be thoroughly investigated. It is of interest to note that the [Ca] × [P] products at which crystals are formed in the presence of lead are as low as those obtained formerly with the most active nucleating collagens(2, 4). It is thus tempting to speculate that the local concentration of lead might play some role in the regulation of calcification. This is supported by the fact that lead does induce ectopic calcification. This has been demonstrated by Selye, who placed this element in his category of "direct calcifiers"(9,10,11).

Summary. Physiological concentrations of lead have been shown to decrease the minimum product [Ca] × [P] necessary to form calcium phosphate crystals *in vitro*. The possible importance of this activating property in biological calcification is discussed.

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Histamine Metabolism in the Pregnant and Non-Pregnant Hamster. (29998)

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It has been shown in this laboratory that the foetuses of all the mammals investigated form histamine, *i.e.*, are endowed with histamine forming capacity (HFC), which in some species attains very high levels(1). Part of this histamine of foetal origin is excreted in the urine as the free amine. Even maternal tissues may exhibit greatly elevated HFC during pregnancy, for instance the mouse kidney. In the present investigation a hitherto unknown site of singularly high HFC in pregnancy has been disclosed.

Methods. The experiments were done on the female golden hamster, *Cricetus auratus*; only 2 animals (No. 2 and 4 in Table V) were of an inbred strain of white hamsters.

Free non-isotopic histamine was determined in 24-hour urine samples collected with the animal in a metabolic cage. The animal was fed a paste of ground pellets mixed with water, 10-12 g of this diet daily, which contained only about 1 μg histamine/g, and water was allowed *ad libitum*. Hydrochloric acid, sufficient to give the urine a pH value below 2, was added to the collecting vessels to prevent formation of histamine by bacteria during the collecting periods. The histamine content of the urine samples was assayed on the guinea pig ileum by the method originally devised by Guggenheim and Löffler(2). The figures for excretion of free non-isotopic histamine are given as μg base/24 hours. To ascertain the identity of the substance with histamine, a histamine antagonist was used according to Reuse's procedure(3).

The histidine decarboxylase activity *in vivo* was determined by injecting ^{14}C-histidine and measuring urinary excretion of radioactive histamine and methylhistamine, a catabolite of endogenously formed histamine. ^{14}C-L-Histidine, 100 μg, labelled in the 2-position of the imidazole ring, was injected subcutaneously, and the urine sam-

TABLE I. Urinary Excretion of Histamine in Hamsters Before, During and After Pregnancy. Values refer to μg of free histamine excreted in 24 hours. Duration of pregnancy for all 9 hamsters in this Table was 16 days.

No. of young in litter	Histamine excreted: Before pregnancy (mean value)	First 10 days of pregnancy (mean value)	Day of pregnancy: 11	12	13	14	15	16	Days after delivery (mean value)
9	3 (8)*(1- 4)	4 (10) (3- 5)	7	40	85	570	890	120	6 (7) (4-12)
10	1 (5) (1- 2)	3 (10) (2- 5)	13	91	240	680	550	68	7 (10) (3-15)
10	3 (1)	4 (10) (2- 8)	11	46	67	770	1300	670	10 (6) (5-13)
7	8 (26) (4-13)	6 (10) (2- 8)	6	10	15	190	750	160	8 (9) (6-11)
12	8 (9) (6-10)	6 (10) (4- 8)	9	14	83	420	750	92	7 (8) (4-13)
9	8 (9) (5-10)	11 (10) (5-25)	13	38	170	600	1100	850	10 (10) (7-17)
15	5 (4) (4- 7)	6 (9) (4-10)	14	7	63	420	870	200	9 (7) (6-11)
12	7 (9) (5- 9)	8 (10) (5-13)	13	14	210	750	800	960	9 (10) (6-18)
16	7 (4) (6- 8)	7 (10) (3-15)	14	15	100	360	1200	190	11 (7) (5-36)

* Figures within parentheses after mean values refer to number of observations; those following in parentheses are extreme values noted.

ples collected during the subsequent 3 days were each analyzed for their content of ^{14}C-histamine and ^{14}C-methylhistamine (1-methyl-4-β-aminoethyl-(^{14}C)-imidazole). The isotopic methods used in this laboratory for determination of ^{14}C-histamine and ^{14}C-methylhistamine in the urine and of the histamine forming capacity (HFC) of excised tissues have been described(4,5).

One μg ^{14}C-histamine formed from the ^{14}C-histidine used corresponded to about 5000 counts/min. In determinations of the HFC of the various layers of the placenta only 10-20 mg tissue were available, whereas with other tissues 200 mg were used for the incubation. To facilitate comparison all values are expressed as counts/min/g.

To investigate the intramural distribution of HFC, placentae were mounted on the block of a freezing microtome and were cut in serial sections from the basal decidua onwards parallel to the uterine wall. By this procedure the placenta was separated into 7 to 10 layers, each consisting of 10 sections of 30 μ thickness. The pooled sections from each layer were weighed and the HFC determined. In addition, one section, 10 μ thick, was taken from the middle of each layer for histological examination. This section was fixed in alcohol and ether, and stained with hematoxylin and eosin.

Results. Excretion of histamine in urine. Urinary histamine was followed continuously in 9 hamsters before, during and after pregnancy (Table 1). In the non-pregnant state the excretion of free histamine was low, 1-13 μg/24 hours. Obvious changes from the non-pregnant values in the amine excretion were not seen during the first two-thirds of the gestation period, which totals 16 days. However, on the 11th day of pregnancy histamine excretion was elevated and rose to very high values during the 3 days prior to term. On the day of parturition a steep fall in the amine excretion occurred, and from the first day after delivery the level was almost as low as before mating.

As to the catabolism of histamine in hamsters, Schayer, Kennedy and Smiley(6) chromatographed the material obtained from the urine of a hamster which had been injected with ^{14}C-histamine. Most of the radioactivity was found in what they referred to as "peak 2" and "peak 3," which they later showed to correspond to histamine and its methylated compounds. In the present study ^{14}C-histamine, 10 μg base/animal, was injected subcutaneously in 3 pregnant and 3 non-pregnant hamsters. About 10% of the injected histamine appeared unmetabolized in the urine. The major part of the radioactivity was recovered in the fractions containing methylhistamine and methylimidazoleacetic acid. No significant difference in catabolism of histamine was observed between the pregnant and the non-pregnant hamsters.

It is thus apparent that in the pregnant as well as in the non-pregnant animal part of the histamine is methylated. For this reason

TABLE II. Urinary Excretion (counts/min) of ^{14}C-Histamine (Hi) and ^{14}C-Methylhistamine (Met-hi) After Injection of 100 μg ^{14}C-Histidine Subcutaneously on 14th Day of Pregnancy and on 7th Day *post partum*. Hamster A bore 9 and B 10 young on 16th day of pregnancy. Urine was collected for 3 days after the injection.

	1st day		2nd day		3rd day	
	Hi	Met-hi	Hi	Met-hi	Hi	Met-hi
Hamster A						
Pregnant	240	530	100	180	10	30
Post partum	10	30	0	0	0	0
Hamster B						
Pregnant	350	740	10	250	10	30
Post partum	10	20	10	0	0	0

the excretion of ^{14}C-methylhistamine, besides that of ^{14}C-histamine, was investigated after injections of ^{14}C-histidine. In 2 female hamsters ^{14}C-histidine, 100 μg/animal, was given subcutaneously in the non-pregnant and pregnant states, and excretion of radioactive histamine and methylhistamine was determined on 3 consecutive days. The values given in Table II show that in the pregnant state much larger amounts of histamine and methylhistamine are excreted than in non-pregnancy.

Histamine forming capacity in vitro. In attempts to identify the site(s) of high HFC in pregnancy, various tissues were excised and examined for their histidine decarboxylase activity *in vitro*. The results are presented in Table III. A slight HFC was observed in the lung of the pregnant animals during the days before term, whereas none was detectable in this tissue from non-pregnant animals. However, none of the 8 extrauterine tissues examined exhibited in pregnancy an elevated HFC which could reasonably account for the excessive amounts of urinary histamine. Further exploration revealed that the large amount of histamine excreted originates within the uterus, predominantly in the placenta (Table IV). The uterine wall and the foetal membranes (placenta removed) also showed a rather high HFC. However, the occurrence of microscopical placental fragments in the uterine specimens cannot be entirely excluded. The various foetal tissues were not separately investigated for their HFC.

TABLE IV. HFC (counts/min/g) of Uterus and Its Contents.

Day of pregnancy	Uterus wall	Placenta	Foetal membranes (placenta removed)	Whole foetus
11	2900	120000	1600	0*
	150	48000	—†	—
14	800	820000	900	100
	560	1100000	2500	260
	250	860000	1200	60
	—	920000	—	—
	—	930000	—	—
15	4400	830000	3800	—
	1900	950000	—	270
	250	1000000	1300	70
	—	1100000	—	170
16	160	970000	400	100
	150	580000	630	40
Non-pregnant	40			
	30			
	10			

* 0 not detectable. † —not determined.

To investigate whether histidine decarboxylase was uniformly distributed in the placenta or was confined to some particular

TABLE III. HFC of Various Tissues of Adult Female Hamsters. Figures represent counts/min/g.

Day of pregnancy	Abd skin	Lung	Liver	Spleen	Gastric mucosa	Small intest	Kidney	Skeletal muscle
11	20	0*	0	40	5000	150	2100	0
14	80	90	50	100	3100	90	1900	20
	150	100	100	90	2200	—†	1300	—
15	80	30	0	30	4500	—	1200	—
	50	150	—	90	1400	170	1200	110
16	80	210	40	170	3700	210	1000	0
Non-pregnant								
	100	0	70	70	9000	140	1000	30
	40	0	80	30	7300	100	810	0
	120	0	50	40	17000	130	940	10
	110	0	10	60	1100	—	1200	—

* 0 not detectable. † — not determined.

TABLE V. Vertical Distribution of Placental HFC (counts/min/g) in 5 Hamsters on 14th (No. 1-3) and 15th Days (No. 4-5) of Pregnancy. Roman figures refer to number of the layers as described in text. Layer I is the one facing the uterine wall.

	Hamster No.				
	1	2	3	4	5
I	390000	920000	1300000	810000	590000
II	1400000	1900000	1900000	1700000	1400000
III	1900000	1100000	1300000	1600000	1900000
IV	1600000	430000	400000	1700000	2100000
V	870000	140000	85000	730000	1100000
VI	460000	62000	54000	120000	250000
VII	220000	10000	41000	21000	100000
VIII	85000			—*	21000
IX	72000				
X					

* Lost.

layer(s), placentae from 5 pregnant hamsters were cut in serial sections as described. Thus, the placenta was divided into 7 to 10 layers, parallel to the uterine wall. Each layer was examined for its HFC, and its histological structure was studied. The figures for enzyme levels are presented in Table V. In Fig. 1 the histidine decarboxylase activity of the 10 layers of the placenta from hamster No. 5 (Table V) is represented in relation to a microphotograph of a transverse section through these layers.

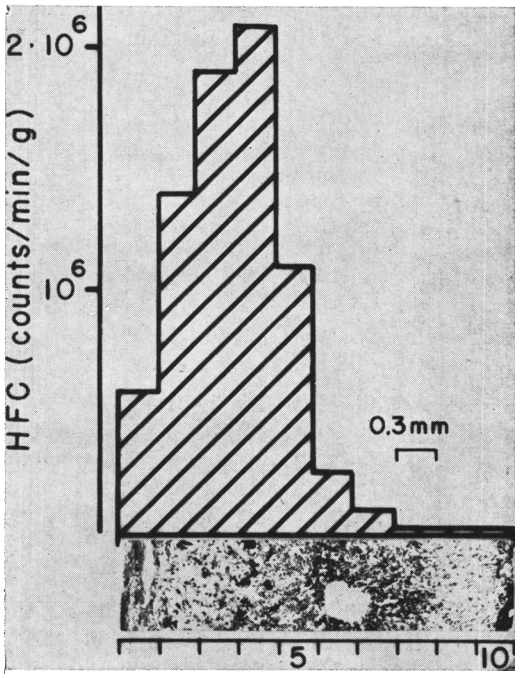

FIG. 1. HFC profile and microphotograph of hamster placenta on 15th day of pregnancy.

The enzyme is preferentially distributed, with peak activity in layers II, III and IV. These layers are distinguished by the presence of particular kinds of cell, among them giant cells(7).

Discussion. The non-pregnant hamster, like all mammalian species studied in this laboratory, forms histamine, and in this animal the gastric mucosa has the highest HFC. Part of the histamine formed is excreted in the urine. The principal pathways of histamine inactivation in the hamster are methylation and oxidative deamination. In this respect the hamster is similar to the mouse.

There is no considerable difference in the tissue HFC, outside the uterus, between the pregnant and non-pregnant hamster, except possibly for the lung where a small amount of activity could be observed. This is in contrast to the findings in the rat and mouse, in which the gastric mucosal HFC is elevated in pregnancy. The latter species shows, in addition, a very high elevation in kidney HFC during pregnancy. The fact that the sites of increased HFC differ from species to species cannot now be explained.

The striking height of placental HFC which by far exceeds the levels observed in any normal tissue so far studied presents a puzzling problem. A clue might be found in the fact that the enzyme concerned is preferentially localized in definite placental layers carrying cells of characteristic structure. Obviously, attempts should be made to isolate these cells and to examine their HFC.

Also the time course of the elevated rate

of histamine formation invites speculation. In the first place, the time course of increased histamine excretion parallels the one previously found to occur in the pregnant rat(8); in both species the onset of elevated HFC is at a late stage of gestation, and the rate of formation falls to about the non-pregnant level just prior to, or at term. This fact may suggest involvement in the complex machinery which controls the contractility of the uterus. It should be recalled here that, in the rat, histamine inhibits uterine contractility and thus appears to contribute to maintaining the uterus quiet under the mechanical stimulus of the growing foetuses. Studies on the effect of histamine on the uterus of the pregnant hamster are in progress. However, a clear answer seems to be remote due to experimental difficulties. Lastly, the possibility of the high placental HFC being part of a specific metabolic function must be considered. The placenta of mammals has been shown to be endowed with an impressively wide range of enzymatic capabilities(9).

Summary. In the hamster the histamine forming capacity (HFC) of various tissues was determined in the non-pregnant and pregnant states. In pregnancy, the HFC, as reflected in urinary histamine, was greatly elevated during the last 4-5 days of pregnancy. The placenta, and furthermore certain layers therein, were recognized as the sites of the increased rate of histamine formation.

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Incorporation of Tritium from Succinate-2,3-H^3 into Long Chain Fatty Acids by Aortic Mitochondria.* (29999)

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It was observed in this laboratory that long chain fatty acid synthesis from acetate in rabbit aortic intima-media strips was greatly stimulated by succinate(1). In fact, in the intact tissue, succinate was a greater stimulant on a molar basis than citrate.

Citrate and isocitrate are the most potent stimulants of fatty acid synthesis by the non-mitochondrial or "malonyl CoA pathway" in liver, adipose tissue, and mammary gland (2,3,4). The mechanism of stimulation by these Krebs Cycle intermediates is still uncertain, and the best available evidence indicates that citrate somehow causes a direct "activation" of the enzyme acetyl CoA carboxylase(5).

The mitochondrion from rabbit aorta is the subcellular unit in which most fatty acid synthesis occurs in this tissue. In isolated, washed aortic mitochondria citrate is a more potent stimulant of fatty acid synthesis than succinate. However, succinate does stimulate by itself and a combination of succinate and citrate will stimulate to a greater extent than equimolar quantities of either separately. Hülsmann(6) has described isocitrate and succinate stimulation of fatty acid synthesis

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