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COMPARISON OF ISOTOPIC AND NON-ISOTOPIC METHODS OF ESTIMATING HISTIDINE DECARBOXYLASE ACTIVITY

BY

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Several methods for the estimation of mammalian histidine decarboxylase activity *in vitro* have been described in recent years (Waton, 1956 ; Schayer, Rothschild & Bizony, 1959 ; Kahlson, Rosengren & Thunberg, 1963 ; Kobayashi, 1963 ; Håkanson, 1966 ; Levine & Watts, 1966). The isotopic methods involve the counting either of radioactive histamine formed from ring-^{14}C-histidine or of radioactive carbon dioxide liberated from carboxyl-^{14}C-histidine. In the non-isotopic methods, histamine formed from histidine is estimated either biologically (Telford & West, 1961) or fluorometrically (Håkanson, 1963).

The present work was designed to compare the results obtained by the $^{14}CO_2$ method of Kobayashi (1963) with those by the dibenzenesulphonyl histamine (or BSH) method of Kahlson *et al.* (1963), and then to compare the results obtained by the $^{14}CO_2$ method with those by the non-isotopic method of Waton (1956).

METHODS

Groups of four or more male Sprague-Dawley rats (body weight 120–150 g) obtained from Fisons Ltd. (Holmes Chapel) were used in most experiments. Pregnant albino mice from the Agricultural Research Council's Field Station at Compton provided the foetal material. Animals were killed by a blow on the head, and tissues were dissected out, cleaned and weighed.

Preparation of tissue extract

Pooled tissues from freshly killed animals were cut into small pieces and homogenized in 0.9% saline (5 ml./g) in a glass homogenizer. The solution was then centrifuged at 5,000$\times g$ for 15 min in a refrigerated centrifuge, and aliquots of the supernatant fluid were removed for the incubation experiments using different methods. Comparisons were therefore made on the same tissue extracts.

Estimation of histidine decarboxylase activity

(a) *$^{14}CO_2$-method.* This was originally devised by Kobayashi (1963). Briefly, the tissue extract (equivalent to 100–400 mg tissue) was incubated with carboxy-labelled-^{14}C-histidine in a closed system in an atmosphere of air and the $^{14}CO_2$ liberated was estimated in a Packard Tri-Carb liquid

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scintillation counter. Details of the method have been described by Radwan & West (1967a). Histidine decarboxylase activity has been expressed as c.p.m./g tissue; 1 μg histamine formed is equivalent to about 10,800 c.p.m.

(b) *BSH method.* This was devised by Schayer et al. (1959) and then modified by Kahlson *et al.* (1963). Briefly, it involves incubation of the tissue extract with ring-2-^{14}C-L-histidine and estimation of the ^{14}C-histamine formed after conversion to the derivative, dibenzenesulphonyl histamine. Details of the method have been described by Radwan & West (1967b). The radioactivity of the BSH crystals is counted directly in a gas flow counter (Nuclear Chicago) and corrected to zero thickness by comparison with a standard correction curve; 1 μg ^{14}C-histamine formed is equivalent to about 3,100 c.p.m. (counted at zero thickness). For highly active enzyme preparations, 100 mg of tissue are required for incubation; for less active material, 400 mg of tissue are necessary.

(c) *Non-isotopic method.* The method of Waton (1956) as modified by Telford & West (1961) was used. Histamine formed from histidine by the action of histidine decarboxylase (in aliquots of 400 mg of tissue) was assayed biologically on the isolated atropinized guinea-pig ileum. Specificity of the responses was checked with mepyramine maleate. Parallel blank incubations were performed, omitting the substrate or the tissue homogenate. The histidine decarboxylase activity was expressed in μg histamine formed/g of tissue/3 hr after subtracting the blank value.

Estimates of histidine decarboxylase activity recorded in the Figures and Table are the mean values of 4 experiments.

RESULTS

Comparison of two isotopic methods

Figures 1, 2 and 3 illustrate the effects of changing the pH values of the incubation mixtures on the histidine decarboxylase activities of extracts of the two portions of rat stomach and of mouse foetus respectively. There is agreement between the results obtained by the two methods—for example, the activity of the fundic portion of rat stomach is 7.8 μg/g with the $^{14}CO_2$ method and 8.1 μg/g with the BSH method, whereas

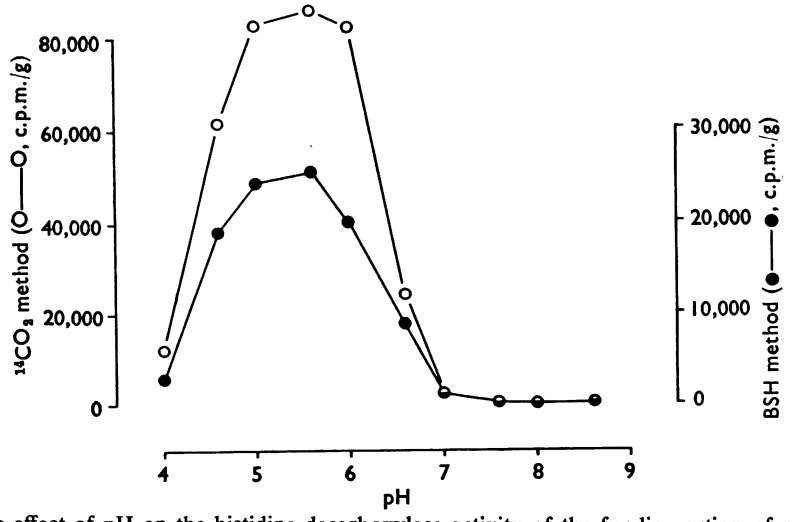

Fig. 1. The effect of pH on the histidine decarboxylase activity of the fundic portion of rat stomach, determined by the $^{14}CO_2$ method (○——○) and the BSH method (●—●) simultaneously. Note the insignificant activity at pH values over 7.

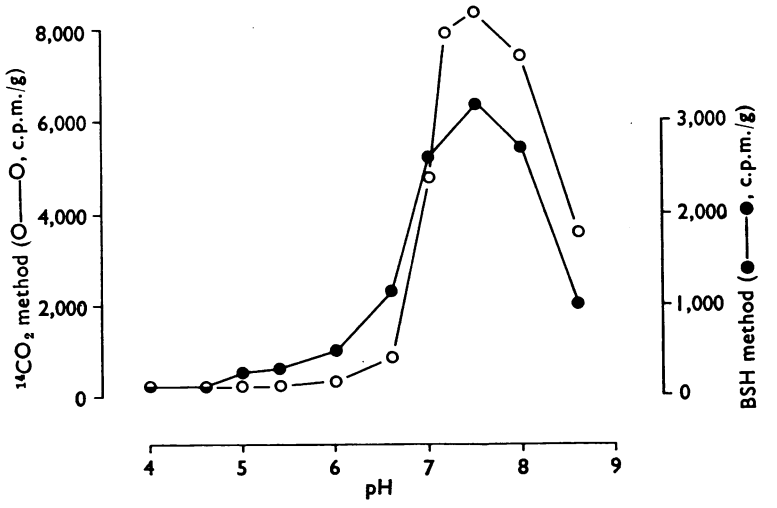

Fig. 2. The effect of pH on the histidine decarboxylase activity of the pyloric portion of rat stomach, determined by the $^{14}CO_2$ method (○——○) and the BSH method (●——●) simultaneously. Note the insignificant activity at pH values below 6.

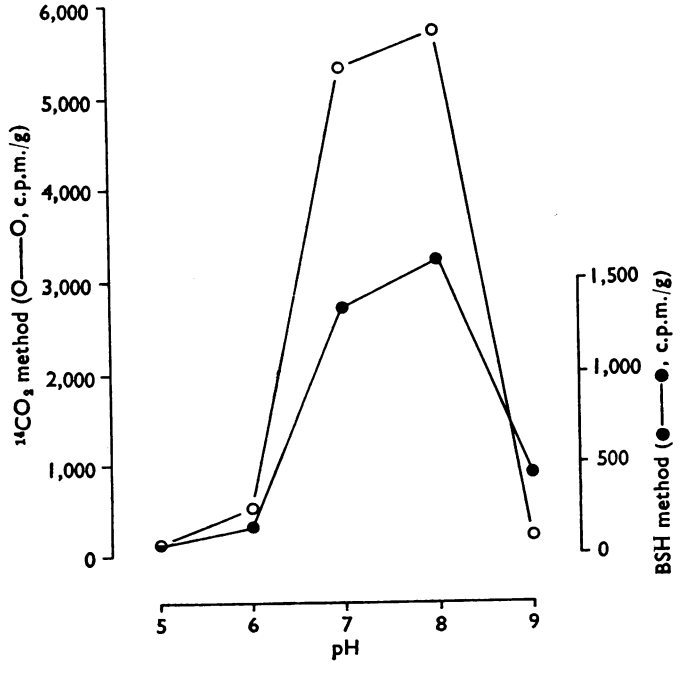

Fig. 3. The effect of pH on the histidine decarboxylase activity of mouse foetal extract, determined by the $^{14}CO_2$ method (○——○) and the BSH method (●——●) simultaneously. Note the insignificant activity at pH values below 6.

the corresponding values for the pyloric portion are 0.7 and 0.9 μg/g respectively. By both methods, therefore, the fundic activity is about 10 times that in the pyloric portion. The $^{14}CO_2$ method has the advantage of speed, simplicity, and moderate sensitivity (limit of detection of about 20 ng histamine≡216 c.p.m., based on a blank value of about 108 c.p.m.), but it has the major disadvantage that it cannot be applied to studies in the intact animal. The BSH method has the advantage of great sensitivity (limit of detection of about 3 ng histamine), although it is very complicated and time-consuming to carry out; it can however be applied to *in vivo* studies.

Comparison of non-isotopic and isotopic methods

Figure 4 shows the histidine decarboxylase activity of several tissues of rat and mouse when determined simultaneously by the non-isotopic and the $^{14}CO_2$ methods. The two methods give comparable results.

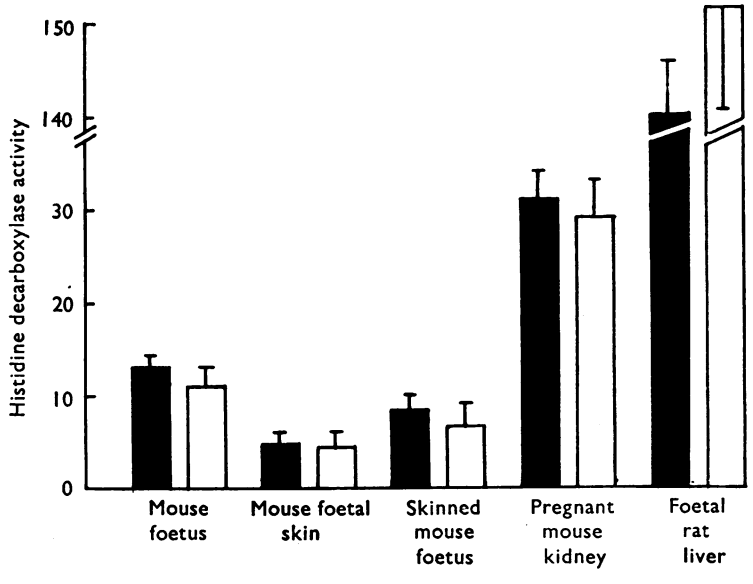

Fig. 4. The histidine decarboxylase activity of mouse and rat tissues as determined by the $^{14}CO_2$ method (closed columns) and the non-isotopic method (open columns). Enzyme activity is expressed as histamine formed (μg/g tissue±S.E.) in 3 hr. Substrate concentrations were identical in each experiment.

Tissues with low histidine decarboxylase activity

The histidine decarboxylase activities of lung, heart, small intestine and spleen of rat, when determined isotopically by the BSH method, are shown in Table 1. The activities in the spleen and lung when determined by the $^{14}CO_2$ method (155 and 98 ng/g respectively) agree with those found by the BSH method (149 and 85 ng/g), but estimates of activity in the heart and small intestine are not possible by the $^{14}CO_2$ method. No enzyme activity is detected in any of these tissues when the non-isotopic method is used. Although the non-isotopic method has the advantage of being relatively inexpensive to perform, it is much less sensitive than the isotopic methods.

TABLE 1

HISTIDINE DECARBOXYLASE ACTIVITIES OF RAT TISSUES

The enzyme activity is expressed as ng histamine formed/g tissue in 3 hr (BSH method)

Tissue	Histamine formed
Heart	25
Small intestine	28
Lung	85
Spleen	149

DISCUSSION

The present work shows that agreement usually exists between the results of the methods examined for the estimation of histidine decarboxylase activity *in vitro*, especially when tissues contain considerable amounts of enzyme activity and have a low catabolism of histamine. The non-isotopic method has the advantage of simplicity and economy, and is the method of choice for highly active enzyme preparations. This conclusion contradicts the view of Kahlson (1962) that non-isotopic *in vitro* methods are unsuitable for the determination of histidine decarboxylase activity of a tissue. When using tissues of low enzyme activity, however, the isotopic methods have to be used.

The most sensitive method for the determination of histidine decarboxylase activity is said to be the BSH method as it has a low blank value; it is the one of choice for measuring very low enzyme activities, as, for example, in rat heart and small intestine. The $^{14}CO_2$ method has a degree of sensitivity which is intermediate between that of the BSH method and that of the non-isotopic method. It has a higher blank value, probably because of spontaneous decarboxylation occurring during the incubation (Callingham, Kobayashi, Maudsley & West, 1965), but it measures simply the rate of decarboxylation whereas both the BSH and non-isotopic methods measure the histamine in the product, regardless of any catabolism occurring during the incubation.

The behaviour of the fundic part of rat stomach is different from that of other tissues examined. Whereas Telford & West (1961), using the non-isotopic method, found only traces of enzyme activity over a pH range of 6–9, in the present work, using both isotopic methods, it is the most active histamine-forming tissue in the rat, having an optimal pH value of about 5.6 (Fig. 1). Radwan & West (1967a) showed that the fundic enzyme is probably of the specific type and it is affected by physiological stress such as starvation. This draws attention to the fundic histidine decarboxylase as a major source of gastric histamine.

SUMMARY

A direct comparison between the different methods for the estimation of histidine decarboxylase activity *in vitro* shows that results are comparable in most cases. The non-isotopic method cannot be used for tissues with low enzyme activity, while the isotopic BSH method is more sensitive but more complicated than the $^{14}CO_2$ method.

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