953), which might in part persist even under the influence of aminoguanidine in the doses used in this study.

After the sham operation performed in the rats of Table 2 the onset and pattern of increased formation of histamine, as inferred from the urinary content, is essentially the same as in undisturbed pregnancy.

A fourth pregnant rat was operated on at the 17th day of pregnancy with the purpose of removing all the foetuses. The subsequent fall in urinary histamine to the low expected level did not, however, occur. At a second operation 3 days later is was found that out of a total of seven foetuses one had been left in place. After removal of the remaining foetus the urinary histamine rapidly fell below the pre-pregnant level.

From these experiments it appears that the increased formation of histamine during the last third of pregnancy depends on the presence of the foetuses and not on other structures characteristic of pregnancy.

*Histamine formation in vitro in the foetus and in various
tissues from pregnant and non-pregnant rats*

In this part of the study the site of histamine formation was investigated by a more direct approach. Various tissues of the pregnant mother were examined at times when the urinary excretion of histamine was high. Foetuses were removed and examined during the period of high histamine excretion and during the period of decreasing excretion. The histamine formation was also estimated in new-born young. In addition, determinations were made on three non-pregnant female rats. The results are summarized in Table 3 from which it will be seen that in pregnant as well as in non-pregnant rats the stomach is most potent in the formation of histamine, followed next by the lungs, whereas

TABLE 3. Histamine formation in rat tissues, foetuses and new-born young.
The figures are counts/min/g tissue

|  | Non-pregnant females | | | Mothers | |
|---|---|---|---|---|---|
| Lung | 89 | —* | — | 90 | 150 |
| Stomach† | — | 690 | 450 | 980 | 370 |
| Kidney | 16 | 3 | — | <4 | 3 |
| Uterus | <44 | 5 | — | <7 | 6 |
| Maternal placenta | — | — | — | <10 | — |
| Foetal placenta | — | — | — | <5 | 9 |
| Brain, total | 26 | 20 | 35 | — | — |
| Abdominal skin | — | — | — | — | 9 |

Foetuses
Day of pregnancy

| | Day of pregnancy | | | New-born | |
|---|---|---|---|---|---|
| 1. | 17 | 1110 | 1. | <3 hr old | 76 |
| 2. | 17 | 2470 | 2. | <1 hr old | 76 |
| 3. | 17 | 2360 | 3. | Collected from the | { 210‡ \ 400 |
| 4. | 19 | 1510 | | vagina | |
| 5. | 20 | 2480 | | during | |
| 6. | 21 | { 290‡ \ 380 | 4. | parturition | { 460‡ \ 970 |
| 7. | 22 | 410§ | | | |
| 8. | 23 | 520 | | | |
| 9. | 23 | { 1080‖ \ 1090 | | | |

* Not determined.    † Total stomach wall.    ‡ Different foetuses.
§ Two foetuses pooled.    ‖ Duplicates.

the uterus, the maternal and foetal placentas and the other tissues examined show low activities. In the tissues investigated there was no conspicuous difference between pregnant and non-pregnant female rats as regards histamine formation. The observations on stomach and lung are in agreement with those of Schayer (1956) who found that in the rat the stomach is the most active in decarboxylating ^{14}C-histidine, followed by the lung.

The foetus, during the period of increased urinary excretion of histamine (nos. 1–5 in Table 3), is very potent in producing histamine. The new-born rat, on

the day after birth, produces histamine at a low rate. A few observations were made during the time between the expected peak values of histamine excretion and low values at term. The foetuses were removed 1 and 2 days respectively before the expected term. The advent of parturition was recognized by a fall in histamine excretion. The results are summarized in Table 3 (nos. 6–9), from which it will be seen that in these rats the rate of histamine formation in the foetuses appears somewhere intermediate between the peak figures and the low values at term.

*Effect of inhibitors of histidine decarboxylase.* It appears likely that the formation of histamine by the foetus under the present conditions results from the action of histidine decarboxylase. Accordingly, the effects of two inhibitors of this enzyme, semicarbazide and hydrazine, were studied in a few experiments. Three foetuses were minced in one pool and divided into six portions, two serving as controls. The samples were incubated with $^{14}C$-histidine as described above and the formed $^{14}C$-histamine was measured. The control samples yielded approximately 1600 counts/min/g. With hydrazine sulphate in the concentration $10^{-4}M$ in the incubation mixture the corresponding counts in duplicate determinations were approximately 280, and with semicarbazide hydrochloride $0.6 \times 10^{-3}M$ the counts were approximately 90.

## DISCUSSION

It was shown recently (Kahlson, Rosengren & Westling, 1958) that in the rat, at about the 15th day of pregnancy, a distinct and steep rise occurred in the urinary excretion of histamine with peak values at 1–2 days before the birth of the young. On the day before parturition the histamine excretion fell steeply towards the pre-pregnant level. Yet the content of histamine in the mother's body did not change appreciably during pregnancy. It was noted further that the larger the number of young in the litter the greater the increase in histamine excretion, and this suggested that an excessive formation of histamine during the last third of pregnancy takes place in the uterus and its contents.

The present experiments show more directly that the excessive formation of histamine during the last third of pregnancy is due to an increase in the rate of histidine decarboxylase activity. During the last third of pregnancy injected $^{14}C$-histidine is decarboxylated at a much higher rate than in non-pregnant females as indicated by the urinary excretion of $^{14}C$-histamine.

Our experiments further demonstrate that the foetus is the site of the increased histamine formation. Removal of the foetuses with the least possible interference with the subsequent course of pregnancy regularly and rapidly abolished the excessive excretion of histamine. Since in the rat removal of the foetuses does not interrupt the further course of 'pregnancy' it appears that

among the uterus and its contents the foetus is the sole structure responsible for the excessive histamine formation.

The experiments on histamine production *in vitro* give information on three points. First, they show in a most direct way that foetal tissue decarboxylates histidine at a very high rate. The rate of histidine decarboxylase activity of the foetus even exceeds that of the stomach which is richer in histidine decarboxylase than any other tissue investigated in the rat. Secondly, the *in vitro* experiments reveal that during the last 1–2 days of gestation the rate of histamine formation in the foetus regresses towards a level which, in the newborn, is low as compared with the peak level. Lastly, the experiments show a relationship between foetal histamine formation and urinary histamine excretion. This finding supports the claim that in the female rat raised on a synthetic histamine-free diet the urinary excretion of free histamine faithfully reflects the rate of endogenous histamine production (Gustafsson, Kahlson & Rosengren, 1957).

The rat foetus does not contain much histamine. At the 19th-20th day of gestation, when the histidine decarboxylase activity and the urinary histamine excretion are at peak levels, the foetus contains about 3–5 $\mu$g/g tissue. At this stage the excess histamine produced by a set of 6–10 foetuses in 24 hr may even equal the total amount of histamine present in the whole body of the mother rat (Kahlson, Rosengren & Westling, 1958). A foetus weighing 2·5 g and containing about 10 $\mu$g histamine may produce 100 $\mu$g of the amine in 24 hr. In the relationship between rate of secretion and actual content of the elaborated product the foetus resembles certain endocrine organs, e.g. the adrenal cortex.

No attempt was made in the present study to examine whether and at what rate the embryo produces histamine during the 2 first weeks of gestation. By the methods used the excess production is manifest from about the 15th day onwards. At this day the single foetus has an average weight of 0·35 g, increasing within the short period of a week to about 5 g at term. At the 9th day of gestation the embryo weighs 0·006 g, at the 11th day 0·02 g and at the 14th day 0·27 g (Misrahy, 1946). It appears that from the 15th day onwards until about the 20th day the formation of histamine, as reflected by its urinary excretion, very roughly corresponds to the increase in weight of the foetuses during this period. It is noteworthy that in no instance was increased excretion observed before the 15th day, not even in animals given a histaminase inhibitor. This is suggestive of a low rate of histamine formation until the 15th day of pregnancy. This day marks the beginning of a large daily gain in weight of the foetuses which, from the 15th to the 16th day, increase in weight by about as much as during the previous 2 weeks. In this connexion it is of interest to note that in the developing egg of the hen, where the duration of embryonic development is about the same as in the rat, the histamine

concentration of the embryo increases tenfold between the 13th and the 19th day (Misrahy, 1946).

Whereas the time of the first appearance of high histidine decarboxylase activity in the embryo is uncertain, the time of its disappearance in the foetus is rather well defined. About 1 day before term the high enzyme activity subsides. The factors responsible for the timing of this event and for the loss of enzyme activity are unknown. The time clock controlling the rate of histamine formation might be linked up with the machinery which controls the duration of gestation.

There is evidence of a regulatory mechanism controlling the concentration of histamine in the gastro-intestinal tract where the histamine content is not correlated to the number of mast cells. Haeger, Kahlson & Westling (1953) found that in cats the concentration of histamine is uniform in the stomach wall of the mother and her foetuses and also in the intestinal wall among foetuses of the same mother, whereas after birth the young go their own way and establish individual levels of histamine scattered within the wide range of variation characteristic of the feline species. This was taken to indicate the existence of a regulatory mechanism operating via the blood stream. Little is known about this, except that removal of the pituitary gland or the adrenals does not influence the concentration of histamine in the gastro-intestinal tract (Haeger, Jacobsohn & Kahlson, 1952).

Within the family of enzymes involved in histamine metabolism it has been shown that the activity of histaminase and histidine decarboxylase is influenced by adrenocortical hormones. In cats, even 6–7 hr after adrenalectomy, at least 75 % of the histaminase activity of the whole body disappears (Haeger & Kahlson, 1952). As to histidine decarboxylase, pre-treatment of rats for 3 days with cortisone or its analogues causes a reduction to less than half of the histidine decarboxylase activity of the lung (Schayer, 1956). The actual level of activity of these enzymes seems to depend on factors which operate within the normal physiological range. The analogy to the sudden loss of enzyme activity in the foetus before term is merely on principle. It should be recalled that the fall in the foetus is on a much larger scale. Even this scale holds under the unlikely proposition that the enzyme is uniformly produced by all tissues of the foetus. It appears more likely that the increased histamine formation takes place in certain tissues yet to be identified. In this case the loss of activity before term would appear on a still more remarkable scale. Only further studies can show whether the striking change in histamine formation is due to diminished rate of synthesis of histidine decarboxylase, the appearance of an inhibitory factor, the disappearance of an activator or other circumstances.

Another relationship between histamine and pregnancy is indicated by the appearance of a high histaminase activity in the placenta. This occurs in several species but seems to be most pronounced in man and the rat. The

histaminase resides mainly in the maternal parts of the placenta (Swanberg, 1950). In the rat even the uterine muscle is rich in histaminase (Roberts & Robson, 1953). As to the function of placental and uterine histaminase it is believed that it protects the foetus and the uterus against deleterious actions of histamine. Since, at least in the rat, the foetus produces much histamine, it appears rather that the mother requires protection against histamine excreted by the foetus: the placental histaminase may provide a barrier between the foetus and the circulation of the mother. The functional contact between histamine leaving the foetus and placental histaminase is, however, not efficient enough to prevent considerable amounts of histamine from entering the mother's circulation, whence it is excreted in the urine. Even without aminoguanidine large amounts of histamine formed in the foetus appear in the urine. As to the function of histamine produced by the foetus, it would seem on *a priori* grounds to lie within the foetus and/or the placenta. Further information, in particular from other animal species, is required on this point.

## SUMMARY

1. The excess formation of histamine in pregnant rats was investigated by three different criteria: (a) the urinary excretion of $^{14}$C-histamine after sub-cutaneous injection of $^{14}$C-L-histidine; (b) the effect of removal of the foetuses on the urinary excretion of histamine; and (c) histamine formation from $^{14}$C-L-histidine *in vitro*.

2. During the last third of pregnancy the rate of formation of $^{14}$C-histamine from injected $^{14}$C-histidine is greatly increased as indicated by the urinary excretion of $^{14}$C-histamine.

3. Removal of the foetuses without otherwise interfering with the course of pregnancy abolishes the increased urinary excretion of histamine.

4. The rate of histamine formation *in vitro* in foetuses removed at the 17th–20th day of gestation exceeds that in any other tissue examined. At about one day before term a fall occurs in the rate of histamine formation in the foetus. The formation of histamine by the foetus *in vitro* is inhibited by hydrazine sulphate and semicarbazide hydrochloride, inhibitors of histidine decarboxylase.

5. It is concluded that in rat pregnancy the foetuses produce large amounts of histamine which escapes into the mother's circulation and raises her urinary excretion of histamine.

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