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Histamine: Entering Physiology

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1. *Introduction*

Acetylcholine, adrenaline, noradrenaline and histamine, representing a class within what DALE originally referred to as Autopharmacology, were known for their actions, when injected, already at the beginning of this century. In the period 1920–1950 experiments were designed to show that acetylcholine and the catecholamines served physiological purposes by their release from preformed stores, thus providing for neurohumoral transmission, to excite or inhibit specific target organs. Similarly launched searches for release of histamine in physiological circumstances proved conspicuously and disappointingly unrewarding. At the massive 'Symposium on Histamine' in honour of Sir HENRY DALE, held in London in 1955[1], there was little material to portend the imminent watershed in histamine research. At that time, naturally, the biogenesis of histamine, its association with mast cells, and its release from these cells by various non-physiological means were the central topics.

It would now appear that the stalemate in the search for a place for histamine in normal physiology has been overcome. This breakthrough was achieved by: 1. elucidation of the origin of tissue and urinary histamine, 2. elaboration of sensitive and specific means to determine the rate of histamine formation in vivo and in vitro, 3. the realization that changes in the rate of formation of histamine in tissues, not its release, provide a clue to the physiological significance of this amine, 4. the observation that great changes in the rate of histamine formation may occur without corresponding changes in tissue histamine content, and 5. the discovery of procedures to inhibit or enhance the rate of histamine formation in vivo.

This new approach and unbiassed views brought as the prime harvest the discovery that striking increases in histamine formation occur in the growing embryo of some species and that significant interrelations exist between hormones and histamine metabolism.

2. *Terminology*

In the course of current trends in studies on histamine a number of terms and definitions are used among which three are novel: histamine forming capacity, non-mast-cell histamine, and nascent histamine.

Histidine decarboxylase (E.C. 4.1.1.22): intracellular enzyme, instrumental in the formation of endogenous histamine. The prefix 'specific' appears superfluous since the affinity of this enzyme is more than 2000 times higher[2] than the affinity of the enzyme(s) currently given the prefix 'non-specific' which should not be designated as histidine decarboxylase, but rather, for the sake of clarity, as DOPA-decarboxylase or its cognates[3].

Histamine forming capacity (HFC): expression introduced 1960[4], denoting histidine decarboxylase activity in numerical terms.

Non-mast-cell histamine: this concept was introduced in 1960[4] and the distinction between mast cell and non-mast-cell histamine was explored in 1963[5].

Nascent histamine: term employed in our laboratory since 1962. Denotes intracellular histamine, formed at high rates, presumably non-mast-cell histamine, seemingly involved in certain kinds of rapid tissue growth and repair. Its action appears to be linked to the very process of its formation, conceptually operating by way of some 'metabolic target(s)'; not to be confused with the liberation of preformed histamine, notably not with the histamine liberated and acting 'intrinsically', as envisaged 1948 by DALE[6]. The action of nascent histamine is presumably not achieved by injected histamine, and its action is not prevented by antihistaminics.

Mobilization of histamine: implies transient loss of intracellular histamine and its shift from one compartment to another; this has in normal physiology been shown to occur in one instance only, in the parietal cell containing region of the gastric mucosa.

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3. *Origin of histamine contained in tissues*

The occurrence of histamine in mammalian tissues has been known since 1911[7] and its distribution in various tissues has subsequently been determined in many tissues and species, determinations which have been summarized by ROCHA E SILVA[8]. An unresolvable dilemma existed even at the time of the Symposium in 1955[1], in that histamine forming enzymes were seemingly not present in any of the tissues of cats and dogs. In these animals, the tissue histamine was believed to be absorbed from the gut, thus acquiring, as it were, a vitamin-like status. It had further been reported that treatment of rats with compounds that inhibit the growth of intestinal bacteria was accompanied by a substantial decrease in urinary histamine excretion and tissue content of the amine. The consensus in 1955 was that in some species tissue histamine was wholly of exogenous origin, whereas in other species, such as the rat, the tissue supply of histamine was dual in origin, at least in part endogenous. If this view were correct, histamine of endogenous origin would be swamped by exogenous histamine and determination of histamine in tissues and urine would be of little physiological significance.

The conundrum of the origin of tissue histamine was finally resolved by two entirely different approaches. The activity of the histamine forming enzyme is so low in most tissues that the activity could not be measured by the methods employed before 1955. By about this time SCHAYER[9] in USA had elaborated a sensitive and specific method for assaying histidine decarboxylase. In whatever tissue SCHAYER looked for histamine formation he found it to occur. At about the same time the present authors[10] undertook a study of germ-free reared rats with the object of establishing 1. whether germ-free rats differ from ordinary rats in the histamine content of their various tissues, 2. whether the urinary excretion in ordinary rats, as in the germ-free ones, reliably indicates the rate of endogenous whole-body histamine formation, and, by implication, physiologically occurring alterations in the rate of histamine formation. The germ-free rats, neither in any particular tissue examined, nor in the whole animal, contained less histamine than the non-germ-free ones. These observations show that even in non-germ-free rats the histamine contained in the various tissues is endogenous in origin.

In the course of this work an additional observation was made that came to bear beneficially in further studies. A sex difference in histamine excretion was noted which was unexpected at the time of these experiments. The female rat, but not the male, excreted large amounts of free histamine that could be assayed directly on a segment of isolated guinea-pig ileum, as previously done by WILSON[11]. In a study of histamine excretion as influenced by X-ray irradiation, LEITCH, DEBLEY and HALEY[12] noted that the female rat excretes much more free histamine than the male.

The principal outcome of the study of germ-free rats is the recognition that the whole-body endogenous histamine formation and changes therein can be followed continuously day after day in the female rat by determining the urinary excretion of histamine. The female rat, with its urinary excretion of histamine, was at once used and is still employed, like an Aladdin's lamp, to reveal overall changes in histamine formation. The first fruit to be gathered by this means was the discovery that in the pregnant rat histamine is formed at exceedingly high rates.

4. *Methods to determine rate of histamine formation*

The methods to determine rate of histamine formation, i.e. histidine decarboxylase activity in individual tissues, or in the whole animal, are of these types:
1. Non-isotopic determination of newly formed histamine;
2. Isotopic: a) whereby newly formed labelled histamine is measured; b) determining $^{14}CO_2$ evolved from carboxyl labelled histidine. The general principles of these methods, and the methods for histidine decarboxylase assay have been comprehensively described by SCHAYER[13].

Non-isotopic whole-body histamine formation. By determining histamine excretion the amine was brought into the realm of normal physiology in that in the pregnant rat we found high rates of histamine excretion, the elevated HFC residing mainly in the foetus, suggestive of a causal connection between increased histamine formation and rapid tissue growth.

Non-isotopic in vitro method. In this method minced tissues are incubated with non-isotopic histidine at approximately 100 times the concentration used in the isotopic method. This method is of little avail in measuring histidine decarboxylase activity for which the method is of low sensitivity and non-specific.

Isotopic in vivo determination. This method, devised by SCHAYER, measures the amount of ^{14}C-histamine excreted in the urine following the injection of a known amount of ^{14}C-histidine. The method determines whole-body HFC and can also be employed in measuring the in vivo HFC of individual tissues.

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Isotopic in vitro determination. Excised tissues are minced with scissors, not homogenized or extracted, since destruction of cell structure is detrimental to histidine decarboxylase activity, as emphasized by SCHAYER[13]. The amount of substrate employed, 40 µg ^{14}C-histidine, incubated with about 0.2 g of tissue, is far too small for decarboxylases other than histidine decarboxylase to participate significantly in the formation of histamine as determined by this in vitro method. There are no standardized units for expressing histidine decarboxylase activity. In reports from our laboratory, tissue HFC is expressed in terms of the amount of histamine formed by 1 g of tissue in 3 h.

Isotopic $^{14}CO_2$ method of Kobayashi. The isotopic techniques employed by SCHAYER and in our laboratory are laborious, time consuming and require a considerably period of training. A simpler procedure for measuring histidine decarboxylase could be based on determining $^{14}CO_2$ formation from ^{14}C-carboxyl labelled histidine as described by KOBAYASHI[14]. GRAHN and ROSENGREN[15] compared results obtained with the $^{14}CO_2$ method with results obtained with SCHAYER's method. The results obtained by the two methods differed. The usefulness of the $^{14}CO_2$ method was found to be limited, seriously so, by the finding that histidine apparently is transformed into some derivative which gives off CO_2 from the carboxyl group without concomitant formation of histamine. Work is in progress aiming at overcoming the inconstancy of the time-saving $^{14}CO_2$ method.

5. *Some characteristics of mammalian histidine decarboxylase*

Ubiquitousness of histidine decarboxylase (HD). This enzyme has been found in all mammals and in every tissue examined by adequate technique for HD activity. Among the tissues indicated in Table I are some rich sources of the enzyme.

HD synthesis can be rapidly accelerated. Under the influence of various hormones, e.g. gastrin, or catecholamines, the HD activity of target tissues rises rapidly. A similar steep rise occurs with stimuli which provoke inflammation. The rise in HD activity is prevented in the presence of inhibitors of protein synthesis[16,17], suggesting that elevated HD activity springs from an increase in the amount of enzyme formed. In our laboratory, endogenous histamine associated with metabolic processes, e.g. growth and protein synthesis, is referred to as 'nascent histamine'. This term serves the purpose, among others, of emphasizing the de novo formation of histamine at elevated HD activity, as distinct from the release of preformed histamine.

Co-enzyme of histidine decarboxylase. ROTSCHILD and SCHAYER[18,19], on subjecting rat peritoneal mast cells to dialysis, noted a large loss in HD activity that could be restored by the addition of pyridoxal phosphate. Similar results were obtained by ONO and HAGEN[20] with HD extracted from mouse mastocytoma. This co-enzyme function has also been shown in vivo: on omitting pyridoxal phosphate from the diet of female rats, the whole-body histamine formation falls to about 20% of normal, as judged by its rate of excretion[5].

Inhibitors of histidine decarboxylase. α-methylhistidine has been recognized as a specific inhibitor of HD[21] and is used to distinguish HD from DOPA-decarboxylase.

6. *Catabolism of histamine*

The principal routes involved in the enzymatic degradation of histamine have been clarified, mainly by the work of SCHAYER[22,23], the results of which are summarized in Figure 1. The Figure shows the various ^{14}C-labelled catabolites that have been identified in the urine after injection of a small amount of ^{14}C-histamine. The principal means of catabolism are oxidative deamination (diamine oxidase, alias histaminase) and methylation (methyl-N-transferase). These enzymes are widespread in mammals, and species differences in their distribution exist.

It is assumed, although difficult to prove, unless non-isotopic methods for identifying urinary endogenous histamine metabolites have been devised, that small amounts of exogenous histamine are catabolized in the same way as endogenous histamine.

7. *Inhibition of histamine formation and what it revealed*

Much could be learnt of the physiological significance of histamine if it were possible to inhibit its formation in vivo. Only recently have two methods for in vivo HFC determinations become available: 1. SCHAYER's isotopic method in which ^{14}C-histamine formed from injected ^{14}C-histidine is measured, and 2. the urinary excretion of free histamine in the female rats. Employing these methods, the following results were obtained.

Pyridoxine deficiency and semicarbazide. Feeding the female rat a pyridoxine-deficient diet and injecting semicarbazide depresses the whole-body histamine

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formation to about 10% of normal[5] as seen in Figure 2. This Figure also shows the agreement of results obtained with SCHAYER's and our method (aminoguanidine was given to minimize histamine inactivation by histaminase). No other means so far deviced equals semicarbazide superimposed on the pyridoxine-deficient diet in its power to inhibit histamine formation in vivo, as testified by various workers, among them JOHNSON[24]. This state of strong inhibition enabled rewarding observations.

Relation between tissue histamine content and HFC. At the time of our report on inhibition[5] it had been recognized by SCHAYER, and in our laboratory, that a consistent relation between histamine content and HFC of tissues did not exist. This disclosure was helpful in altering the course in histamine research: the flood of reports on histamine content waned and studies of histamine formation came to the fore.

Feed-back relation. On instituting inhibition of histamine formation, observations were made indicating that a lowering of tissue histamine content elevates tissue HFC by a feed-back relation between histamine content and histidine decarboxylase activity. This phenomenon will be further described in the section discussing gastric secretion.

Lifetime, mode of binding, turnover rate. SCHAYER et al.[25] injected ^{14}C-histidine into guinea-pigs and rats and determined the amount of ^{14}C-histamine present in excised tissues at various time intervals. In rat abdominal skin the lifetime of a histamine molecule was about 15 days. We[5] determined turnover rate by examining various tissues for histamine content at 1 to 199 days of strong inhibition of histamine formation. In tissues rich in mast cells, inhibition of histamine formation for several weeks was not followed by a significant fall in histamine content, whereas in non-mast-cell tissues, e.g. the gastric mucosa, the content fell to low levels within 24 h.

The determinations summarized in Table II show that tissue histamine with regard to lifetime resides in at least three pools, firmly bound in mast cells (skin) and in non-mast cell compartments of the lung (lung is poor in mast cells), loosely bound in the gastric mucosa and in certain portions of the lung and small intestine.

Rebound, overshoot. On discontinuing inhibition treatment, a state of supernormal histamine formation ensues, as seen in Figure 2. It represents a state of elevated HFC maintained in the absence of drugs, a quasi-physiological state in which valid information can be sought on the physiological significance of 'nascent histamine', as will be discussed in the paragraph on wound healing.

Table I. Comparison of histidine decarboxylase activity (HFC) in some tissues of different species determined in vitro in our laboratory

	Skin	Gastric mucosa	Maternal kidney	Placenta	Whole foetus
Rat	20	8000	10	10	3000
Mouse	30	1000	20,000	10	10,000
Hamster	20	1000	300	20,000	20
Guinea-pig	< 10	300	100	20	400
Cat	10	40		20	20
Man	10	40		< 10	

The values are approximate and expressed as ng histamine formed/3 h/g tissue[3].

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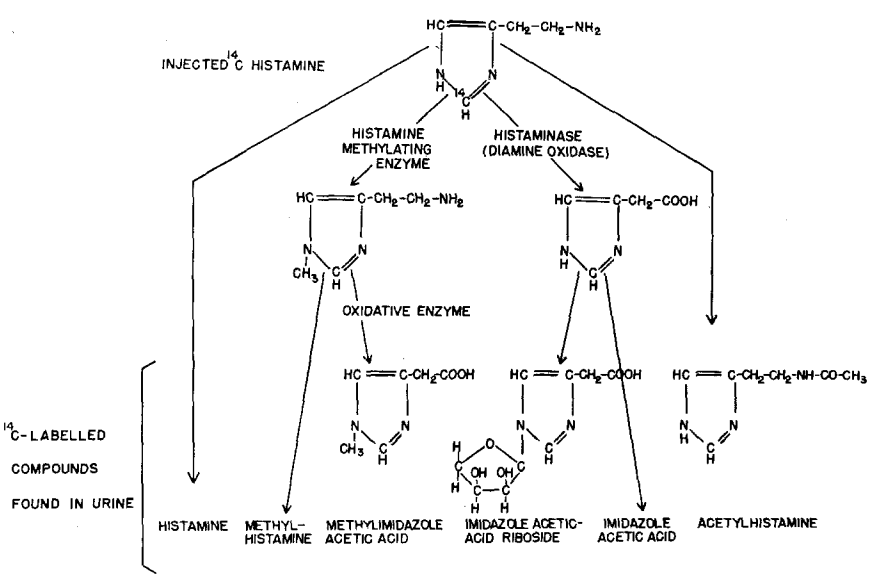

Fig. 1. Principal pathways for the catabolism of injected ^{14}C-histamine.

α-methylhistidine. This compound was first synthesized by ROBINSON on a μg scale and investigated by ROBINSON and SHEPHERD[27]. For unexplained reasons, they did not recognize the powerful inhibitory action of this compound which was later assessed in vitro in a study in which the compound was defined as a specific competitive inhibitor of histidine decarboxylase. The usefulness of this compound in studies in vivo is limited by the fact that it is inactivated by decarboxylation and consequently produces strong inhibition only on continuous infusion. α-methylhistidine is useful in distinguishing histidine decarboxylase from DOPA-decarboxylase which is not inhibited[5].

4-bromo-3-hydroxybenzyloxyamine (NSD-1055). In vitro, this compound strongly inhibits both histidine decarboxylase and DOPA-decarboxylase[28], nearly 100% inhibition being obtainable. In vivo, the compound is less active presumably owing to rapid destruction. In the authors' laboratory about 50% inhibition was obtained employing SCHAYER's isotopic method[29], injecting ^{14}C-histidine and determining ^{14}C-histamine formed, whereas SCHAYER[30], employing the same method, found NSD-1055 ineffective in vivo. In vivo studies with this compound revealed an interesting situation: JOHNSTON and KAHLSON[29] found that NSD-1055 inhibited the decarboxylation of injected ^{14}C-histidine, whereas the decarboxylation of endogenous histidine, a natural constituent of cells, was not inhibited. The complex problem of in vivo inhibition of histamine formation has been discussed in a current Monograph[3]. The present authors hold the view that in vivo, 100% inhibition of histidine decarboxylase will not be attainable owing to the feed-back relation, i.e. lowered tissue histamine content will promote the synthesis of the enzyme.

8. *Histamine metabolism in pregnancy*

Having noted that in the female rat the urinary excretion of free histamine reflects the rate of endogenous histamine formation, the discovery that the rat produces excessive amounts of histamine during a certain phase of pregnancy lay at the door. The elevated HFC was found to reside mainly in the rapidly growing foetus. This finding sowed the seed from which grew the idea of an association between high HFC and certain types of rapid tissue growth.

Rat. On the 15th day of pregnancy a steep increase in histamine excretion occurs that subsides around the day of parturition, as shown in Figure 3, which also shows that if the foetuses are removed, the excess histamine excretion ceases. In vitro determinations revealed that the foetal liver produces histamine at an enormously high rate with an HFC about 100 times higher than in the rest of the foetal body in which the HFC is also relatively high until 2–3 days after birth. The foetal histamine is non-mast-cell in natur, its rate of turn-over is high and the histamine content of the foetus is low, i.e. the first discovered example of 'nascent histamine'.

Mouse. As pregnancy proceeds histamine excretion rises to values sometimes 100 times the pre-pregnant, falls after delivery, the pre-pregnant level not being attained until a few weeks after delivery. In this species the elevated HFC of the foetus resides mainly in the skin[32]. In contrast to the rat and hamster the kidney

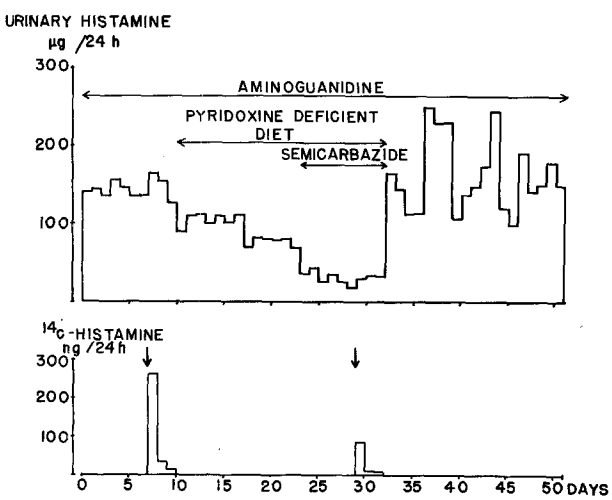

Fig. 2. Female rat fed on a histamine-free diet. Upper section, urinary excretion of free histamine; lower section, excretion of ^{14}C-histamine after s. c. injection of 220 μg ^{14}C-histidine. Semicarbazide was injected s. c., 1st day 50 mg/kg twice daily, and 2nd day onward 75 mg/kg twice daily. Note rebound (overshoot) and similarity in results obtained with the non-isotopic and isotopic methods[3].

Table II. Turnover-rate of histamine in various tissues. The estimates denoted (A) are those by SCHAYER[25]; (B) ours[5]; (C) BJURÖ et al.[26].

Abdominal skin	Slow (A), (B), (C)
Ear	Slow (B)
Gastric mucosa	Rapid (A), (B), (C)
Kidney	Rapid (C)
Liver	Rapid (C)
Lung	Slow in one portion Rapid in a different portion } B
Small intestine	2 different rates, as in lung (B)
Tongue	Slow (B)

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of the pregnant mouse displays high HFC, a phenomenon which, as will be shown, renders the kidney of the non-pregnant mouse singularly useful in exploring agencies and circumstances conducive to elevation of histidine decarboxylase activity[32].

Hamster. The gestation period in this species is only 16 days. On the 11th day of pregnancy histamine excretion increases, rises to very high levels and falls steeply on the day of parturition[33]. Persistent exploration revealed that the high HFC resided in the uterus, predominantly in the placenta in which a particular kind of giant cells, described by WARD-ORSINI[34], displayed a HFC which by far exceeds the levels observed in any normal tissue so far studied.

Man. Information is scanty. With the foetus in uteru (legal abortion), blood was collected from the umbilical artery and vein and from a carotid artery after the foetus had been removed. In foetal carotid artery and umbilical artery plasma histamine was present in easily demonstrable amounts, whereas maternal plasma did not contain histamine in measurable concentrations. There was more histamine in the umbilical artery plasma than in the umbilical vein plasma[35]. Similar results have been reported by BJURÖ, LINDBERG and WESTLING[36].

Histaminase in pregnancy. The presence of a histamine-inactivating factor in the human placenta[37] and in the blood of pregnant women[38] represents the first recorded instance of a major change in histamine metabolism in normal physiology. Pregnant women dispose of injected histamine more efficiently[39], but the means by which this is achieved are not fully understood. It would appear that in the human foetus methylation is a principal histamine metabolizing route[40]. Among the conundrums of histaminase only two will be mentioned. In the rat, even when not subjected to inhibition of histaminase by aminoguanidine, large amounts of histamine produced by the foetus do traverse the seemingly powerful histamine-destroying placental and uterine barriers. Further, the pattern of urinary metabolites of injected ^{14}C-histamine in the pregnant and non-pregnant human females[41] is mainly the same as the pattern found by SCHAYER and COOPER[42] in human males. There is no evidence that histaminase is essential to the normal course of pregnancy and parturition in man and animals.

9. *Relations between hormones and histamine metabolism*

A connection of this kind is evident from the observations of histaminase in pregnancy. Yet, this lead has been of little avail to students of the physiology of histamine.

Sex differences were found in our study of the biogenesis of tissue histamine in germ-free rats. In the male, methylation is a principal catabolic pathway[43], and testosterone administration increases the proportion of methylhistamine in the urine of castrated male and female rats[44]. A target organ, specifically responsive to some unknown circumstance conducive to raising histidine decarboxylase activity, exists in mice, in which the kidney of the female is endowed with a much higher HFC than that of the male[45]. Oestradiol, administered to ovariectomized mice produced a 10- to 50-fold elevation of kidney HFC[3].

Testosterone, injected in female mice, produces the strongest abolition of histamine formation obtainable in vivo by any means available [46]. Histamine and its metabolites practically disappear from the urine. Instead, an other amine, seemingly putrescine, appears in the urine. It will be discussed below that histamine and putrescine appear to be linked to metabolic pathways in which they serve similar functions related to tissue growth.

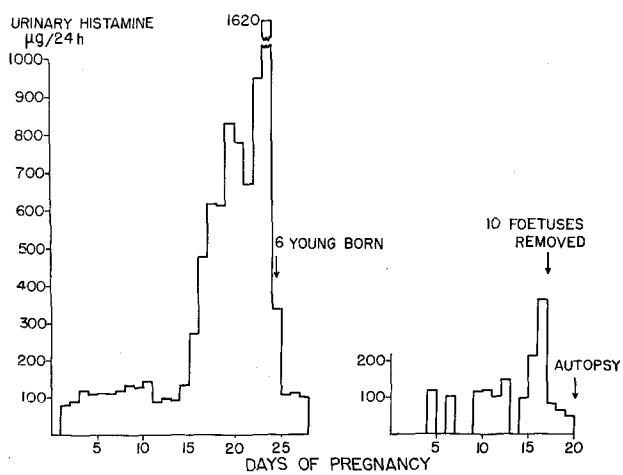

Fig. 3. Urinary excretion of histamine in undisturbed pregancy (left side of the figure) and in a rat where the foetuses were removed at the 17th day of pregnancy (right side). Throughout the whole course of the observations the rats were under the influence of the histaminase inhibitor aminoguanidine[31].

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10. *Tissue growth in healing wounds*

Studies of the histamine metabolism in the reparative growth in healing skin wounds appeared attractive because in the skin of the rat the rate of histamine formation can be lowered or raised experimentally. The pertinent observations are as follows[47,48].

Index of the rate of repair. The tensile strength (TS) of the healing wound in situ, reflecting the rate of repair, was determined in terms of grammes of pull, 5 days after wounding, employing a tensiometer designed at our institute[3].

HFC of wound and granulation tissue. Granulation tissue was allowed to grow into plastic sponges placed between the skin and underlying muscle. Wound tissue, as sampled, included a few mm of the edges of the wound. Granulation and wound tissue were obtained at various intervals after infliction of the wound, and their HFC was compared with contralateral abdominal skin from the same rat. In wound tissue, excised 24 h after wounding, the level of HFC was 50–60 times that in control skin. In the granulation tissue, the HFC reached a peak at the 5th day and then fell steeply, the peak level being about the same as in wound tissue.

Histamine content of excised tissue. This was much lower than that of the intact skin, indeed, in granulation tissue the histamine content is lower than in any other tissue of the rat so far examined. The histamine formed so rapidly is not held by these tissues, indicative of nascent histamine, a situation pertaining also to embryonic tissues.

Repair at lowered HFC. As set out in section 7, semicarbazide in conjunction with a pyridoxine deficient diet strongly inhibits histamine formation in vivo (c. 80%) in the rat. Subjecting rats to inhibition by this means retarded rate of healing, in terms of TS, by about 40%.

Repair in the phase of 'overshoot'. In this phase, described in section 7, histamine formation is increased 2–4 times for a week or longer. During this phase the tissues are cleared of drugs, i.e. the elevated HFC constitutes the sole relevant tissue change. Figure 4 illustrates the singularly great increase in rate of healing in the period of elevated HFC.

Ineffectiveness of extracellular histamine and anti-histamine drugs. Flushing the healing wound with extracellular histamine, derived from 'long-acting' histamine in the form of an oily suspension deposited under the skin, did not significantly influence the rate of repair, nor did anti-histamine drugs alter the rate of healing. That is to say, the mode of action of nascent histamine cannot be fulfilled by extracellular histamine, nor is its action antagonized by anti-histamines.

The effect of cortisone. It is well known that cortisone in large doses inhibits the rate of wound healing. SCHAYER et al.[49] have shown that cortisone inhibits histamine formation in the rat skin, and SANDBERG[50] found that under the influence of cortisone the amount of granulation tissue growing into implanted polyvinyl sponges was greatly reduced. Further, SANDBERG and STEINHARDT[51] demonstrated that administration of cortisone restrained the elevation of HFC which normally is engendered in the tissues of the wound; they inferred that cortisone retards healing by way of its restraining effect on HFC.

HFC in human wound tissue is considerably higher than in specimens of intact skin[48].

11. *Malignant and regenerative growth*

From the above findings it appeared likely that a similar elevation of HFC might be found in malignant growth.

Mastocytomata. A specimen was found incidentally in a dog in 1958 in our laboratory and investigated for its HFC. It was high, whereas normal mast cells, although rich in histamine, do not display particularly high histidine decarboxylase activity. For discussion of this subject, see KAHLSON and ROSENGREN[3].

Rat hepatoma. MACKAY et al.[52], on implanting a hepatoma subcutaneously in female rats, noted that the urinary histamine rose steeply from the normal

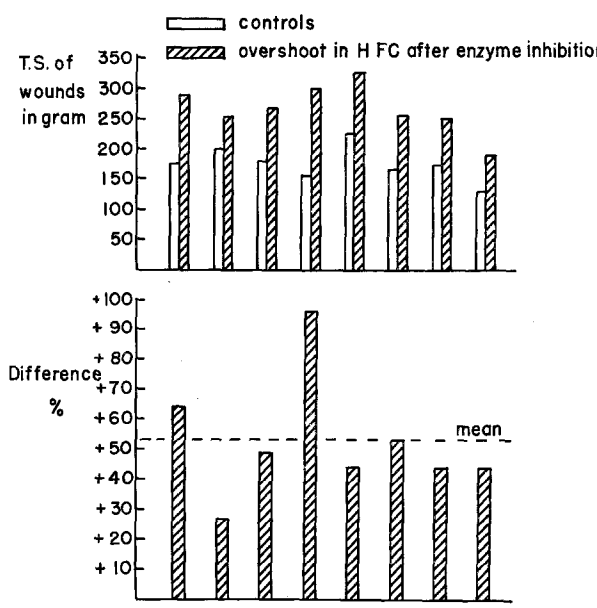

Fig. 4. Healing during the 'overshooting' phase[47].

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30–40 μg per rat in 24 h to more than 1 mg. SHEPHERD and WOODCOCK[53] examined in vitro seven various kinds of rat hepatoma and found exceedingly high HFC values in all but one.

Virus-induced rat sarcoma. This has been thoroughly investigated[3]. Histamine excretion increased steadily as the tumour grew and its HFC, determined in vitro, was high. There was a tendency for a correlation between height of HFC and mitosis rate in the tumour. The histamine content was very low. The liver of the tumour-bearing host engendered conspicuously high histidine decarboxylase activity.

Walker carcinosarcoma. This tumour was investigated in great detail by MARIAN JOHNSTON in our laboratory[54], and the results are discussed in a Monograph[3]. The urinary excretion of histamine rose as the tumour grew as shown in Figure 5. The tumour tissue displayed HFC levels of a height that is exceeded only by the HFC of the gastric mucosa and foetal tissues. The liver was enlarged, as in rats with virus-induced tumours, and its HFC was very much higher than in non-tumour-bearing controls. In parabiosis experiments it was shown that the HFC was elevated in the liver of the non-tumour-bearing partner also, indicating the involvement of a blood-borne agent(s) in the mechanism causing elevation of HFC at sites distant from the tumour.

Ehrlich ascites tumour in mice. The histamine metabolism of this tumour has been more thoroughly studied in our laboratory than any other rapidly growing tissue[3]. Cell multiplication in this tumour was high during the first three days after inoculation, as indicated by the mitotic index and the growth curve. During this phase, the histidine decarboxylase activity per unit mass of tumour was very high, whereas in later stages, with decreasing mitotic index, the HFC fell correspondingly and was nearly zero when cell multiplication was in abeyance.

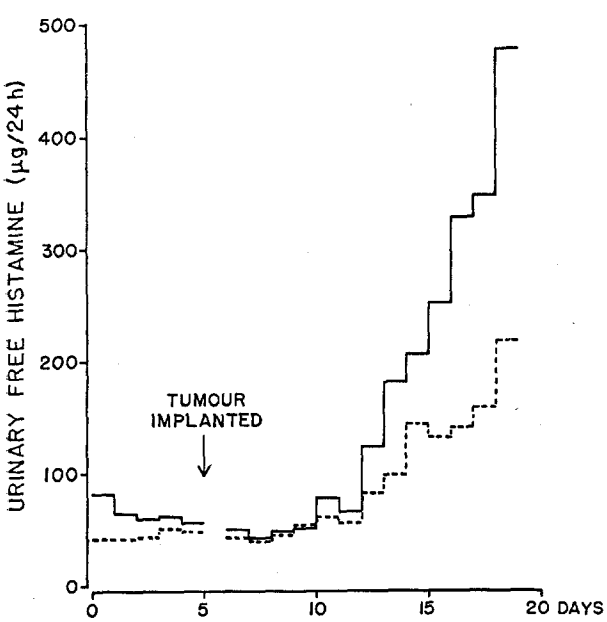

Fig. 5. Urinary histamine excretion patterns in two female rats implanted at the arrow with the Walker carcinosarcoma[54].

Nature of the tumour enzyme. On all criteria tested, e.g. strong and weak inhibition by, respectively, α-methylhistidine and α-methyl-DOPA, and pH optimum, the enzyme in all tumours investigated displayed the behavior characteristic of embryonic histidine decarboxylase.

Significance of nascent histamine. In the virus-induced sarcoma, the high HFC persisted throughout a long line of re-transplantation. It is known that after several transplant generations generally only those enzymic systems essential to energy production, growth and reproduction are retained in the tumour[55].

Putrescine is formed in some rapidly growing normal and malignant tissues. In these instances, the regenerating rat liver[56] and the STAT-1 rat sarcoma[57], histidine decarboxylase activity is low and replaced by ornithine decarboxylase the product of which is putrescine, a diamine, as is histamine. It appears reasonable to assume that nascent histamine and putrescine are capable of substituting for each other in a process of linkage to metabolic pathways essential to growth.

12. *Gastric secretion*

BABKIN[58], in a lecture 1938, conjectured that stimulation of the vagal supply to the gastric glands may liberate acetylcholine which, in turn, would release histamine from some site in the vicinity of the oxyntic cell. This prophetic pronouncement incited to a long line of experiments to test the hypothesis. This work has in places been confounded by emotions, as once the theory of acetylcholine as the agent in neuromuscular transmission. 'Another nail in the coffin' has been hammered for histamine as an agent in gastric secretion[59] mostly driven by more feeling than understanding. Now, at last, the occasionally maltreated amine has been restored. The evidence of this will be briefly explained (a full account is given by KAHLSON and ROSENGREN[3], and by KAHLSON, ROSENGREN and SVENSSON[60]).

Histamine formation in the gastric mucosa. The gastric mucosa of all species studied, man, dog, cat, rat, hamster, mouse and frog, forms histamine by the

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agency of histidine decarboxylase. The enzyme activity is higher the lower the sensitivity of the parietal cell to injected histamine. The histamine content and the HFC are not contained in the parietal cell proper, but in a structure adjacent to this cell, as shown by THUNBERG for the rat[61], illustrated in Figure 7. This implies that mobilized histamine impinges from the outside on the parietal cell which is endowed with receptors for histamine.

Effect of feeding, gastrin and vagal influence. It was discovered in 1964 that feeding and injected gastrin provoked striking events in the status of gastric histamine: histamine contained in the mucosa was mobilized and concurrently the mucosal HFC rose to levels several times the normal[3], as shown in Figure 6. Vagus stimulation per se elevates mucosal HFC, demonstrated in antrectomized rats in which antral gastrin release is eliminated.

The HCl-secretory device, as envisaged by ROSENGREN and SVENSSON in 1969[62], is shown in Figure 7 with slight alterations from the original one[3,60]. It comprises two components, the parietal cell proper, and an adjacent structure in which HFC and histamine stores reside. The HFC cell is stimulated to increased histamine formation by food, endogenous gastrin, vagal discharge and by lowering (mobilization) of its histamine content (interpreted by us as a feed-back relation[3]). Histamine mobilized and formed at increased rate is carried to the parietal cell. This latter cell is presumably under stimulatory influence by the vagus nerves and by some unknown factor(s) that increase the sensitivity of the parietal cell, a facilitating influence.

Clinical implication. Testing acid secretory capability by injecting histamine will provide information on the responsiveness of the parietal cell, but discloses nothing about the functional state of the proximal component of the secretory device. Obviously, administration of gastrin or its active derivatives should be employed.

Kinetics and magnitude of histamine mobilization during secretion. JOHANSSON et al.[63] assessed the rate and amount at which histamine is mobilized in the mucosa by determining in the urine of conscious rats the total amount of histamine and ^{14}C-histamine formed from injected ^{14}C-histidine during infusion of gastrin for 5 h. The ratio ^{14}C-histamine/total amount of urinary histamine, i.e. the specific activity of ^{14}C-histamine in the urine, was taken to indicate the contribution of newly formed histamine to the total amount of histamine excreted. The authors calculated that the amount of histamine mobilized in the mucosa per hour was more than twice the secretorily effective threshold amount on infusing histamine intravenously. From this they find it 'difficult to conceive of an intramural disposition so contrived that histamine mobilized at secretorily superthreshold rates, in close vicinity to the parietal cell, would elude stimulative encounter with this cell'.

Life-time of gastrin in the circulation and duration of secretion. Various authors have determined the half-life of injected gastrin in the cat, dog and human and found values of the order of 10 min. Gastric digestion proceeds in terms of hours, and so does the concomitant elevation of mucosal HFC. This latter phenomenon is an established effect of gastrin whereas no valid evidence of a direct action of gastrin on the parietal cell has been given.

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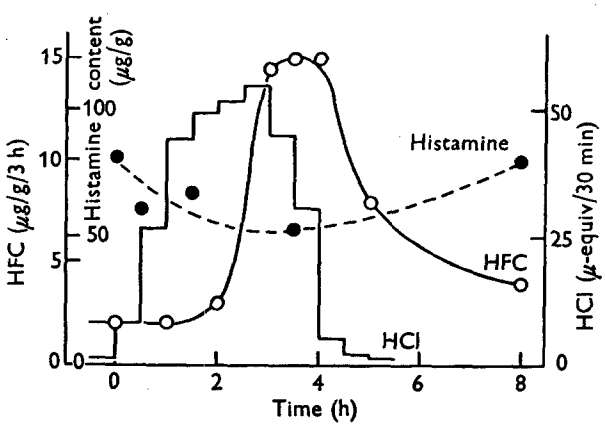

Fig. 6. Changes in HFC (open circles) and in histamine content (filled circles, broken line) of the gastric mucosa related to acid secretory response following re-feeding fasted rats. HFC and histamine content are means of figures obtained in a series of rats. The curve of HCl secretion is based on means from 3 experiments in a rat with a denervated pouch. Feeding was carried out at zero time[3].

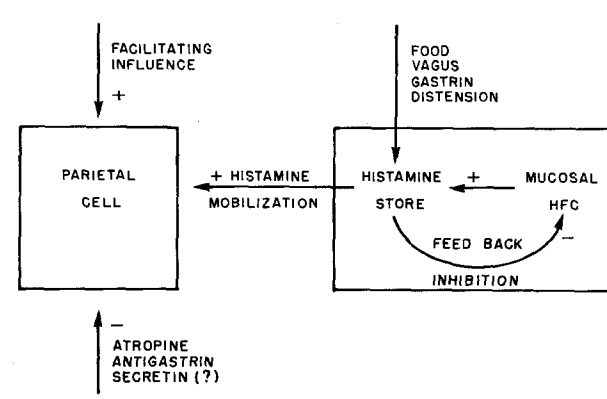

Fig. 7. The HCl-secretory device comprises two distinct components: the parietal cell proper and the HFC cell which forms and contains histamine. Stimulating components of feeding, e.g. gastrin, cause histamine mobilization. Histamine mobilization: a) stimulates acid secretion, b) decreases mucosal histamine content, c) releases histidine decarboxylase formation from restraint by decreasing histamine content, d) contributes to increased urinary histamine[60].

Receptors for histamine in the mucosa, concerned in histamine-stimulated acid secretion, have only recently been defined by BLACK et al.[64]. A specific blocker of mucosal histamine receptors given the trade name burimamide, inhibits to about equal degree acid secretion evoked by histamine, pentagastrin and food. This discovery of a histamine receptor for acid secretion provides strong support for the operation of a single final stimulatory agent, i.e. histamine, and complies with the notion of the secretory device as seen in Figure 7.

Zusammenfassung.

Die hier geschilderte Periode der Histaminforschung ist durch die Erkenntnis gekennzeichnet, dass grosse Veränderungen in der Geschwindigkeit der Histaminbildung unter physiologischen Verhältnissen vorkommen. In der Magenmucosa bedingen Gastrin und Nahrungszufuhr eine Mobilisierung von Histamin und eine Erhöhung der Aktivität der Histidindecarboxylase. Bei verschiedenen Formen von normalem und malignem Wachstum wird in den Geweben «Nascent-Histamin» gebildet, dessen Wirkung exogenes Histamin nicht ausüben kann und auch nicht mit Antihistaminen antagonisiert wird[65].

[64] J. W. BLACK, W. A. M. DUNCAN, C. J. DURANT, C. R. GANELLIN and E. M. PARSONS, Nature, Lond. *236*, 385 (1972).

[65] Acknowledgment is due to editors and publishers of journals for permission to reproduce published figures, and to the Swedish Medical Research Council for supporting our work under grant No. B72-14x-2212-05.

SPECIALIA

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Effects of *s*-Triazines on Protein Synthesis in Leaves of Peas (*Pisum sativum* L.) and Sweet Corn (*Zea mays* L.) and on the Ultrastructure of Pea Cotyledons

Sublethal concentrations of *s*-triazines cause an increase of proteins in leaves and seeds of several species of plants[1–4]. The protein-stimulative properties of simazine have been attributed to its action on nitrate reductase activity[2,5]. However, WU et al. have presented evidence that *s*-triazines influence not only the activity of nitrate reductase but also of transaminase, glutamate dehydrogenase, cytochrome oxidase, starch phosphorylase, pyruvate kinase, and δ-aminolevulinate dehydratase[6,7]. Application of these compounds to bush bean plants has resulted in an increased number of rough endoplasmic reticula and protein bodies in the cotyledons[8].

The present study was undertaken to ascertain the effect of *s*-triazines on protein synthesis evaluated as amino acid incorporation in the leaves of peas and sweet corn and ultrastructure of protein bodies of pea cotyledons.

Material and methods. For amino acid incorporation study, 2 and 5 mg/l solutions were made of *s*-triazines, simazine (2-chloro-4, 6-*bis* (ethylamino)-*s*-triazine), propazine (2-chloro-4, 6-*bis*(isopropylamino), igran (2-methylthio-4-ethylamino-6-isobutylamino-*s*-triazine), and prometone (2-methoxy-4, 6-*bis*(isopropylamino)-*s*-triazine). The 2 mg/l solutions were each sprayed on the leaves of the 27-day-old pea plants and the solutions containing 5 mg/l were each sprayed on the 42-day-old sweet corn plants until run-off. Triton-B 1956 was used as a surfactant. Leaf discs were cut with a cork borer from both the controls and treated plants after spraying. The discs were incubated in a medium containing radioactive amino acids. The rate of incorporation of L-leucine-U-^{14}C into protein of leaf discs was measured by using the method of KEY[9].

For ultrastructural study, 2 mg/l water solution of simazine, containing Triton-B 1956 was uniformly sprayed on the leaves of 40-day-old peas. The developing cotyledons were harvested 16 days later (12 days after anthesis). For the electron microsopic study, slices of developing pea cotyledons, approximately 1 mm^3, were fixed in glutaraldehyde and post-fixed in 3% OsO_4. After fixation, tissues were washed with 0.1*M* phosphate buffer. pH 7.3, dehydrated in graded ethanol and embedded in Epon 812. The blocks were sectioned with a glass knife on a Sorvall

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