# INCREASED FORMATION OF HISTAMINE
# IN THE PREGNANT RAT

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So far it has not been possible to assign with certainty a part for histamine in normal physiology. Considering its omnipresence and wide range of actions, when liberated or injected, this situation is envisaged 'not without an inner feeling of disappointment' (Rocha e Silva, 1955).

There are no methods available for the accurate estimation of small changes in the histamine concentration of blood or plasma such as might possibly occur during increased activity of certain tissues, e.g. the parietal cells of the gastric mucosa. It has been suggested that the estimation of histamine in the urine is more likely to give information about the release of histamine in the body than investigations on venous blood (Adam, 1950; Gaddum, 1951). This promising approach is limited by the fact that the food commonly eaten by man and laboratory animals contains substantial amounts of histamine, part of which is excreted in the urine. Further, with such a diet intestinal bacteria decarboxylate histidine to histamine, constituting an additional source of exogenous histamine (Schayer, Wu & Smiley, 1954). Large amounts of urinary histamine derived from exogenous sources are likely to conceal increases in urinary histamine derived from increased production or release of endogenous histamine. Schayer, Davis & Smiley (1955) showed that in rats exogenous urinary histamine could be diminished by giving the animals a protein-free diet containing antibacteral agents. This procedure, however, seems hardly suitable for experiments over a long period of time.

The complications due to exogenous histamine can now be overcome. It has been observed that in rats raised and maintained on a synthetic histamine-free diet the distribution and urinary excretion of histamine is the same as in germ-free-reared rats, accordingly devoid of intestinal bacteria, and fed on the same diet (Gustafsson, Kahlson & Rosengren, 1957). This similarity indicates that, even in normally kept rats fed as mentioned, histamine from exogenous sources does not contribute detectably to the urinary excretion.

Under these premises it was decided to reinvestigate the problem of liberation and production of histamine in various physiological conditions. In a first series of experiments reported here it was found that the rat, during a definite phase of pregnancy, excretes excessive amounts of histamine. A preliminary report of some of the observations has been given (Kahlson, Rosengren & Westling, 1958).

### MATERIALS AND METHODS

*Animals and diet.* The observations were made on white rats of the same stock as those used in a previous report (Gustafsson *et al.* 1957). The non-pregnant animals weighed 180–235 g and were kept under similar conditions as described in the paper referred to above. Each rat lived in a metabolism cage, which provided facilities for collecting urine and faeces separately. The composition of the synthetic diet is shown in Table 1. This diet was given as a paste. It was essentially histamine-free, the total histamine content being less than $0.002$ $\mu$g/g as estimated according to Code (1937). The rats consumed 10–15 g daily, expressed as dry diet. Additional water was given *ad libitum*. In some cases the food was mixed with the antibacterial agents oxytetracycline ($0.18$ g/100 g dry diet) and succinylsulphathiazole (3 g/100 g dry diet).

TABLE 1. Composition of the diet

| Main constituents (g/kg diet) | | Vitamins (mg/kg diet) | | Vitamins (mg/kg diet) | |
|---|---|---|---|---|---|
| Casein | 220 | Vitamin A | 21,000 i.u. | Nicotinamide | 200 |
| Wheat starch | 630 | Vitamin D | 4,500 i.u. | Choline | 2,000 |
| Arachis oil | 100 | Vitamin E | 500 | Inositol | 1,000 |
| Salt mixture* | 40 | Vitamin $K_1$ | 10 | *p*-Aminobenzoic acid | 300 |
| Vitamin mixture | 10 | Thiamine | 50 | Biotin | 1 |
| | | Riboflavin | 20 | Folic acid | 20 |
| | | Pyridoxin | 20 | Vitamin $B_{12}$ | 0.02 |
| | | Calcium pantothenate | 100 | Ascorbic acid | 1,000 |

* The salt mixture was the same as described by Hubbell, Mendel & Wakeman (1937).

Vaginal smears were taken each morning and stained with methylene blue. For mating, the females were transferred for 24 hr to an ordinary cage containing a male rat kept on the same synthetic diet. The females were allowed to mate when in pro-oestrus or on the first day of oestrus. The day of removal from the male was considered to be the first day of pregnancy. The last full 24 hr urine collection period before parturition was considered to be the last day of pregnancy.

*Assay and identification of histamine.* Urine was collected in 24 hr samples and the estimation of free and in a few cases conjugated histamine was performed using the guinea-pig's isolated ileum in Tyrode solution containing atropine, as described by Gustafsson *et al.* (1957). The Tyrode solution contained (g/l.) NaCl $8.0$, KCl $0.2$, $CaCl_2$ $0.2$, $MgCl_2$ $0.1$, $NaHCO_3$ $1.0$ and $NaH_2PO_4$ $0.5$. All histamine values given in the present paper refer to free histamine expressed as $\mu$g of the base.

The urine from the last 7 days of the first pregnancy of one rat (No. 5) was pooled and the histamine content of the mixture estimated. The pooled urine was then made strongly alkaline and extracted three times with *n*-butanol (McIntire, Roth & Shaw, 1947) using a mechanical shaker. The butanol fractions were combined and shaken with dilute HCl to elute the histamine. The HCl eluate was freeze-dried and the dry residue taken up in a small volume of distilled water. This urine extract was assayed for histamine content and it was found that 76% of the original activity of the urine had been recovered in the extract. The extract was subjected to paper chromatography.

Paper chromatography was carried out in an ascending system of *n*-butanol:ethanol:concentrated ammonium hydroxide in the volume proportions 8:1:3. Whatman No. 1 paper was used. The histamine spots were located by spraying the paper with a diazo reagent (*p*-bromoaniline and sodium nitrite, as described by Urbach, 1949). Elution of histamine from non-sprayed paper strips was achieved by cutting up parts of the strip in Tyrode solution.

The histamine-like effect of rat's urine was also assayed on the blood pressure in cats under chloralose, the arterial blood pressure being recorded by a mercury manometer. Diluted, neutralized urine and histamine were given into a leg vein and the depressor responses compared.

For studies on the effect of histaminase on the gut-contracting agent in rat's urine a purified preparation of pig kidney histaminase was used. The enzyme preparation was made as described by Arvidsson, Pernow & Swedin (1956). Neutralized urine from animals not given aminoguanidine was added to the enzyme solution in an open test tube, kept in a water-bath at 37° C, and the rate of disappearance of the biological activity followed in serial samples. Concomitantly, the action of the enzyme preparation on an amount of pure histamine, corresponding to the activity present in the urine, was estimated. The procedures employed were the same as used by Lindahl, Lindell, Westling & White (1957).

*Extraction of tissues for histamine.* Rats were killed by a blow on the head. The chest was opened widely and a blood sample obtained from the heart. Extraction of histamine from whole blood and from various tissues of the rat was made with trichloroacetic acid according to Code's (1937) modification of the method of Barsoum & Gaddum (1935). Whole rats were extracted with the exclusion of the bony parts. The skin, except that on the tail, was removed, cut in small pieces and a portion from it extracted. The pooled soft parts (viscera and the skeletal muscles) were minced in a Waring blendor with a suitable volume of trichloroacetic acid. Determinations of the histamine content of tissues were carried out as previously described (Gustafsson *et al.* 1957).

*Histaminase inhibitor.* Aminoguanidine sulphate (Eastman Kodak) was used. The drug was injected under the skin in the neck in a dose of 20 mg/kg once daily at 9.00 a.m. The assay of histamine in urine from animals given aminoguanidine was often carried out on a piece of guinea-pig ileum suspended in a Tyrode solution which contained aminoguanidine in concentrations adequate to make the amount of aminoguanidine possibly added with the urine negligible in its effects. The values obtained in such assays were the same as in ordinary assays, indicating that aminoguanidine injected into the animal did not interfere with the assay, e.g. by potentiation of the effect of histamine on the gut.

## RESULTS

### *Histamine excretion during undisturbed pregnancy*

Observations on the amount of histamine excreted in the urine during undisturbed pregnancy have been made on six rats, two of which were not followed continuously throughout pregnancy. In the other four rats daily estimations of histamine in the urine were made before, during and after pregnancy. In all six rats a conspicuous increase in the daily excretion of histamine was found in the last third of pregnancy.

The mean daily histamine excretion before pregnancy varied from 18 to 43 $\mu$g/24 hr in the four rats (Table 2). During the first two weeks of pregnancy the values tended to increase slightly in three rats, but there was no striking change as compared with the values before conception. At the 15th day of pregnancy a distinct and steep increase in the excretion of histamine ensued in all four rats that were followed continuously (Fig. 1). The highest values observed varied from 123 to 835 $\mu$g/24 hr. The peak value in each rat was found 1 or 2 days before the birth of the young.

The increase in histamine excretion seemed to be larger the greater the number of young in the litter. Thus the highest values for histamine excretion were seen in rats No. 1 and 5 (835 and 536 $\mu$g/24 hr, respectively) which had the

largest litters (9 and 8 young, respectively). The increase in histamine excretion was rather small in rat No. 4, which bore one young. From Table 2 it will further be seen that the increase in histamine excretion was out of proportion to the increase in body weight during pregnancy.

TABLE 2. Urinary histamine excretion in four rats and changes during pregnancy. Values for histamine excretion refer to $\mu$g of free histamine excreted in 24 hr; mean values for several days are given. The italic figures in brackets beside the mean values indicate the number of observations; the figures in brackets below the mean values are the extreme values observed

| Rat No.    ... | 1 | 3 | 4 | 5 |
|---|---|---|---|---|
| Body weight before pregnancy (g) | 185 | 230 | 235 | 180 |
| Body weight at end of pregnancy (g) | 250 | 280 | 260 | 230 |
| Approximate duration of pregnancy (days) | 22 | 24 | 23 | 22 |
| No. of young in litter | 9 | 6 | 1 | 8 |
| Mean histamine excretion before pregnancy | 19 (*6*) (13–24) | 18 (*6*) (14–25) | 43 (*3*) (32–48) | 24 (*2*) (20–28) |
| Mean histamine excretion during pregnancy | | | | |
| a, days 1–7 | 26 (*7*) (21–33) | 24 (*6*)* (17–34) | 39 (*7*) (27–53) | 35 (*7*) (25–39) |
| b, days 8–14 | 33 (*7*) (20–65) | 25 (*6*)* (20–31) | 32 (*7*) (18–39) | 32 (*7*) (21–42) |
| c, from 15th day until end | 291 (*8*) (137–835) | 131 (*10*) (52–228) | 74 (*9*) (35–123) | 272 (*8*) (105–536) |

* One sample lost.
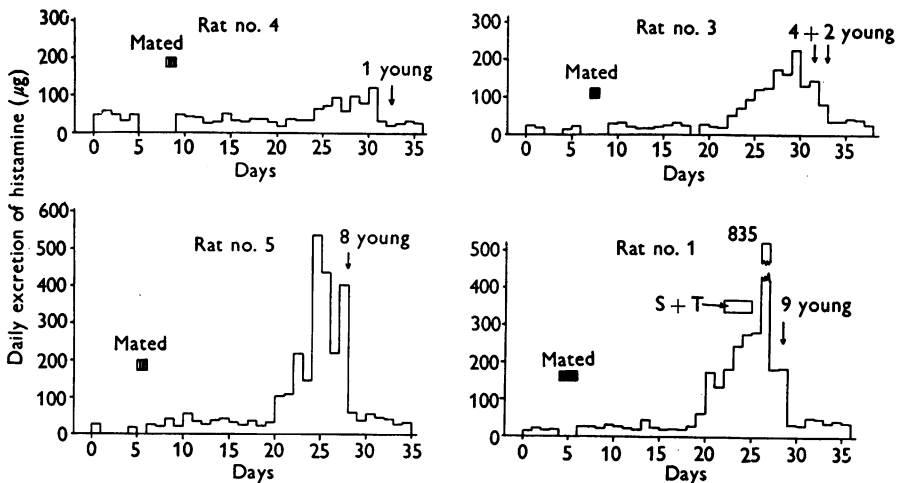


Fig. 1. Urinary excretion of histamine before, during and after pregnancy in four rats. The arrow indicates the day of parturition. Onset of raised excretion occurred at the 15th day following mating; fall in excretion on the day before parturition. Note relationship between histamine output during the last third of pregnancy and number of young. In rat No. 1, S + T indicates administration of antibiotic drugs during 3 days.

In three rats estimations of urinary histamine after thorough hydrolysis with hydrochloric acid were made before and during the last third of pregnancy. The histamine content of the urine did not increase significantly after hydrolysis, indicating that conjugated histamine was not excreted in appreciable amounts. This is in agreement with previous reports on non-pregnant female rats (Gustafsson *et al.* 1957).

The increased histamine content of the urine during the last third of pregnancy could possibly in part have been due to contamination with cellular material from the vagina. Therefore, in one rat, the histamine content was estimated in a sample of urine, voided directly from the urethra into a beaker. This urine was centrifuged thoroughly, diluted and assayed immediately. Its content of histamine was 17·0 $\mu$g/ml. The histamine concentration in the regularly collected urine from the previous 24 hr was 19·5 $\mu$g/ml. It thus seems likely that the increased histamine content of the urine is not due to any contamination during the collection period.

An experiment was made to see whether in the rat intestinal bacteria produced more histamine from the dietary histidine during pregnancy, thus adding an exogenous contribution to the urinary histamine. It has been found by Wilson (1954) and by Schayer *et al.* (1954) that in normally fed rats production of histamine by intestinal bacteria can be depressed considerably by giving antibacterial substances by the mouth. In rat No. 1, during the 17th, 18th and 19th days of pregnancy, the synthetic diet was mixed with oxytetracycline and succinylsulphathiazole in three times the amount used by Schayer *et al.* (1954). This treatment did not materially affect the progressive increase in the histamine excretion. It is therefore unlikely that the increase in urinary histamine is derived from bacterial sources.

*Evidence that the substance in pregnancy urine is histamine*

There is good reason to believe that the substance in normal female rat's urine, which contracts the guinea-pig's ileum under the present conditions, is identical with histamine (Leitch, Debley & Haley 1956; Gustafsson *et al.* 1957). The following results demonstrate this identity also for the gut-contracting agent observed to appear in large amounts in the urine in the last third of pregnancy.

The active agent in the pregnant rat's urine caused the same type of contraction of the guinea-pig's ileum and gave the same dose-response curve as histamine. The contractions caused by pregnancy urine and by histamine were equally depressed and returned in a parallel manner after mepyramine (Reuse, 1948). The biological activity of pregnancy urine disappeared on incubation with a purified histaminase preparation from pig's kidney, and the rate of disappearance was the same as that of an equivalent amount of histamine.

Diluted urine from one rat was assayed for histamine-like activity on the

cat's blood pressure. The values for histamine content of urine samples collected before, during and after pregnancy agreed well with those found on the guinea-pig ileum. When mepyramine had been injected to the cat the depressor effect of urine was diminished to the same degree as were the depressor effects of histamine.

On paper chromatography of freeze-dried urine from rat No. 5 in the last third of the first pregnancy and spraying the paper with a diazo reagent, a spot appeared which had approximately the same $R_F$ value as histamine. If histamine and the freeze-dried urine were put together on the chromatogram only one spot emerged. On elution of non-sprayed strips of paper it was found that the gut-contracting agent could be recovered from an area corresponding to the diazo-coloured spot on the sprayed strips. The other parts of the non-sprayed strip yielded no biological activity on elution.

The sum of evidence presented thus strongly indicates that the agent appearing in the urine of pregnant rats is identical with histamine. Additional, indirect, evidence of the identity is presented in the following section, where it is shown that the excretion of the active agent was increased under the influence of aminoguanidine, a histaminase inhibitor.

### The effect of aminoguanidine on the excretion of histamine in the pregnant rat

The effect of the histaminase inhibitor aminoguanidine on the pattern of histamine excretion during pregnancy was studied for various reasons. In the rat histaminase appears to be the most important enzyme for histamine inactivation (Schayer, 1953). Aminoguanidine is capable of inhibiting histaminase activity in the living rat in doses which seem to have no untoward effects (Schayer, Kennedy & Smiley, 1953). Further, the effect of aminoguanidine was of interest because of the high histaminase activity in the uterus and placenta of pregnant rats, observed *in vitro* by Roberts & Robson (1953). Roberts (1954) also noted that large doses of aminoguanidine, given to rats during pregnancy, had a deleterious effect on the foetuses and/or the mother.

The dosage of aminoguanidine used in the present experiments, 20 mg/kg once daily, is twice as large as that reported to give almost complete inhibition of histaminase during 24 hr in the intact rat (Schayer *et al.* 1954). We found that in non-pregnant rats an increase in dosage to 50 mg/kg twice daily did not materially increase the urinary histamine above the level obtained by the single 20 mg/kg dose. Thus the 20 mg/kg dose appears to be sufficient for complete inhibition of histaminase. In the pregnant rat the situation may possibly be different, but no attempts have been made to elucidate this point further. Considering the reported harmful effect of aminoguanidine on pregnancy it was not thought advisable to increase the dose.

The basal excretion of histamine in non-pregnant rats was increased three to four times by aminoguanidine (compare the figures of Tables 2 and 3). In seven pregnant rats to which aminoguanidine was given, extremely high values of histamine excretion were found during the last period of pregnancy. In three of these rats (Nos. 3, 4 and 5) excretion was followed continuously and the results are shown in Table 3. In these three rats histamine excretion had been followed during a preceding pregnancy without aminoguanidine treatment and the results are given in Table 2. In Fig. 2 the daily urinary excretion of histamine is also given for rat No. 5 before, during and after the two successive pregnancies. Essentially the same pattern of histamine excretion occurred in the pregnant rats whether they had received aminoguanidine

TABLE 3. Urinary histamine excretion in pregnant rats under the influence of aminoguanidine. Same rats as in Table 2. For explanations see Table 2. Rat No. 5 received aminoguanidine (20 mg/kg once daily) before and throughout pregnancy, rats No. 3 and 4 for 2 days before pregnancy, for 2 days during the first week of pregnancy and from the 10th or 11th day of pregnancy, respectively, until after parturition

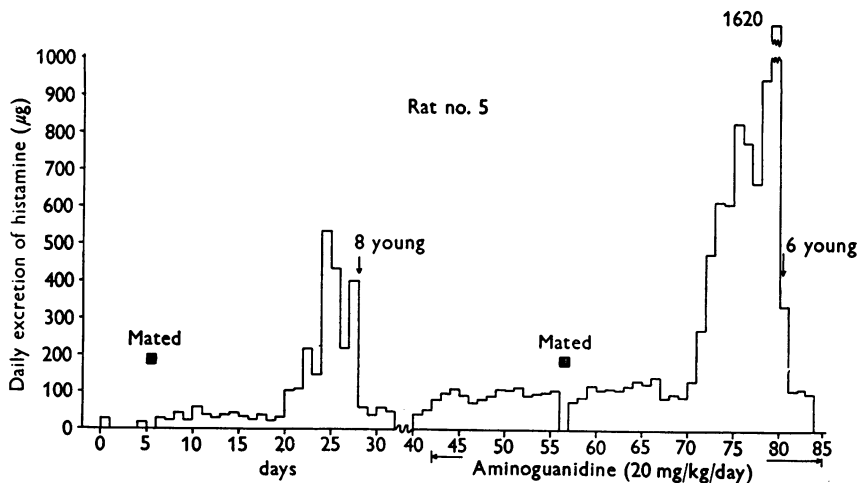| Rat No. ... | 3 | 4 | 5 |
|---|---|---|---|
| Body weight before pregnancy (g) | 235 | 235 | 180 |
| Body weight at end of pregnancy (g) | 270 | 260 | 235 |
| Approximate duration of pregnancy (days) | 23 | 22 | 23 |
| No. of young in litter | 4 | 4 | 6 |
| Mean histamine excretion before pregnancy | 76 (2) | 86 (2) | 104 (7) |
| | (64, 87) | (77, 95) | (93–115) |
| Mean histamine excretion during pregnancy | | | |
| a, days 1–7 | 79 (2) | 142 (2) | 105 (7) |
| | (65–94) | (131–152) | (77–120) |
| b, days 8–14 | 96 (5) | 119 (4) | 116 (7) |
| | (84–108) | (94–128) | (86–144) |
| c, from 15th day until end | 503 (9) | 381 (8) | 760 (9) |
| | (230–748) | (168–495) | (274–1620) |



Fig. 2. Urinary excretion of histamine in rat No. 5 before, during and after pregnancy, in two successive pregnancies; the first without, the second under the influence of aminoguanidine, the first with 8 and the second with 6 young; arrow indicates day of parturition.

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or not. The increase in histamine excretion did not begin earlier under the influence of aminoguanidine but, as seen in Fig. 2 for rat No. 5, started also on the 15th day. A possible ·exception occurred in rat No. 4, in which two high values were observed during the first week of pregnancy with amino-guanidine treatment. In the second week, however, the histamine excretion was again much the same as that obtained before or after the pregnancy. A comparison of the results given in Tables 2 and 3 shows that in all three rats aminoguanidine greatly increased the histamine excretion during the last third of the pregnancy. The increase is particularly striking in rats Nos. 3 and 5, because these rats had smaller litters in their second pregnancies when amino-guanidine was given. In the small number of rats studied aminoguanidine in the given dosage appeared to have no harmful effect on the course of pregnancy.

### Distribution of histamine in pregnant and non-pregnant rats

Four pregnant and four non-pregnant rats were examined, the pregnant ones at periods when the urinary histamine was known to be at high levels. All rats had been given aminoguanidine, 20 mg/kg, once daily, for at least 10 days before they were killed.

The various tissue samples and the foetuses were removed and extracted for histamine. The values obtained are given in Table 4. The histamine content of the soft tissues in pregnant rats was $7 \cdot 2 \pm 0 \cdot 33$ (mean $\pm$ S.E.) and in non-pregnant rats $5 \cdot 6 \pm 0 \cdot 42$ $\mu$g/g. The difference is probably significant $(P < 0 \cdot 05)$. The pregnant uterus on the other hand contained less histamine $(2 \cdot 0 \pm 0 \cdot 25$ $\mu$g/g$)$ than the non-pregnant uterus $(8 \cdot 5 \pm 1 \cdot 6$ $\mu$g/g$)$, the difference being significant $(P < 0 \cdot 01)$.

TABLE 4. Distribution of histamine ($\mu$g/g) in pregnant and non-pregnant rats

| | Pregnant | | | | Non-pregnant | | | |
|---|---|---|---|---|---|---|---|---|
| Rat No.    ... | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
| Day of pregnancy | 20 | 20 | 19 | 20 | — | — | — | — |
| No. of foetuses | 9 | 7 | 10 | 9 | — | — | — | — |
| Urinary histamine in preceding 24 hr | 700 | 440 | 670 | 870 | 120 | 67 | 73 | 137 |
| Placenta (foetal)* | 4·4 | 4·8 | 2·3 | 3·5 | — | — | — | — |
| Uterus | 1·6 | 2·5 | 1·6 | 2·4 | † | 8·1 | 11·4 | 6·1 |
| Foetus (mean of two) | 5·1 | 5·1 | 4·2 | 3·3 | — | — | — | — |
| Whole blood | 2·8 | † | 1·3 | 1·9 | 1·1 | 2·9 | 0·9 | 0·3 |
| Skin (total) | 26·0 | 14·4 | 18·7 | 15·9 | 17·7 | 19·6 | 14·0 | 15·0 |
| Soft tissue (mean of duplicates) | 7·3 | 8·0 | 6·5 | 6·8 | 6·4 | 4·8 | 5·0 | 6·3 |

* 3–5 placentas were pooled and extracted; † not examined.

Neither the foetal placenta, the uterus proper or the foetuses are conspicuously rich in histamine; they rank rather among tissues where the histamine concentration is low. For comparison, figures for some other tissues from six non-pregnant rats fed on a histamine-free diet and examined in this laboratory are given (Gustafsson et al. 1957): lung 3·5–8·0, small intestine 9·4–28·5, gastric mucosa 28·5–53·9, gastrocnemius muscle 1·9–3·2 $\mu$g/g.

DISCUSSION

The increased excretion of histamine in the pregnant rat reported in the present paper is the result of an increased production of the amine. This can be inferred from simple arithmetic: when histamine is protected from destruction by inhibiting the histaminase the urinary excretion in 24 hr may exceed the amount of histamine present in the whole body. The actual rise in production is probably greater than the increase in urinary excretion, since even with complete inhibition of histaminase a considerable and so far unknown proportion of histamine endogenously produced is not excreted in the urine (Schayer *et al.* 1954).

The distribution and whole-body content of histamine appear to be the same in pregnant and non-pregnant rats. Neither the uterine tissues nor the foetuses were conspicuously rich in histamine. However, the relationship between the number of young in the litter and the amount of urinary histamine points towards the uterus and its contents as the source of the increased histamine formation.

In non-pregnant rats fed with synthetic histamine-free diet the urinary histamine is of endogenous origin. In the pregnant rat also it appears that histamine from exogenous sources does not contribute to the urinary excretion, since antibacterial drugs added to the diet did not alter the pattern of increased histamine excretion. In the present experiments the excess urinary histamine could be derived from one or more of three possible sources: (1) maternal tissues specifically concerned with the elaboration of histamine during pregnancy; (2) foetal tissue; (3) the kidneys. Little is known about the capacity of the kidney to decarboxylate histidine and its contribution to urinary histamine. It has been reported that in the rat aminoguanidine does not significantly increase the amount of $^{14}$C-histamine in the urine following injection of $^{14}$C-histidine, which is taken to indicate that this histamine was not exposed to histaminase, which again suggests formation of histamine in the kidney followed by immediate excretion (Schayer *et al.* 1954). This is in agreement with the original observations by Holtz & Credner (1944) who injected histidine in guinea-pigs and found an increased excretion of histamine in the urine. In pregnancy the possibility of histamine formation in the kidney deserves consideration on account of the increased urinary excretion of histidine during pregnancy (for references see Christensen, Date, Schønheyder & Volqvartz, 1957). However, the significant increase in the pre-pregnant level of urinary histamine and the enormous rise during pregnancy under the influence of aminoguanidine indicate increased production mainly of extrarenal origin.

Substantial evidence of changes in histamine metabolism in pregnancy is provided by observations on the histaminolytic power of various tissues (for

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references see Roberts & Robson, 1953). In the rat Roberts & Robson (1953) found a large increase in histaminase activity of the uterus and placenta during pregnancy. These authors speculate that the function of uterine and placental histaminase is to protect the uterus against histamine liberated as a result of break-down and degeneration of tissues related to the uterus. It has further been reported (Roberts, 1954) that in the rat aminoguanidine in doses producing more than about 50% inhibition of the maternal placental histaminase leads to a general disturbance of the course of pregnancy; large doses tended to produce death of the mother, smaller doses tended to kill part of or all the litter and some of the mothers. According to Roberts this again suggests that histaminase protects the mother against histamine and diamines during pregnancy.

A suggestion that histamine may be the chemical stimulus of the ovum responsible for initiating placentation in the rat comes from Shelesnyak (1952, 1954, 1957). He found that various antihistamines when instilled into a uterus horn suppressed the decidual cell response and inhibited normal ova-implantation of pregnancy; when given subcutaneously on successive days the antihistamine drugs interfered with foetal development but did not inhibit decidua formation or nidation. The effect was attributed specifically to the antagonism of the drugs to histamine and not to some other known side action of the antihistamines.

A suggested relationship between histamine metabolism and the thyroid gland is mentioned on account of reports on increased thyroid activity during pregnancy. Parratt (1957) found up to a fivefold increase in urinary excretion of free histamine in rats given daily subcutaneous injections of sodium-L-thyroxinate (1 mg/kg) for 1–3 weeks. Whether and to what extent increased thyroid activity plays a part in the increased histamine production in rat pregnancy can only be revealed by specially designed experiments.

There is evidence suggesting that during pregnancy the activity of the adrenal cortex is increased. Among the influences responsible for this augmented activity the increased production of histamine may possibly provide auxiliary stimulus. It has been shown that in rabbits histamine, when carried in the general circulation, constitutes an effective stimulus to increased secretion of ACTH from the adenohypophysis (Fuche & Kahlson, 1957).

It is of interest to consider what changes occur in the rat at about the 15th day of pregnancy and the day before parturition, where the two turning points in histamine excretion were observed. From the 11th to 15th day after mating vascular connexions between maternal and foetal placentas develop; the 15th day marks the onset of rapid growth of the foetus. As to the last day of pregnancy it has been shown in the rabbit that on this day the progesterone level falls sharply (Schofield, 1957; Bengtsson, 1957). Corresponding experiments on the rat are not known to the present authors.

It is not known whether the increased histamine excretion in pregnancy is specific for the rat or occurs in other species as well. The increased histamine excretion reported by Ungar & Pocoulé (1937) in human pregnancy could not be confirmed by Werle & Effkemann (1940), nor could Wicksell (1949) find any significant changes in the levels of plasma histamine and urinary excretion of histamine before, during and after parturition.

The function of the increased production of histamine in rat pregnancy is unknown. One useful purpose could be served by the fact that in the rat histamine inhibits the smooth muscle of the uterus (for literature see Feldberg & Schilf, 1930). For the pregnant uterus not to contract under the stimulus of distension by the growing foetuses requires some sort of block or inhibition of its smooth muscle. Such inhibition appears requisite mainly during the period of rapid growth of the foetuses and should be withdrawn just before parturition. This time course coincides with the period of increased production of histamine.

## SUMMARY

1. In rats fed on a synthetic histamine-free diet the urinary excretion of histamine was examined before, during and after pregnancy.

2. At about the 15th day of pregnancy a distinct and steep rise occurred in the excretion of histamine. The peak values occurred 1 or 2 days before the birth of the young.

3. On the day before parturition the histamine excretion fell steeply towards the pre-pregnant level.

4. There was a relationship between the increase in histamine secretion during pregnancy and the number of young in the litter. The greater the number of young the greater the increase in histamine secretion. This suggests that the excessive formation of histamine during the last third of pregnancy takes place in the uterus and its contents.

5. Under the influence of the histaminase inhibitor aminoguanidine, the general pattern of histamine excretion in non-pregnant and pregnant rats remained unchanged, but the total amount excreted increased. At the end of pregnancy the daily excretion occasionally exceeded the histamine content of the whole body.

6. Aminoguanidine in the doses employed did not detectably interfere with the course of pregnancy and parturition.

7. The distribution of histamine in the body did not change during pregnancy.

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