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THE FORMATION OF HISTAMINE IN THE RAT FOETUS

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The foetus in the rat and man, the two species so far investigated, produces histamine at a high rate (Kahlson, Rosengren & Westling, 1958; Kahlson, Rosengren, Westling & White, 1958; Kahlson, Rosengren & White, 1959). This has been shown in the rat by (1) measurement of the urinary excretion of endogenous histamine, (2) study of the rate of urinary excretion of ^{14}C-histamine after injections of ^{14}C-histidine, (3) determination of the rate of histamine formation in foetal tissues *in vitro*, and (4) investigation of the effect of removing the foetus, without removing the placenta, on the excretion of histamine in the pregnant rat. The urinary excretion shows that in the rat the increase in histamine formation occurs at the 15th day of pregnancy and climbs to a peak in the last 1–2 days before term, when a precipitous fall in the rate of formation ensues. It would seem that the foetal formation of histamine during the last third of pregnancy is due to an increase in histidine decarboxylase activity, because the formation of the amine by foetal tissues *in vitro* is inhibited by semicarbazide and hydrazine in concentrations which are known to inhibit this enzyme.

The rat foetus contains little histamine. Even at the peak of histidine decarboxylase activity and urinary histamine excretion (19th–20th day of gestation) the average concentration of histamine in the foetus is less than in the mother. It thus appears that foetal tissues produce the amine at a high rate, but bind histamine only loosely; and, since the histamine is in a state of rapid turnover, the amount found in the tissue is small.

As the significance of the histamine production in the foetus is not known, it seemed desirable to identify the tissue(s) responsible for the high histamine-forming capacity. Further, to understand the discrepancy between histamine content and its rate of formation in the foetus, it appeared essential to study the binding of histamine in foetal tissues. A preliminary communication of these results has been given to the Physiological Society (Kahlson, Rosengren & White, 1959).

METHODS

Measurement of histamine formation in vitro. The method developed by Schayer and his co-workers, and described in detail by Kahlson, Rosengren, Westling & White (1958; see

note, p. 137) was used. A brief outline only will be given here. The tissues were minced and suspended in 0·1 M sodium phosphate buffer of pH 7·4. Where possible 1·5 g of tissue was used but only 0·5–0·6 g of liver in the experiment of Table 1, column 1; the total volume of the suspension was 3 ml. The samples were incubated for 3 hr at 37° C under nitrogen with 40 μg ^{14}C-histidine. The amount of ^{14}C-histamine formed during the incubation was determined by isotope dilution with inert histamine dihydrochloride as carrier. The carrier was added at the end of the incubation and trichloroacetic acid was added to precipitate protein. The sample was filtered and the filtrate was made alkaline and saturated with Na_2SO_4. The filtrate was then extracted with butanol and the butanol fraction was shaken with hydrochloric acid. This procedure was followed by evaporation of the acidic part to dryness and solution of the residue, mainly histamine dihydrochloride, in water to which picric acid in alcoholic solution was then added. The histamine dipicrate crystals formed were isolated and spread on a plate, and their radioactivity was determined. For further purification the histamine, in the form of dihydrochloride, was treated with *p*-iodobenzenesulphonyl chloride and the crystals of pipsyl histamine were isolated and counted in the same way as the histamine dipicrate.

The measurement of radioactivity was made under infinite-thickness conditions in a gas flow-counter. The crystals were deposited on plates of equal size, so that the exposed surface was equal for all samples. The criterion of purity was constant radioactivity after repeated recrystallizations with different charcoal adsorbents; three to six recrystallizations were needed to obtain constant radioactivity. After each recrystallization at least 1000 counts were recorded from each sample.

Extraction of tissues for histamine. Rat foetuses were removed under ether anaesthesia. The tissues required for study (liver, heart, lungs, etc.) were dissected from each member of a litter and pooled. About 30 min elapsed between the death of the foetuses and the beginning of the tissue extraction. Determinations of the histamine content of tissues were made as described by Gustafsson, Kahlson & Rosengren (1957).

RESULTS

Histamine formation in vitro

The results are presented in Table 1 in terms of counts/min/g tissue and signify the amount of ^{14}C-histamine which is formed in 3 hr from ^{14}C-L-histidine; 1 μg ^{14}C-histamine gives 600 counts/min. The results for the organs were obtained by pooling the tissues in each litter. Three litters were examined on the 19th, 20th and 21st days of gestation, respectively. Histamine excretion by the mother is at a peak on the 19th or 20th day of gestation, and tends to fall on the 21st day.

It will be seen that the foetal liver produces histamine at an enormously high rate. The histamine-forming capacity per gram of tissue is about 100 times higher in the liver than in the rest of the body. It is far greater than in the stomach, which is the most potent manufacturer of histamine in the adult; the foetal stomach itself is much more active in forming histamine than that of the adult. In fact, all foetal tissues investigated are potent producers of histamine. At the 19th day of pregnancy, when the figures are very high, organs small in mass could not be examined because of the limitations of the method used. From the available figures it can be

calculated that the liver accounts for about 80 % of the histamine formed by the foetus. However, it should be emphasized that all foetal tissues examined produce histamine at a rate which is very high when compared with the infantile and adult animal.

On the day before term, when the maternal excretion of histamine is on the wane, the activity of the foetal liver has fallen to the low level found in the new-born, and within 2–3 days the histidine decarboxylase activity of the liver regresses from an unparalleled height to the low level found in the new-born and adult animal.

TABLE 1. Rate of histamine formation in 3 hr in rat foetuses, new-born, and adults, expressed as counts/min/g tissue. 1 μg ^{14}C-histamine gives 600 counts/min

	Foetus 19 days	Foetus 20 days	Foetus 21 days	New-born < 3 hr	Young 2–8 days	Adult pregnant
Whole body	—	3,440	340	860	15	—
Liver	14,000 (23,000)*	8,730	2,280	2,240	5–40	5
Stomach	2,800	—	—	—	—	370–980†
Lungs	1,900	930	150	60	5	90–150†
Heart	—	—	140	30	0	—
Kidneys	—	—	190	—	0	< 5†
Stomach + intestines	—	1,250	150	—	—	—
Brain	10	5	20	5	0	—
Rest of the body (muscle, skin, bone)	130 (220)*	360	70	60	30	—

* Figures in brackets are the means of determinations in three litters; the other figures in this column are from a single litter. All the results in the second column are from one litter, and so also those in the third and fourth.

† Figures quoted from Kahlson, Rosengren, Westling & White, 1958.

Histamine content of foetal tissues

Since the published figures for the histamine content of the foetus are somewhat inconsistent, and because our rats were fed on a special histamine-free diet, it was considered necessary to study the histamine content of the foetus anew. In the developing rat embryo histamine is present at the 9th day of gestation in measurable amount. Subsequently the content of the whole foetus increases progressively, slowly at first and more rapidly after the 12th day (Misrahy, 1946). Dixon (1959) was able to detect histamine only at the 11th day of gestation, a time when Misrahy found 1·3 μg/g in the whole foetus, but confirmed the subsequent continuous increase in histamine content. According to Misrahy the foetus at the 20th day contains histamine in a concentration of 4·6 μg/g, whilst Dixon found less than half this amount; at the 19th–21st day we found 1·3–2·1 μg/g (Table 2) and at the 19th–20th day about 3–5 μg/g (Kahlson, Rosengren, Westling & White, 1958). Greater discrepancies exist in the figures reported for the histamine content of various rat foetal organs. Parratt & West (1957) found a striking increase in the histamine

concentration of the lung (25 times) and of the liver (12 times) at birth as compared with the pre-natal figures and a return to near foetal levels by about the sixth day of extra-uterine life. On the other hand, Dixon noted in the lung a low and constant level during intra-uterine life, climbing rapidly after birth to reach a maximum about the third hour post partum. According to this author the ante-partum levels in the liver vary, the content at birth being high and maintained for the first few hours after birth and then falling precipitously to a low level which does not change after the second day. Misrahy found the content to be the same in the lungs of new-born and adult individuals, whereas the corresponding ratio in the livers was about 4:1.

TABLE 2. Histamine content of tissues of rat foetuses and new-born young. The figures denote μg histamine/g tissue

	Whole foetus	Foetus without liver	Liver	Lung
Foetus, 19th day of pregnancy	—*	2·3	18·6	—
Foetus, 19th day of pregnancy	2·1	1·4	23·5	0·7
Foetus, 21st day of pregnancy	1·3	1·3	4·6	0·7
Young, 6 hr after birth	—	8·5	1·0	1·0
Young, 8 hr after birth	—	10·1	0·9	0·8
Young, 8 days after birth	—	11·7	0·8	—
Adult females (Gustafsson *et al.* (1957)	5·1–6·9	—	—	3·3–8·2

* Not examined.

We have therefore examined two litters on the 19th day of pregnancy, one on the 21st day and one litter at 6 hr, one at 8 hr and one 8 days after birth, pooling the livers, lungs, etc., from the members of each litter. As recorded previously, at the 19th day the histamine-forming capacity of the foetus is about maximal, whilst the 21st is the day of steep regression in the rate of histamine formation. The results obtained for histamine content are shown in Table 2. At the time of maximum histamine production in the whole foetus, although the rate of production *in vitro* is about 10 μg/g tissue/hr, the average total concentration in the body is not greater than a few micrograms per gram of tissue. It is evident that the newly formed histamine is not bound in the foetus but is transferred rapidly to the mother.

Binding of newly formed histamine

To see what proportion of newly formed histamine remains bound, in the sense that it cannot be easily washed away, the following experiments were done *in vitro* on whole foetuses, liver, lung and skin. The minced tissue was incubated with ^{14}C-histidine as already described. After 3 hr incubation the mixture was centrifuged at 1000 *g* for 10 min. The supernatant fluid was decanted and the residue was washed with about 6 ml. of buffer solution

and centrifuged twice. The ^{14}C-histamine content of the tissue mince and the combined supernatants were determined separately. The results are presented in Table 3, which shows that in the whole foetus about 12% of the newly formed histamine is bound, in the liver 20%, in the lung 40% and in the skin 45%. It should be recalled that of the total formation of histamine in the foetus about 80% takes place in the liver.

TABLE 3. Binding of newly formed histamine in rat foetuses and in foetal tissues expressed as counts/min/g tissue. A high ratio in the last column indicates a low histamine-binding capacity and vice versa

Tissue	Day of pregnancy	Supernatant (*a*)	Tissue (*b*)	Ratio (*a*)/(*b*)
Foetus (3) * same litter	17	2100	350	6·0
Foetus (4) same litter	17	2100	310	6·8
Foetus (2) same litter	17	2000	220	9·1
Foetus (2) same litter	17	2200	270	8·1
Foetal liver	20	3490	780	4·5
Foetal lungs	20	210	150	1·4
Foetal skin	20	140	120	1·2

* The figures in brackets denote the number of foetuses pooled in one sample.

These experiments show that the histamine formed in foetal tissues easily diffuses into the surrounding fluid and that only a small proportion of it is retained in the tissue. By contrast, in a similar type of experiment on the skin of the adult female rat, only about 1/3 of the formed histamine was found in the supernatant whilst 2/3 was retained in the tissue (Schayer, Davis & Smiley, 1955).

DISCUSSION

Foetal histamine is predominantly of non-mast-cell origin. In the rat foetus mast cells have been found only in the skin (for references, see Dixon, 1959); yet the histamine-forming capacity of the skin is very low. On the other hand, the ability of non-mast-cell tissues to form histamine in the foetus is unequalled. In the adult the most potent manufacturers of histamine are the stomach wall and the mast-cell tumour; yet a mast-cell tumour of a dog investigated in our laboratory (Lindell, Rorsman & Westling, 1959) was about 100 times less active per unit weight in forming histamine than the foetal liver.

The histamine-forming cells and the intracellular location of histidine decarboxylase remain to be identified. It is considered that the histamine-forming capacity of foetal tissues depends on the activity of this enzyme, since the formation of histamine is abolished by inhibitors of histidine decarboxylase (Kahlson, Rosengren, Westling & White, 1958; Kahlson & Rosengren, 1959*a*). These inhibitors are rather unspecific and do not furnish precise evidence, but the findings agree with the assumption that we are dealing with histidine decarboxylase.

Foetal tissues, in general, do not contain much histamine and the rather

high values in the liver are exceptional, but the rate of histamine formation in this tissue is outstandingly high. Histamine is likely to be formed in the liver in considerable amounts during the dissection and preparation of the samples for extraction and the estimates may not represent the actual level of histamine content at the moment of death. The same holds true for other foetal tissues where the rate of histamine formation is high. These circumstances may in part account for the discrepancies in the figures for histamine content in foetal tissues which have been reported by various authors.

In adult tissues rich in mast cells histamine is not easily extracted and rather drastic procedures, which destroy cell structures, are required. In foetal tissues the situation is entirely different. The histamine, so abundantly formed, diffuses freely into the surrounding fluid; the differences in histamine-binding capacity are shown in Table 3. Only in the skin are mast cells present, and this may explain the position which skin occupies at the upper extreme in binding capacity. From the present observations a generalization can be made concerning non-mast-cell histamine: the amount of histamine yielded on extracting a tissue bears no relationship to its histamine-forming capacity. Similar reflexions have occurred to Schayer *et al.* (1955).

Foetal tissues endowed with a high histamine-forming capacity and a low binding capacity are subjected to histamine in a state of rapid turnover. Little is known about the physiological meaning of this process and whether it operates for the benefit of the foetus, or of the mother or of both. Fortunately it has now become possible to depress histamine formation in experiments on the foetus. Under the combined influence of pyridoxine deficiency and rather small doses of semicarbazide, a carbonyl reacting agent which inhibits a variety of enzymes, including histidine decarboxylase, the histamine-forming capacity can be lowered to about 20 % of normal. As a consequence foetal growth is promptly arrested, the foetus dies and becomes mummified. In controls fed on the pyridoxine-deficient diet for the same period of time, or simply injected with semicarbazide, no abnormalities in the course of pregnancy and foetal development were noted (Kahlson & Rosengren, 1959*a*, *b*). These authors (1959*b*) also found that rat liver tissue regenerating after partial hepatectomy displays a substantially elevated histamine-forming capacity. Further, the histamine-forming capacity is also considerably raised in the tissues of healing skin wounds (G. Kahlson, K. Nilsson, E. Rosengren & B. Zederfeldt, unpublished). Thus, histamine-forming capacity has been found to be associated with three types of growth: foetal, regenerative and reparative. The phenomenon would therefore seem to be related in some way to the general process of growth.

The physiological meaning of the histamine-forming capacity and the consequences of reducing or abolishing it will be further elucidated when inhibitors of histidine decarboxylase which are more specific than semicarbazide become available. The discovery of rich sources of mammalian histidine decarboxylase, such as foetal tissues and gastric mucosa, should be helpful in the search for such inhibitors.

SUMMARY

1. The histamine-forming capacity, the histamine content and the histamine-binding capacity have been determined in a variety of tissues from rat foetuses at various stages of gestation.

2. The histamine-forming capacity is very high until about one day before term and is particularly great in the liver, where it ascends to levels greater than any previously recorded.

3. The histamine content of foetal tissues is below that of the mother in most of the tissues investigated.

4. The histamine-binding capacity of foetal tissues is low: newly formed histamine diffuses freely into the surrounding fluid.

5. As a result of a high histamine-forming capacity and a low histamine-binding capacity foetal tissues are exposed to histamine in a state of rapid turnover.

6. In tissues in which the histamine is of non-mast-cell origin the content bears no relation to the histamine-forming capacity.

7. The physiological significance of the histamine-forming capacity is discussed.

We wish to correct a misprint which appeared in the earlier paper by Kahlson, Rosengren, Westling & White (1958): on p. 338, line 23 for ^{14}C-histidine read ^{14}C-histamine.

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