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COMPARATIVE STUDY OF TWO ISOTOPIC METHODS DETERMINING HISTAMINE FORMATION *IN VITRO*

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Abstract—The merits of two isotopic procedures determining histamine formation, Schayer's isotope dilution method (pipsyl method) and Kobayashi's $^{14}CO_2$ method, both adapted for use in our laboratory, were compared in various tissues of rats and mice. Histidine decarboxylase activity determined in rat gastric mucosa after sham operation, antrectomy or vagotomy, spleen and lung by both methods was in fairly good agreement. In these tissues, except for antrum resection, the $^{14}CO_2$ estimate was approximately 20–30 per cent higher than with the pipsyl method. The higher values were presumably due to the production of $^{14}CO_2$ without formation of corresponding amounts of histamine. In rat small intestine, the $^{14}CO_2$ method was not reliable and with both rat and mouse liver the method entirely failed. In the mouse kidney, however, results obtained by the $^{14}CO_2$ method were fairly reliable. In the $^{14}CO_2$ method the incubation should be carried out under nitrogen as erratic results were obtained in some tissues under aerobic conditions. In conclusion the $^{14}CO_2$ method is suitable for tissues with high or moderately high histidine decarboxylase activity.

BY THE late 1950s it was recognized that great changes in the rate of histamine formation occurred without corresponding changes in tissue histamine content.[1] It was also realized that the non-isotopic methods for determining histidine decarboxylase activity available at that time were lacking in sensitivity and specificity and were not suitable for the determination of activity in tissues with a low or moderate rate of histamine formation. At that juncture in histamine research, sensitive and specific isotopic methods were developed which greatly furthered progress in this field. These methods fall in two classes: the method of Schayer[2] in which ^{14}C-histamine formed from ^{14}C-histidine is measured and the method of Kobayashi[3] that determines $^{14}CO_2$ evolved on the decarboxylation of ^{14}C-carboxyl labelled histidine. These methods will henceforth be referred to as the pipsyl and $^{14}CO_2$ methods, respectively. The pipsyl method, and its successive modifications, is time consuming and complicated and hence has been used in relatively few laboratories. The $^{14}CO_2$ method is rapid and simple and is widely used in its various modifications.

There are a few reports comparing the two isotopic methods[4,5] but a comprehensive comparison has hitherto not been undertaken. In addition to rat liver, lung, spleen and small intestine, histidine decarboxylase activity was measured by both methods in the gastric mucosa in which histamine formation is reduced after antrectomy[6,7] and increased after truncal vagotomy,[8] and the female mouse kidney in which histamine formation is substantially reduced after testosterone administration.[9] The gastric mucosa and the mouse kidney thus provide the possibility to test the performance of the two methods on tissues in which the enzymic activities were experimentally lowered or increased.

MATERIAL AND METHODS

Animals and tissue sampling. Adult female rats of the Sprague-Dawley strain and adult female and male mice of the NMRI strain were used. They were fed a standard diet and had tap water. The rats were fasted for 18 hr before they were killed.

Surgical procedures. Vagotomy in rats, under ether anaesthesia, was performed through a midline abdominal incision wherefrom the anterior and posterior vagal trunks were cut and resected for about 1 cm. To prevent gastric distension, a pyloroplasty was established by an incision 1 cm along the anterior wall of the pyloroduodenal junction and closing the incision transversally. Antrectomy was carried out by resecting the stomach a few millimeters proximal to the clearly recognizable antral-corpus border and, in addition, including 3–4 mm of the proximal duodenum in the resection. Gastro-intestinal continuity was provided for by a gastro-duodenostomy end-to-end. The post-operative treatment is important and has been described previously.[6,10] Sham operation involved a laparotomy. There was a postoperative recovery period of seven weeks before histamine formation was determined in the gastric mucosa.

Determination of formation of 2-ring ^{14}C-histamine (pipsyl method). This method, as adapted for use in our laboratory, included the following steps. Minced tissues were incubated for 3 hr at 37° under nitrogen in beakers usually containing 100 mg of tissue, 40 μg of 2-ring ^{14}C-L-histidine, 10^{-4} M aminoguanidine sulphate, 10^{-3} M EDTA, 5×10^{-4} M glutathione, 10^{-5} M pyridoxal-5-phosphate, 0·1 M sodium phosphate buffer (pH 7·4) and 0·2% glucose, the total volume made up to 3·0 ml. The specific activity of the substrate was 9·8 mCi/m-mole in all determinations except for the gastric mucosa of sham operated or vagotomized rats and on the kidney of untreated female mice when the specific activity of the substrate was 2·0 mCi/m-mole. EDTA and glutathione were added in order to attain the same composition of the incubation mixture as in the $^{14}CO_2$-method. In the blanks, 10^{-2} M semicarbazide, was used to inhibit decarboxylase activity.[11] The incubation was arrested by adding carrier histamine (40 mg base) and perchloric acid to a final concentration of 0·4 M. Histamine was separated from histidine on a column of ion exchange resin (Dowex 50 W-X4, 100–200 mesh). After conversion of histamine to pipsyl histamine, the radioactivity of formed histamine was determined at infinite thickness in a gas flow counter. Recrystallization of the pipsyl histamine was carried out until activity was constant. With ^{14}C-histidine of specific activity 9·8 mCi/m-mole and the measuring equipment used, 1 ng of ^{14}C-histamine formed corresponded to 5 cpm. Before use the commercial ^{14}C-histidine was purified to remove traces of ^{14}C-histamine.[11] This procedure reduced the amount of ^{14}C-histamine in the blank samples to a negligible value.

Determination of histamine formation from ^{14}C-carboxyl-labelled histidine (CO_2-method). The original procedure was only slightly altered in the present determinations. Figure 1 shows the incubation vessel used by us. If not otherwise stated, the incubation procedure was virtually the same as in the pipsyl method. Unless otherwise stated, nitrogen was the gas phase in the incubations. The specific activity of the ^{14}C-carboxyl-L-histidine used was either 104·9 μCi/m-mole or 1049 μCi/m-mole. The incubation was stopped by gentle tipping of 1 ml 2 M citric acid from the side arm into the incubation mixture. Maximum $^{14}CO_2$ trapping by Hyamine 10-X (0·1 m-mole) on a filter paper wick was achieved by continuing mechanical shaking

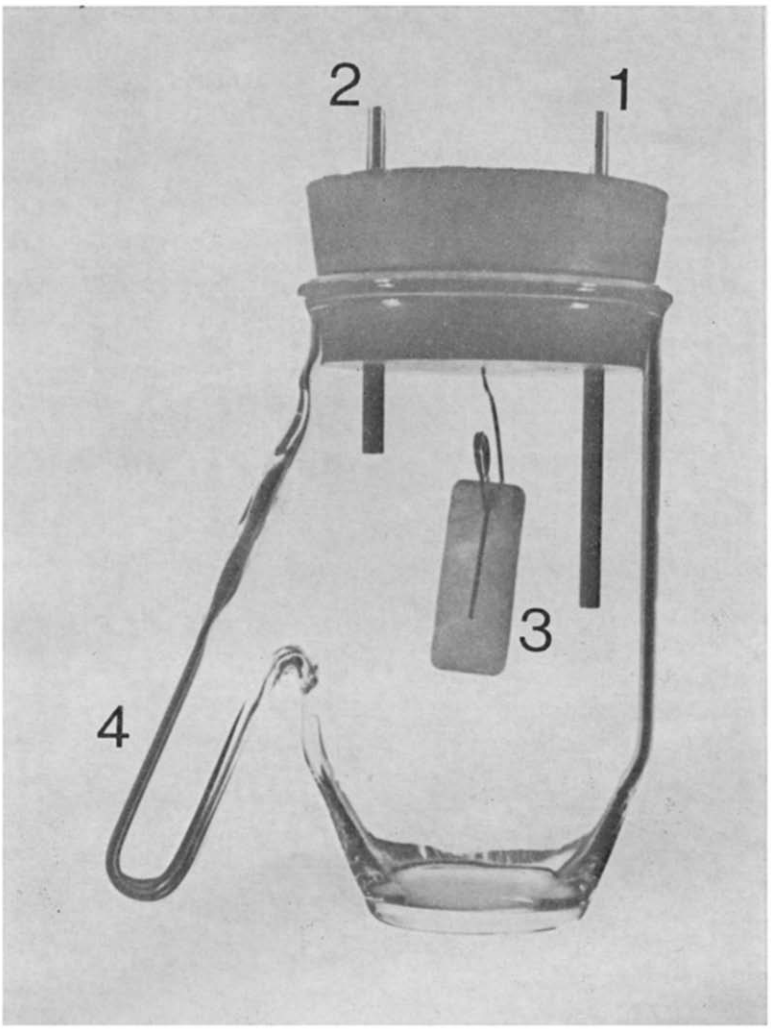

FIG. 1. Incubation vessel employed in the $^{14}CO_2$ method. The figures represent: 1, inlet for N_2; 2, outlet for N_2; 3, filter paper; 4, side arm for introducing citric acid.

for another 45 min. Using ^{14}C-labelled sodium bicarbonate, a recovery of 97 per cent was recorded. Finally, the filter paper was placed in a counting vessel containing 10 ml of Bray scintillation solution[12] and the radioactivity was determined in a liquid scintillation spectrometer (Packard TRI-CARB, 2002) at a counting efficiency of 73 per cent with a background of about 20 cpm. With the counting equipment used and a substrate specific activity of 1049 μCi/m-mole, 1 ng histamine formed corresponded to 15 cpm.

Materials. The compounds employed were purchased as follows. Hydroxide of Hyamine 10-X,*p*-(diisobutyl cresoxyethyl)dimethyl benzyl ammonium hydroxide (Packard), L-histidine-carboxyl-^{14}C (New England Nuclear), L-histidine-ring-^{14}C (Radiochemical Centre, Amersham), pipsyl(*p*-iodobenzenesulfonyl) chloride (Sigma), testosterone (British Drug House Ltd).

RESULTS

Comparative tissue assays. Histamine formation by gastric mucosa was determined by both methods in samples from rats subjected to sham operation, antrectomy or vagotomy. With both methods gastric mucosal histamine formation was found to be substantially altered by antrectomy or vagotomy, diminished after the former and increased after the latter operation. Values of histamine formation by the three kinds of mucosa are detailed in Table 1 from which it is apparent that on the whole there

TABLE 1. HISTAMINE FORMATION (ng/g/3 hr) IN SAMPLES OF RAT GASTRIC MUCOSA DETERMINED BY THE $^{14}CO_2$ AND THE PIPSYL METHODS

	Sham operated			Antrum resected			Vagotomized		
	CO_2 method	Pipsyl method	Ratio CO_2/pipsyl	CO_2 method	Pipsyl method	Ratio CO_2/pipsyl	CO_2 method	Pipsyl method	Ratio CO_2/pipsyl
	21780	16920	1·28	1950	1940	1·00	42910	38600	1·11
	5920	4800	1·23	2280	2390	0·95	15630	12220	1·27
	7980	6080	1·31	1500	1520	0·98	72230	56860	1·27
	5060	3670	1·37	2240	2180	1·02	48230	38180	1·26
	12890	10190	1·26	1440	1390	1·03	57000	39380	1·44
	6580	5150	1·27	1540	1450	1·06	22710	16450	1·38
				1280	1270	1·00	65800	48190	1·36
							99360	67200	1·47
Mean	10040	7800	1·29	1750	1740	1·01	52980	39640	1·32
S.E.	±2607	±2041		±153	±164		±9579	±6577	

Six rats were subjected to sham operation, seven to antrectomy and eight to vagotomy
Gastric mucosa from individual rats was investigated concurrently by the two methods.

is a fair accordance in the results obtained by the two methods. The values in the sham operated and vagotomized groups were about 30 per cent higher with the $^{14}CO_2$ method than with the pipsyl method. In the antrectomized group, however, there was no difference between values obtained with the two methods.

Gastric mucosal histamine formation is high compared to most other tissues. It seemed of interest to employ the two methods in tissues with low or moderately high histamine formation. The results are summarized in Tables 2 and 3 which also show blank values (cpm) for different tissues. A reasonable agreement in results was found in lung and spleen of rats and in female mouse kidney. On the other hand, in the

TABLE 2. HISTAMINE FORMATION (ng/g/3 hr) IN SAMPLES OF RAT LUNG, SPLEEN, SMALL INTESTINE AND LIVER DETERMINED BY THE $^{14}CO_2$ AND PIPSYL METHODS

	Lung			Spleen			Small intestine			Liver		
	CO_2	Pipsyl	CO_2/pipsyl	CO_2	Pipsyl	CO_2/pipsyl	CO_2	Pipsyl	CO_2/pipsyl	CO_2	Pipsyl	CO_2/pipsyl
	104	137	0·75	171	205	0·83	0	9	—	0	37	—
	348	208	1·67	740	496	1·49	34	12	2·83	251	62	4·04
	134	162	0·82	708	513	1·38	46	37	1·24	0	76	—
	325	194	1·67	693	413	1·67	85	21	4·04	29	82	0·35
	201	181	1·11	663	496	1·33	19	57	0·33	0	65	—
	188	160	1·17	430	329	1·30	21	30	0·70	0	57	
Mean	217	174	1·20	568	409	1·33	34	28	1·83	—	63	—
S.E.	±40·7	±10·5		±91·4	±49·7		±12·0	±7·3			±6·5	
Mean blank value (cpm)	787	6·3		762	5·7		637	6·3		1661	6·0	
n	(3)	(3)		(3)	(3)		(3)	(3)		(3)	(3)	

A zero value of enzyme activity corresponds to the blank value or less.

TABLE 3. CONCOMITANT ASSAY OF HISTAMINE FORMATION (ng/g/3 hr) IN FEMALE MOUSE TISSUES MEASURED BY THE $^{14}CO_2$ AND PIPSYL METHODS

	Kidney						Liver		
	Normal			Testosterone					
	CO_2	Pipsyl	CO_2/pipsyl	CO_2	Pipsyl	CO_2/pipsyl	CO_2	Pipsyl	CO_2/pipsyl
	810	1130	0·70	0	10	—	55	16	3·43
	2750	2580	1·06	0	0	—	18	12	1·50
	4160	3260	1·27	0	2	—	74	12	6·16
	1760	1750	1·00	0	7	—	0	9	—
	510	560	0·90	0	4	—	0	14	—
	11710	8550	1·36	0	11	—	0	14	—
Mean	3610	2970	1·05		5·7		25	12·8	
S.E.	±1708	±1184			±1·80		±13·2	±0·98	
Mean blank value (cpm)	152	3·0		774	5·0		1766	5·5	
n	(6)	(2)		(1)	(1)		(6)	(2)	

Testosterone, at a dose of 0·5 mg, was administered daily for 5 days.
A zero value of enzyme activity corresponds to the blank value or less.

rat and mouse liver, in which histamine formation was consistently demonstrated with the pipsyl method, the $^{14}CO_2$ method failed. In these tissues the $^{14}CO_2$ method was inadequate since the blank values were very high, sometimes even exceeding the values of the actual samples. In another tissue with low activity, the rat small intestine, the $^{14}CO_2$-estimates were unreliable, apparent from the erratic CO_2/pipsyl ratios. The high histamine formation of normal female mouse kidney was drastically lowered after testosterone injection which could be shown with both methods.

Incubation in air or nitrogen. This is relevant only to the $^{14}CO_2$ method. Determinations were done on lung, spleen and gastric mucosa of non-operated rats and kidney of female and male mice. Aminoguanidine, an inhibitor of diamine oxidase,

TABLE 4. HISTAMINE FORMATION (ng/g/3 hr) IN VARIOUS TISSUES MEASURED BY THE $^{14}CO_2$ METHOD UNDER NITROGEN OR AIR

	Rat						Mouse kidney			
	Lung		Spleen		Gastric mucosa		Female		Male	
	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
	170	301	259	330	6050	6020	1070	1120	46	220
	185	257	627	560	6730	7820	479	727	54	180
	154	232	485	668	12820	7970	2510	2510	58	154
	153	150	261	312	6320	7170			78	171
	261	354	374	379	6440	6540			50	207
	362	301	550	643	4980	4670				
	258	350	487	521	7970	7700				
Mean	220	278	435	488	7330	6840	1350	1450	57	186
S.E.	± 29·2	± 27·2	± 53·5	± 55·8	± 975	± 451	± 603	± 540	± 5·6	± 12·0
Mean blank value (cpm)	642	824	635	763	44	50	64	186	693	1739
n	(6)	(6)	(6)	(6)	(6)	(6)	(3)	(3)	(6)	(6)

was omitted from the incubation mixture. In four of the tissues included in Table 4, similar values were obtained with nitrogen or air as the gas phase. The values for the blanks were higher with air than with nitrogen but this disadvantage was compensated for by a correspondingly increased value in the actual samples. In the fifth tissue, male mouse kidney, incubation in air yielded significantly more $^{14}CO_2$ than in nitrogen ($P < 0.001$). It is, therefore, more reliable to use nitrogen as the gas phase.

Histamine formation as affected by substrate concentration. In both isotopic methods, a substrate concentration of 0.9×10^{-4} M (final concentration in the incubate) was regularly used. This concentration is considered to be physiological as pertaining to many mammalian tissues.[13] To ascertain the significance of the substrate concentration, minced rat gastric mucosa and female mouse kidney were incubated

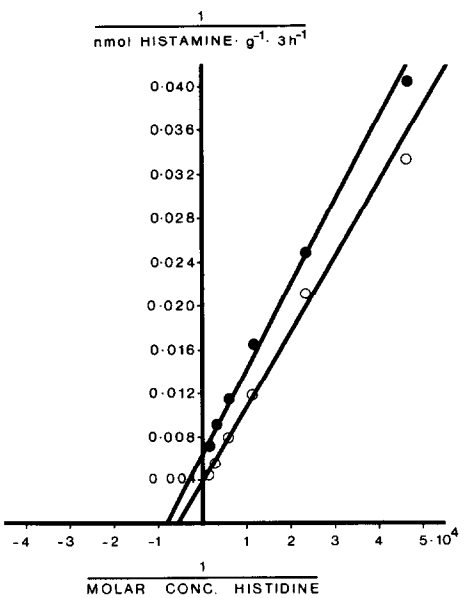

FIG. 2. A double reciprocal plot of the concentration of added histidine against histamine formation. The apparent K_m value for rat gastric mucosa (●) is 1.2×10^{-4} M, and for female mouse kidney (○) is 1.8×10^{-4} M. Each point is the mean of two determinations.

with various concentrations of ^{14}C-carboxyl-labelled histidine for 3 hr. Aminoguanidine was not added to the incubation mixture. A linear relationship between substrate concentration and rate of histamine formation was demonstrated by a double reciprocal plot as in Fig. 2 which indicates that endogenous histidine does not interfere to any significant extent with the determinations. The apparent K_m for the enzyme at pH 7·4 was about the same for the two tissues examined, 1.2×10^{-4} M for rat gastric mucosa and 1.8×10^{-4} M for female mouse kidney.

DISCUSSION

Isotopic methods are indispensible in tissues with a low rate of histamine formation. The merits and limitations of the $^{14}CO_2$ method have not been ascertained since, to our knowledge, only two investigations are on record specifically comparing the current isotopic methods. Maudsley *et al.*[4] determined histidine decarboxylase

activity in a few samples of gastric mucosa, lung, spleen, small intestine and heart of the rat. They found good agreement between the $^{14}CO_2$ and the pipsyl method except for the intestine and the heart in which tissues the enzyme activity was too low to be measured with the former method. On the other hand, Grahn and Rosengren, investigating histamine formation in minced mouse lung, disclosed that under certain circumstances, such as the presence of reducing agents, i.e. adrenaline or sodium ascorbate, non-enzymatic formation of $^{14}CO_2$ from ^{14}C-carboxyl-labelled histidine occurred without concurrent formation of histamine.[5] Non-enzymatic formation of $^{14}CO_2$ without concurrent histamine formation has been confirmed by other workers.[14]

In the present study, the $^{14}CO_2$ method, as employed appeared acceptable for most of the tissues investigated although the values obtained were 20–30 per cent higher than with the pipsyl method. This deviation between the two methods is unimportant in experiments in which great changes in histidine decarboxylase activity are brought about and expressed in terms of per cent change against controls. In the small intestine of the rat the deviations from the pipsyl method are great and in the liver of rats and mice the $^{14}CO_2$ method appeared unavailing.

In attempting to explain divergent results obtained with the two isotopic methods the following circumstances should perhaps be taken into account. Even with the pipsyl method, there is a measure of uncertainty in that the amount of histamine present when the incubation is stopped might be less than the amount primarily formed owing to uncontrollable catabolism, e.g. by imidazole-*N*-methyl-transferase for which enzyme no specific inhibitor has been described. The distribution of imidazole-*N*-methyl transferase has been thoroughly studied in various tissues of rats and mice.[15,16] We found no correlation between the methylating activity of the tissues investigated and the difference in histamine formation obtained with the two methods used in the present study.

In gastric mucosa of sham operated or vagotomized rats histamine formation was 30 per cent higher as assayed with the CO_2 method. On the other hand, following antrectomy almost identical figures were obtained with the two methods. This could be explained by the fact that the metabolic state of the mucosa following antrectomy is reduced.[17] The non-specific contribution of $^{14}CO_2$ may be subsequently diminished, since $^{14}CO_2$ may be evolved by processes other than decarboxylation of histidine to histamine, e.g. transamination of histidine to imidazole puruvic acid and a subsequent decarboxylation of this compound.

A few tissues where variations in histamine formation can be induced have been included in the present study, among them the rat gastric mucosa after antrectomy. In this tissue, workers in this laboratory, employing the pipsyl method, found substantial histidine decarboxylase activity, although lower than in controls.[6] In the present study these results have been confirmed by the two isotopic methods available. By contrast, workers, using a modification of the $^{14}CO_2$ method, in subsequent publications reported this enzyme activity as absent, unaltered and lowered after antrectomy.[8,18,19] Other examples will emphasize the present discordance in the pertinent methodology. With the $^{14}CO_2$ method, histidine decarboxylase activity could not be demonstrated in the gastric mucosa of the cat, rhesus monkey and the pig.[20] Employing the same method, the enzyme has been reported to be present in the rhesus monkey,[21] with the pipsyl method significant activity was found in

cats,[22] and in pigs, with a non-enzymatic procedure, the highest rate of mucosal histamine formation ever noted, has been reported.[23] It would appear that in instances of overt discordance, employing the two isotopic methods concomitantly would dispel the prevailing confusion and thereby secure adequate interpretation of results.

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