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Histamine formation from ^{14}C-L-histidine in man

B. BERG, G. GRANERUS, MAJ-BRITT JOHANSSON, H. WESTLING AND T. WHITE

Departments of Clinical Physiology and Medicine, University of Lund, Lund, Sweden

Summary

1. ^{14}C-L-histidine was given i.v. to one normal subject and two patients with chronic myelocytic leukaemia. Urinary excretion of histamine and two of its metabolites methylhistamine and methylimidazoleacetic acid, total as well as ^{14}C-labelled, was measured, as well as blood ^{14}C-histamine. In addition the total urinary and pulmonary elimination of ^{14}C was followed.

2. Total ^{14}C elimination was high during the first days, then declined slowly except for a plateau at the 10th–14th day in the two patients. There was a measurable elimination even after some months.

3. ^{14}C-histamine appeared in the blood of the leukaemic patient, whereas in the normal subjects the values were hardly measurable.

4. The leukaemic patients excreted much more of the two ^{14}C-labelled histamine metabolites than the normal subject. The difference in excretion of the ^{14}C-labelled metabolites was largest around the 12th day after the infusion of ^{14}C-L-histidine.

5. The results indicate that the leukaemic patients formed at least 20 times more histamine daily than the normal subject.

Introduction

To our knowledge it has so far not been possible to demonstrate the formation of histamine from C^{14}-L-histidine in man *in vivo* (Brown, Silva, McDonald, Snyder & Kies, 1960; Lindell & Westling, 1966). Especially remarkable is the failure to demonstrate ^{14}C-activity in urinary histamine metabolites after giving labelled histidine to patients with mastocytosis (Demis & Brown, 1961), since this disease is held to be associated with an increased rate of histamine formation.

It has been known for a long time that blood from patients with chronic myelocytic leukaemia contains much histamine (Code & McDonald, 1937) but only recently has it been shown that such patients have other abnormalities in their histamine metabolism. Thus Berg, Granerus, Westling & White (1971) found a considerably elevated urinary excretion of not only histamine but also its main metabolites methylhistamine and methylimidazoleacetic acid. These observations indicated that some 100 mg histamine per day could be formed daily in patients with chronic myelocytic leukaemia. However, it was felt that definite proof of increased histamine formation required direct demonstration of histamine synthesis from ^{14}C-labelled histidine. Moreover, it could not be shown whether the increased urinary excretion of histamine metabolites in leukaemia was due to an increased turnover rate or to an increased 'histamine pool', of which the same

fraction as in the normal subject was renewed. A study of the excretion of ^{14}C-labelled histamine metabolites after injection of ^{14}C-L-histidine would give some information also on this point.

For reasons of cost the present study was limited to three subjects, two with chronic myelocytic leukaemia and one normal subject. All were given ^{14}C-L-histidine i.v. and the urinary excretion of ^{14}C-labelled and non-labelled histamine metabolites was followed over several months. In addition, some observations were made on the pulmonary elimination of radioactive carbon dioxide and on radioactive histamine in the blood.

Methods

Three subjects were studied:

Normal: Male, 41 years, healthy. Smoker. White blood cell count 7,000–9,000 cells/mm^3. Normal differential count. Subject B in Granerus (1968).

CML 1: Male, 67 years, chronic myelocytic leukaemia (case 4 in Berg *et al.*, 1971). White blood cell count 113,000 cells/mm^3. 5% Basophilic cells in bone marrow aspirate. Splenomegaly. No treatment at the start of the study.

CML 2: Female, 45 years, chronic myelocytic leukaemia (case 1 in Berg *et al.*, 1971). White blood cell count 224,000 cells/mm^3. 5% Basophilic cells in bone marrow aspirate. Splenomegaly. No treatment at the start of the study.

Permission to administer up to 500 μCi of ^{14}C-L-histidine to patients with chronic myelocytic leukaemia was granted by the Hospital's Isotope Committee. After the first observation in patient CML 1 it was apparent that it was possible to demonstrate ^{14}C-histamine formation from ^{14}C-L-histidine in man. Since it would be necessary for interpreting the findings in leukaemia, a study was also made on a normal subject. This subject was fully informed of the risks involved in giving radioactive histidine and volunteered freely.

Administration of ^{14}C-L-histidine. This was infused slowly by vein during about 30 min in the morning of day 1. The subjects had received breakfast two hours before. Urine was collected over 24 h periods, the first period including the time of infusion. ^{14}C-L-histidine was purchased from the Radiochemical Centre, Amersham, England (Batch 26, specific activity 150 μCi/mg). It was dissolved in 0·1 M sodium phosphate buffer, pH 7·4. Appropriate aliquots of the infusates were kept as a standard solution and for the estimation of ^{14}C-histamine and total content of ^{14}C. The amounts of histidine given to the subjects are shown in Table 1.

Although highly pure according to the manufacturer's specification, the ^{14}C-L-histidine was contaminated with ^{14}C-histamine. The contamination was diminished by passing the histidine solution through a Dowex 50W-X4 column, as described by Kahlson, Rosengren & Thunberg (1963). After this procedure some ^{14}C-histamine still remained (Table 1). Previous observations have shown that ^{14}C-histamine is rapidly and completely eliminated from the body (e.g. Lindell & Westling, 1966). Accordingly, the contaminating ^{14}C-histamine may only influence the results during the first 24 h after injection. The histamine contamination has quantitative importance only in CML 1, where it can at the most account for 37% of the ^{14}C-labelled histamine metabolites excreted in the first 24 hours.

Elimination of ^{14}C from the body. After injection of L-histidine labelled with ^{14}C in the 2 position of the imidazole ring, ^{14}C is excreted in the urine as unchanged histidine or metabolites thereof, and eliminated through the lungs as $^{14}CO_2$. Additional possible elimination routes (by perspiration and defaecation) were not examined.

Total urinary ^{14}C activity was measured with two different methods: In dried urine samples in a flow counter and in diluted urine mixed with scintillating phosphorous compounds and measured in a liquid scintillation counter. In both procedures appropriate dilutions of the injected solutions were used as internal standards to allow correction for self absorption. The two methods gave similar results. The values presented refer to those obtained by the flow counter method.

Expired $^{14}CO_2$ was measured as described by Kaihara & Wagner (1968). Briefly, the exhaled air was passed through an alkaline hyamine solution containing phenolphthalein, the carbon dioxide in the expired air neutralizing the hyamine solution until the end-point was shown by the indicator. The stock solution of hyamine was titrated with hydrochloric acid, and the amount of carbon dioxide trapped easily calculated.

Measurement of ^{14}C-activity in the hyamine solution was made in a liquid scintillation counter after addition of scintillating phosphorous compounds. The parameter primarily measured is thus the specific activity of expired CO_2. Usually only one sampling of expired $^{14}CO_2$ was made each day, in the morning. Total elimination of $^{14}CO_2$ over 24 h was roughly estimated from the morning value, using an assumed value for the total 24 hours' CO_2-elimination by the lungs, taking the patient's basal metabolic rate into account. In some instances the $^{14}CO_2$-elimination is given for shorter time periods than 24 h; in these instances only the specific activity is given, thus making assumptions about total CO_2-elimination unnecessary.

Measurements of histamine metabolites. Non-labelled histamine, methylhistamine and 1-methyl-4-imidazoleacetic acid were measured as described previously (Berg *et al.*, 1971). ^{14}C-histamine in blood was measured as described by Lindell, Rorsman & Westling (1961), except that the extract was first passed through a Dowex 50W-X4 column to separate ^{14}C-histamine from ^{14}C-L-histidine (Kahlson *et al.*, 1963). The urinary content of ^{14}C-labelled histamine and methylhistamine was measured as described previously (Helander, Lindell, Nilsson & Westling, 1962; Lindell, Nilsson, Roos & Westling, 1960; Wetterqvist & White, 1968a and b) with the addition of passage through a Dowex 50 column, as mentioned above. Previously, it has not been possible to measure ^{14}C-labelled 1-methyl-4-imidazoleacetic acid in urine containing large amounts of ^{14}C-L-histidine and its acid metabolites. To obtain satisfactory specificity in the extraction procedure, the

TABLE 1. *Amounts of ^{14}C-L-histidine, ^{14}C radioactivity and contaminating ^{14}C-histamine given to the subjects*

Subject	^{14}C-L-histidine (μg)	^{14}C activity (μCi)	^{14}C-histamine (% of injected ^{14}C-L-histidine)
Normal	2,090	330	0·0023
CML 1	2,830	447	0·0196
CML 2	2,160	342	0·00098

urine sample containing carrier amounts of non-labelled methylimidazoleacetic acid was subjected to esterification as described by Granerus & Magnusson (1965). The ester was extracted as described by Granerus (1968) and separated as the ester picrate. After hydrolysis of this ester picrate, methylimidazoleacetic acid was crystallized as the picrate, the radioactivity of which was measured. ^{14}C-activity in histamine, methylhistamine and methylimidazoleacetic acid was measured at infinite thickness in a flow counter, with a background of about 25–32 cpm (counts per minute). At least 3,000 counts were taken. After measurement of the radioactivity each sample was recrystallized with charcoal to remove impurities. When the radioactivity was constant over three recrystallizations the value was accepted.

Blood samples (50–60 ml) for estimation of the content of ^{14}C-histamine were taken at two to four day intervals (see Fig. 4). The blood from CML 1 was drained directly into tubes containing carrier amounts of histamine (cf. Lindberg, 1963). After mixing, 0·6 M perchloric acid was added. In the normal subject and in CML 2 blood cells and plasma were first separated by centrifuging for 5 minutes. These blood samples were taken in oxalate tubes. Carrier was thereafter added separately to plasma and the remaining cell mass. After mixing, perchloric acid was added. Plasma contained only small amounts of ^{14}C-histamine. Consequently the value for whole blood was calculated also in the normal subject and CML 2 so as to make comparisons with CML 1 possible.

During the urine collection periods the subjects were usually given a special diet (Granerus, 1968).

Results

1. *Elimination of ^{14}C*

The total ^{14}C excretion in the urine was relatively high during the first 24 h (4, 15 and 7% of the injected amount in the normal, CML 1 and 2, respectively (Table 2). Thereafter a rapid decrease occurred down to 0·3% of the injected amount per 24 h in all three subjects, less than one week after the infusion of ^{14}C-L-histidine. Then there was a clear hump in the excretion curve in CML 2 (Fig. 1) and a tendency to increasing values in CML1, but after three weeks the three subjects appeared to behave similarly again, with a slowly declining excretion of ^{14}C. Even some months after the infusion a small but relatively steady excretion was seen. The values for total ^{14}C-excretion fit well with those of Gaut, Pocelinko & Solomon (1970), who found that 7·7 and 10·5% of the radioactivity of injected ^{14}C-L-histidine was excreted in one and 7 days, respectively.

The 'hump' in the excretion of ^{14}C coincided with a conspicuous increase in excretion of ^{14}C-labelled methylhistamine and methylimidazoleacetic acid (Table 2). In fact, the sum of ^{14}C-histamine and these two labelled derivatives can explain a substantial part of the 'hump' in the ^{14}C-excretion in CML 2 (Fig. 1). On days 10–14 this sum amounts to about 0·08% of the injected ^{14}C-L-histidine per day and this is of the same order of magnitude as the 'hump'.

The pulmonary elimination of $^{14}CO_2$ was followed only in the normal subject and in CML 2. There was a difference between the normal subject and CML 2 in that the estimated elimination of $^{14}CO_2$ was smaller in the normal subject and declined more rapidly, especially on days 8–14. The difference was similar in

TABLE 2. *Urinary excretion per 24 h of total ^{14}C (as % of injected amount), of radioactive histamine, methylhistamine and methylimidazoleacetic acid (^{14}C-labelled compounds in μmoles per mole of injected ^{14}C-L-histidine) in a normal subject and in two patients with chronic myelocytic leukaemia*

	Total C^{14}			Histamine			Methylhistamine			Methylimidazoleacetic acid		
Day	Normal	CML 1	CML 2	Normal	CML 1	CML 2	Normal	CML 1	CML 2	Normal	CML 1	CML 2
Non-radioactive	—	—	—	0·06	0·24	0·11	0·31	5·9	5·2	4·1	41	63
1-14	—	—	—	(0·04-0·11)	(0·14-0·45)	(0·08-0·15)	(0·20-0·41)	(4·9-7·5)	(3·1-8·4)	(1·8-5·1)	(22-59)	(33-94)
Radioactive												
1	4·2	14·6	7·0	27	50	13	22	44	29	130	430	430
2	0·75	1·8	0·70	1	21	4	2	41	25	30	370	280
3	0·48		0·40	0		5	2		31	20		280
4	0·42		0·31	0		1	1		28	24		280
5	0·28	0·27	0·30	0	4	1	4	54	42	16	450	410
6	0·29	0·21	0·25	0	1	2	12	50	42	23	440	420
7	0·19	0·24	0·23	1	5	2	2	66	49	22	570	430
8	0·18	0·21	0·25	1	0	2	3	60	57	20	530	500
9	0·14	0·12	0·30	1	4	6	3	74	72	13	680	570
10	0·13	0·26	0·29	1	2	1	3	95	64	14	760	610
11		0·19	0·26		1	2		77	68		650	720
12			0·28			0			73			720
13	0·13		0·28	2		2	0		60	17		730
14	0·12		0·23	1		3	0		60	14		610
20		0·24	0·13		5	1		65	20		570	210
29		0·12	0·06		0	1		27	16		340	180
42		0·07	0·06		2	0		7	19		80	160
62	0·04	0·02	0·05	2	1	2	0	1	4	5	30	60
115			0·02			2			5			27
159		0·02			0			10			48	

For comparison, urinary excretion of total (non-radioactive) histamine, methylhistamine, and methylimidazoleacetic acid (mg/24 h) is indicated at the top of each column. The mean value during the first 14 days is given. The range is given below in parentheses.

nature to the difference in the urinary excretion of ^{14}C just described. The similar behaviour of pulmonary and urinary elimination of ^{14}C suggests a similar cause; however, it is very unlikely that pulmonary elimination of $^{14}CO_2$ is caused by catabolism of ring-labelled histamine or its metabolites.

The total amount of ^{14}C eliminated during 7 months was roughly calculated using interpolated values for the days not examined. In urine, 16, 30 and 20% of the injected amount was excreted in the normal subject, CML 1 and CML 2, respectively. Corresponding estimated values for $^{14}CO_2$ elimination were similar, or somewhat larger.

These findings could indicate that a substantial part of the infused ^{14}C is retained in the body. A more likely explanation for the low cumulative values are deficiencies inherent in the calculation of the pulmonary elimination of $^{14}CO_2$ during 24 h from one or two single values (see **Methods**). Thus, in CML 2 some observa-

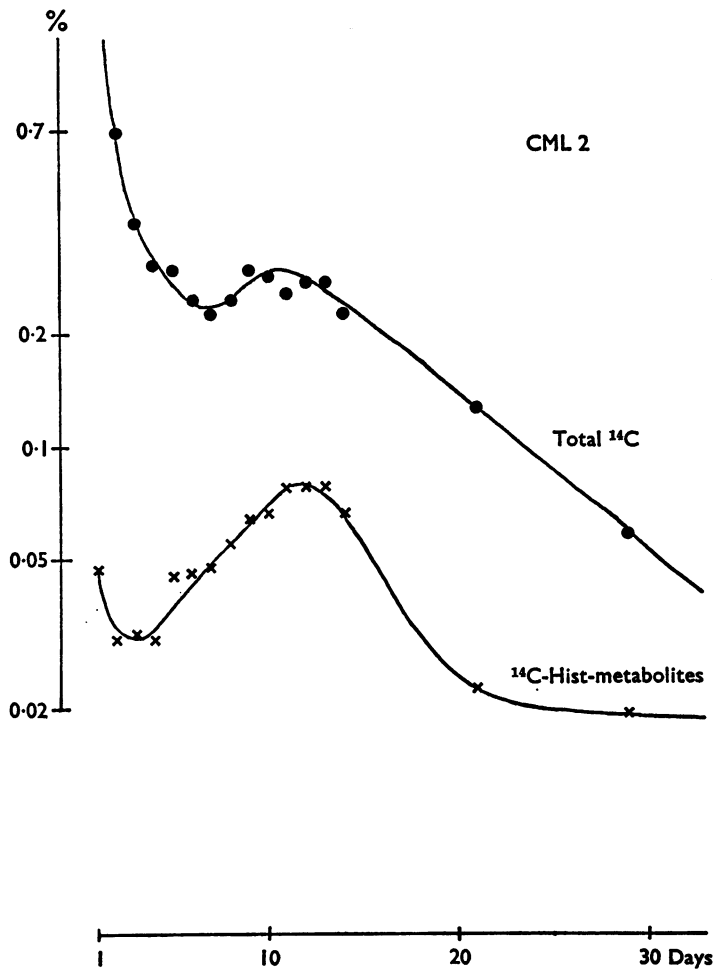

FIG. 1. Urinary ^{14}C activity and urinary ^{14}C-histamine metabolites (sum of histamine, methylhistamine and methylimidazoleacetic acid) in CML 2. The vertical axis indicates per cent of injected ^{14}C activity (Table 1) eliminated in 24 h (log scale). The horizontal axis shows days after infusion of ^{14}C-L-histidine.

tions were made which indicate that the specific ^{14}C activity of CO_2 eliminated by the lungs may vary rapidly. Figure 2 shows that even during the infusion, $^{14}CO_2$ was exhaled and a peak was reached 15 min after stopping the infusion. Figure 3 shows serial determinations of ^{14}C activity in expired CO_2 during two days, and one morning and one afternoon value on a third day. A doubling of the specific activity occurred in the afternoon, presumably due to changes induced by the mid-day meal. It is obvious that calculation of the 24 h elimination of $^{14}CO_2$ from a single morning observation is likely to give too low a value.

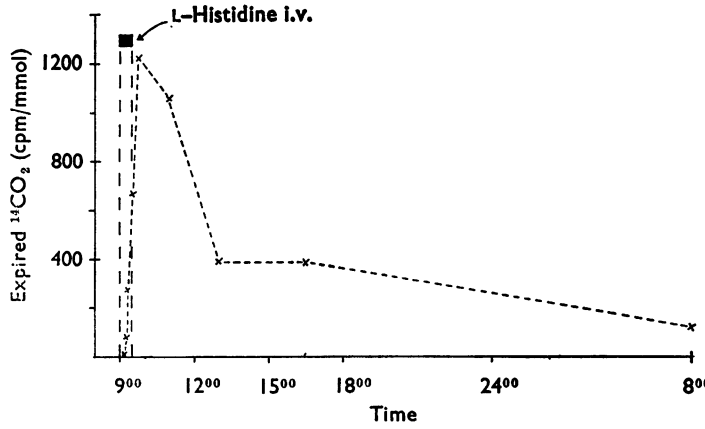

FIG. 2. Specific activity of expired $^{14}CO_2$ (vertical axis) during the first 24 hours after the infusion of ^{14}C-L-histidine. Figures below horizontal axis indicate the time of the day. Period of infusion indicated by black bar. Subject CML 2.

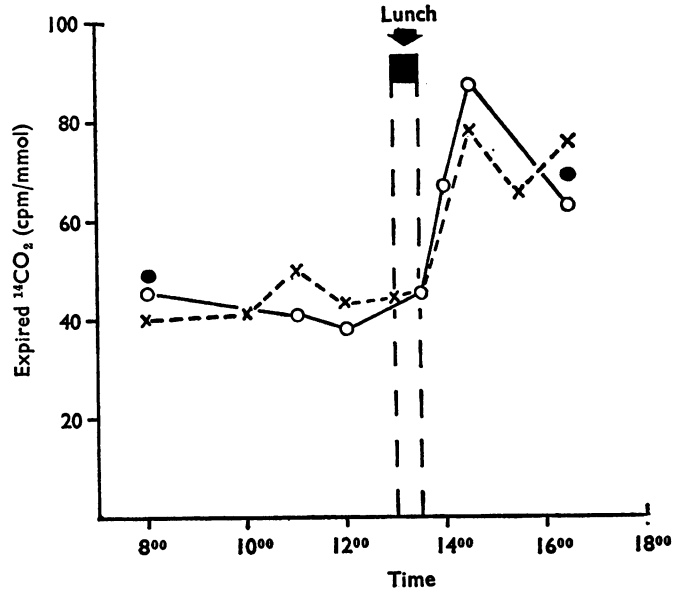

FIG. 3. Specific activity of expired $^{14}CO_2$ in CML 2 (vertical axis). Observations on 3 days are shown. On one day only two observations (filled circles) were made, at 08.00 and 16.30 hours. On two other days (open circles and crosses) serial observations were made during the whole day. Figures below horizontal axis indicate the time of the day.

2. *Excretion of histamine and its metabolites into the urine*

The values of ^{14}C-labelled histamine, methylhistamine and methylimidazolacetic acid are given in Table 2. It is obvious that the two leukaemic patients excrete much more of the three radioactive compounds; the difference is most apparent for methylhistamine and methylimidazoleacetic acid.

A comparison of the sums of all three substances indicate that the amounts of ^{14}C-histamine formed in the leukaemic patients were at least twenty times larger than in the normal subject. The excretion of ^{14}C-labelled histamine varied considerably. On the other hand the time courses of the urinary excretion of ^{14}C-labelled methylhistamine and methylimidazoleacetic acid were very similar, but quite different in the leukaemic subjects from the normal subject. In the latter the highest values were obtained on the first day and then a rapid decline occurred. In the leukaemic subjects higher values were seen even during the first day but the subsequent decline was brief and after a few days the excretion rose to peak values at the 10th–14th day.

Mean values, and range, for the urinary excretion of non-labelled histamine, methylhistamine and methylimidazoleacetic acid during the first 2 weeks after injection are given in Table 2. There were large variations in the excretion of all substances, particularly in the leukaemic patients. The values in the normal subject were in the expected range (Granerus, 1968). The two leukaemic patients excreted abnormally large amounts of methylhistamine and methylimidazoleacetic acid (compare Berg *et al.*, 1971). The urinary histamine was high in CML 1 but only slightly elevated in CML 2.

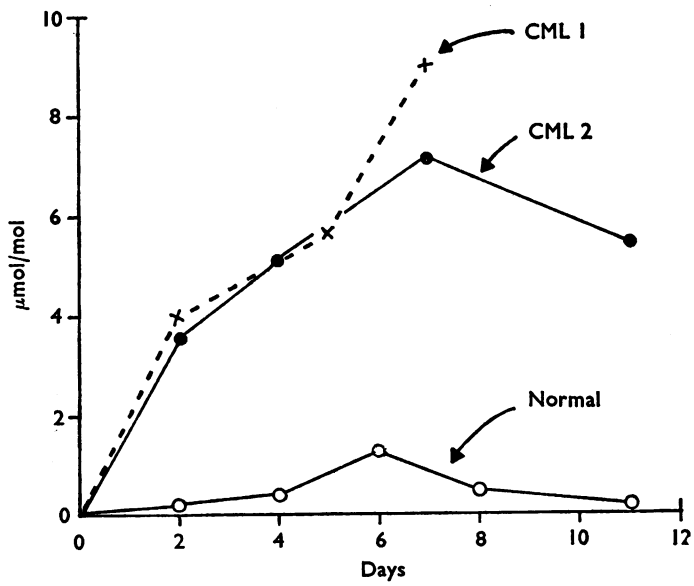

FIG. 4. ^{14}C-Histamine in whole blood (μmol ^{14}C-histamine in one ml of whole blood per mol of injected ^{14}C-L-histidine). The horizontal axis, shows days after infusion of ^{14}C-L-histidine. Total histamine content of whole blood (not shown in figure) was high (12 and 13 μg histamine per ml blood) in the two patients (CML 1 and CML 2) before treatment, and declined during the first month of treatment to 0·8 and 3·9 μg histamine per ml blood. In the normal subject the histamine content of the blood was 0·075 μg/ml.

For ethical reasons treatment with busulphan could not be withheld during the entire study. Busulphan treatment was started on the 6th and 16th day after injection, in CML 1 and CML 2, respectively. The decline in excretion of ^{14}C-labelled histamine metabolites seen in both patients after the peak on the 10th–14th day is thus probably not due to busulphan treatment. On the other hand the later decline, after 2–3 months, of the excretion of endogenous, non-labelled metabolites is likely to be an effect of the treatment (Berg *et al.*, 1971).

3. *Blood histamine values*

The histamine concentration in whole blood was normal in the normal subject and extremely high in the two leukaemic subjects initially, with a decline during treatment. The values of ^{14}C-histamine in blood at various times after infusion of ^{14}C-L-histidine are shown in Figure 4. It should be noted that the values in the normal subject are very small and barely significantly different from the background level of radioactivity. The rise in blood ^{14}C-histamine in the two leukaemic subjects stands out clearly. The ^{14}C-histamine was mainly located in blood cells. The late rise in radioactive blood histamine shows that it cannot be proportional to the plasma level of ^{14}C-L-histidine, which is likely to be high the first day after infusion, and then probably declines very rapidly (compare Borsook, Deasy, Haagen-Smit, Keighley & Lowy, 1950).

Discussion

The present study appears to be the first one in which histamine formation from L-histidine *in vivo* has been demonstrated in man. That this would be possible in chronic myelocytic leukaemia was not surprising in view of the previous observations in this disease (Berg *et al.*, 1971). Initially we did not think it worthwhile to attempt labelling of histamine metabolites in a normal subject but on the basis of the findings in the first subject studied, CML 1, a rough calculation suggested that even a normal subject would yield measurable quantities of urinary ^{14}C-histamine metabolites after ^{14}C-L-histidine infusion.

The histidine intake in the subjects studied is not known exactly, but when the standard diet was given (i.e. most of the days examined) it must have been about 2 g per day. The normal subject excreted about 0·2% of this as methylimidazoleacetic acid daily (i.e. 4 mg) whereas the corresponding figures in the leukaemic subjects were 2–4% (i.e. 40–80 mg) daily. The fraction of injected ^{14}C-L-histidine appearing as ^{14}C-labelled methylimidazoleacetic acid during the first 14 days was about 0·05% in the normal subjects and 0·8% in the leukaemic patients. The difference between the leukaemic patients and the normal subject is thus about the same in the tracer experiment as under ordinary conditions. On the other hand it appears certain that the fraction of the ^{14}C-labelled L-histidine converted to urinary histamine metabolites is smaller than the fraction of the total daily histidine intake that is excreted as histamine metabolites, even if we extrapolate the observations to 3–4 months. This difference is probably due to the intravenous administration of ^{14}C-histidine, giving a very short-lasting elevation of the plasma concentration.

The major difference between the leukaemic patients and the normal subject was the large excretion of ^{14}C-labelled methylimidazoleacetic acid and methyl-

histamine in the leukaemics, whereas the histamine values did not differ so strikingly. The same tendency held for the non-labelled compounds. Obviously, the urinary excretion of unchanged histamine is not a good indicator of changes in histamine metabolism (compare Westling, 1969). The relatively *higher* values for methylated histamine derivatives again indicate a more 'efficient' methylation of the excess histamine in patients with chronic myelocytic leukaemia (Berg *et al.*, 1971). This might be due to adaptation of the histamine-methylating enzyme(s); it is also possible that the excess histamine in myelogenous leukaemia is formed, stored and/or released in such a way that it preferentially methylated.

Rather surprisingly the highest excretion of ^{14}C-labelled methylhistamine and methylimidazoleacetic acid occurred as late as 10–14 days after the infusion of L-histidine in the leukaemic patients, whereas the normal subject showed a steadily declining excretion of metabolites from the highest value on the day of infusion. So far as we know a 'delayed' excretion of ^{14}C-labelled histamine metabolites—as in our leukaemic subjects—has not been observed in animal studies. The only reasonable explanations for this unexpected finding are that the infused ^{14}C-L-histidine was either retained as such in some tissue, or converted to ^{14}C-histamine, that was stored for some time before being released and made accessible for methylation. It is unlikely that methylhistamine or methylimidazoleacetic acid would be stored for several days in the body. The excretion of ^{14}C-labelled histamine metabolites on days 10–14 is so large that it contributes substantially to an increase in the total ^{14}C excretion in the urine. However, the total ^{14}C seems to increase somewhat more than can be accounted for by histamine metabolites, even when allowing for a reasonable amount of ^{14}C-labelled imidazoleacetic acid. (This compound was not measured in the present experiments since it can be formed both directly from L-histidine and from histamine, and is thus not a reliable indicator of histamine metabolism in a tracer experiment where ^{14}C-labelled histidine is used.) Similarly the difference in expired $^{14}CO_2$ between CML 2 and the normal subject on the seventh to the eleventh day indicates that there is also an increased elimination of ^{14}C-containing substances not related to ^{14}C-histamine metabolism.

Our tentative explanation of the findings is therefore as follows: ^{14}C-L-histidine is incorporated in basophilic leucocytes, which are formed in large amounts in the leukaemic subjects. In these labelled cells part of the ^{14}C-L-histidine is decarboxylated to ^{14}C-histamine, which is stored in the basophilic granules, and part of it is retained in the cells as unchanged ^{14}C-L-histidine. The cells appear in the blood after an unknown interval, possibly a few days and succumb after about 10–12 days. The ^{14}C-histamine in them is quickly metabolized and excreted into the urine. These dying cells also release other ^{14}C-labelled compounds, e.g. L-histidine. and these raise the urinary and expired ^{14}C.

If this interpretation is correct our findings suggest that the typical life time of a histamine-containing leucocyte in chronic myelocytic leukaemia is about 10 days.

With a daily excretion of 20 times the normal amount of histamine metabolites in the patients, one might expect a rapid turnover rate of the body histamine, as compared to normal individuals. However, the 'delayed' excretion of labelled histamine metabolites in the patients (with a peak around the 12th day) is evidence against an increased turnover rate, provided that the decarboxylation of histidine occurs 'early' and does not significantly contribute to the delay. If this is

correct, the present findings prove the existence of a large "histamine pool" in the patients (Berg *et al.*, 1971), the turnover rate of which may be normal, or even slower than normal. Of course this fits well with the presence of hyperplastic myeloid tissue in chronic myelocytic leukaemia.

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