Biochemical Pharmacology, Vol. 17, pp. 23–30. Pergamon Press 1968. Printed in Great Britain

# HISTAMINE FORMATION IN NORMAL, REGENERATING AND MALIGNANT LIVER TISSUE OF THE RAT

D. M. SHEPHERD and B. G. WOODCOCK

Department of Pharmacology and Therapeutics, University of Dundee, Scotland

(*Received* 14 *July* 1967; *accepted* 23 *August* 1967)

**Abstract**—No relationship could be demonstrated between the histidine decarboxylase activity and the growth of the liver of partially-hepatectomised rats during the period when the rate of regeneration is maximal. Similarly there was no correlation between the histidine decarboxylase activity and the growth rate of a series of transplantable hepatomas in the rat. These results do not support the view that histidine decarboxylase has an essential role in regard to rapid tissue growth.

PREGNANT rats excrete abnormally large amounts of urinary histamine from about the 15th day of gestation until term. This histamine is formed in the foetal livers by a substrate-specific histidine decarboxylase (L-histidine carboxy-lyase; EC4.1.1.22), the activity of which falls precipitously soon after parturition. Thereafter histamine formation in the liver is low and is due to the presence of aromatic L-amino-acid decarboxylase* (aromatic L-amino-acid carboxy-lyase) for which numerous naturally-occurring and purely synthetic aromatic L-amino acids can serve as substrates.[1, 2] It has been suggested that histamine formation in the foetus might be related to the general process of growth.[3]

Adult rats in which the liver is regenerating after partial hepatectomy also excrete increased amounts of histamine in the urine, and this has been attributed to the growth of the regenerating liver.[3] However, Kameswaran and West[4] were unable to detect an increase in the histidine decarboxylase activity of regenerating liver measured *in vitro*, nor were they able to confirm the reported increase in urinary histamine; similar experiments carried out in this laboratory also gave negative results† possibly because the method used for the measurement of histamine formation was not sufficiently sensitive. In this paper a re-investigation of the histidine decarboxylase activity of rat regenerating liver is reported using a sensitive radio-isotopic method.

Previous investigations in this laboratory have shown that the rapidly-growing malignant hepatoma, F-Hep, growing s.c. in rats of the August strain,[5, 6] and also primary liver tumours resulting from the feeding of diethylnitrosamine (DENA) to Wistar rats,[7] both form histamine at high rates and, like foetal rat liver, contain specific histidine decarboxylases. At first sight these observations seem to provide support for a relationship between histidine decarboxylase and growth. However, further studies of the action of DENA showed that the appearance of the specific

* This enzyme will be referred to as non-specific histidine decarboxylase.

† D. M. Shepherd and B. G. Woodcok, unpublished observations.

histidine decarboxylase in the cancerous liver coincided only with the development of hepatomatous tissue but not with the proliferation of bile-duct epithelium which occurs during the later stages of DENA treatment. Thus it is likely that the rise in the specific histidine decarboxylase activity in the liver of DENA-treated rats is associated with the growth of a particular type of cell, the hepatoma cell, rather than with growth in general. A similar conclusion was reached by Buttle, Eperon, Kameswaran and West[8] who, in studies of several hepatomas, found that only the F-Hep tumour showed a combination of rapid growth with a high rate of histamine formation. Results are now presented for the histidine decarboxylase activity in other rapidly-growing rat hepatomas.

## METHODS

*Partial Hepatectomy.* Female Wistar rats, 210–230 g, were partially hepatectomised as described by Higgins and Anderson.[9] For animals killed after 12 hr the time of operation was 9 p.m.; all other partial hepatectomies were carried out at 10 a.m. Ether anaesthesia was used in all operations, precautions being taken to avoid post-operative sepsis without the use of antibiotics. Sham-operated rats served as controls.

*Preparation of tissue homogenates.* Each rat was killed by a sharp blow on the head, exsanguinated, and the required tissue quickly removed and homogenised in an ice-cooled Potter-Elvehjem homogeniser. Tumour and liver tissue were used as 50% homogenates in 0·9% saline. Owing to its high enzyme activity foetal liver ($19 \pm 1$ days gestation) was prepared as a 15% homogenate.

*Determination of mitotic index of regenerating liver.* Approximately 0·2 g of liver tissue cut from the small caudate lobe was fixed in Susa/10% formalin (1/2) and subsequently stained with celestin blue, Mayer's haemalum and eosin.[10] The mitotic index was determined according to the method of Abercrombie and Harkness.[11] The number of parenchymal cells in the easily recognisable stages of mitosis, early prophase to late telophase, were counted in 100 microscope fields at 1000 × magnification.

*Measurement of histidine decarboxylase activity.* In a final incubation volume of 2·5 ml the concentrations of the various reagents were as follows: Sodium phosphate buffer, $5{\cdot}6 \times 10^{-2}$ M; aminoguanidine bicarbonate, $6 \times 10^{-5}$ M; pyridoxal phosphate, $1{\cdot}62 \times 10^{-5}$ M in incubations with $^{14}$C-histidine or $1{\cdot}62 \times 10^{-4}$ M in incubations with unlabelled histidine; $^{14}$C-L-histidine $6{\cdot}5 \times 10^{-5}$ M or L-histidine, $3{\cdot}22 \times 10^{-2}$ M; tissue homogenate 1 ml. Ring-2-$^{14}$C-L-histidine, 226 $\mu$c/mg (Radiochemical Centre, Amersham, England), was substantially freed from traces of $^{14}$C-histamine by ion-exchange before use as detailed by Kahlson, Rosengren and Thunberg;[12] it was then diluted with unlabelled histidine to a sp. act. of 15 $\mu$c/mg.

Incubations were carried out in duplicate for 3 hr at 37° in 10 ml stoppered centrifuge tubes. The tissue homogenate and freshly prepared pyridoxal phosphate solution were added after preincubation of the other constituents for 5 min at 37°. Benzene, 20 mg (1 drop), used in some experiments to characterize the enzyme, was always added last to avoid loss by evaporation.

At the higher histidine concentrations the incubations were carried out at pH 6·5 (optimal for specific histidine decarboxylase) and pH 8·5 (optimal for non-specific histidine decarboxylase), the histamine formed being measured by biological assay on the atropinised ileum of the guinea pig. Incubations with $^{14}$C-histidine were carried out at pH 7·4 which is optimal for histamine formation at low substrate concentrations

by both enzymes; the $^{14}$C-histamine formed was determined as the dibenzenesulphonyl derivative.[13, 14]

## RESULTS

*Histamine formation during rat liver regeneration after partial-hepatectomy.* Histamine formation by regenerating liver was measured *in vitro* 12, 24, 48, 72 and 96 hr after partial-hepatectomy. During this 96-hr period the rate of growth is at its peak: the weight of the regenerating liver increases from 30 per cent to more than 70 per cent of the pre-operative weight, most of this increase occurring in the 24–48 hr interval following the operation.[9] Two partially-hepatectomised and two sham-operated rats were studied for each time interval. The experiment was carried through three times in all, thus providing measurements on 6 partially-hepatectomised and 6 sham-operated rats for each of the post-operative observation periods.

Both the wet-weight and the dry-weight of the liver increased during regeneration (Fig. 1). The histidine decarboxylase activity and associated changes in the mitotic

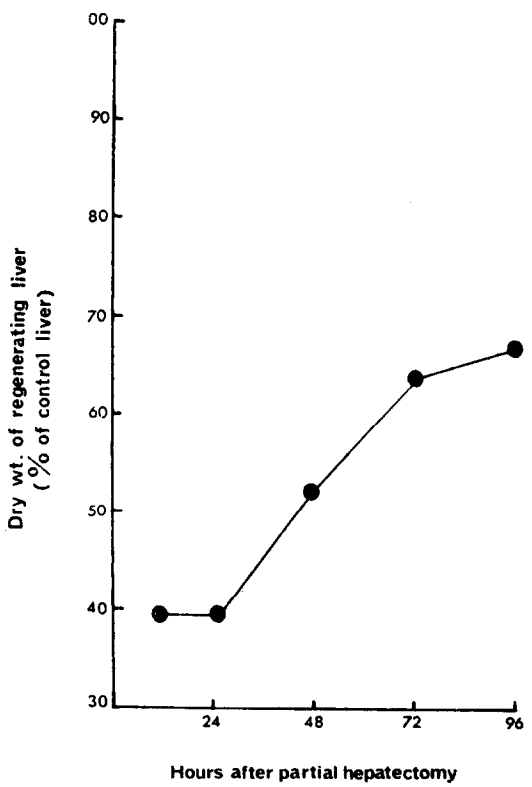


FIG. 1. Changes in dry weight of rat regenerating liver after partial hepatectomy. Each result is the mean of observations on 6 partially-hepatectomised and 6 control animals.

index are shown in Fig. 2. The data for histidine decarboxylase activity were examined statistically by the method of analysis of variance. The interaction factor was found to be non-significant ($F_{4,50} = 2{\cdot}12$), indicating that the histidine decarboxylase activities of normal and regenerating liver change in a similar manner with respect to time. The

histidine decarboxylase activities of the regenerating livers were significantly lower than those of the corresponding controls ($F_{1,54} = 4{\cdot}18$, $P < 0{\cdot}05$) during the first 48 hr following the operation; thereafter there was no significant difference between the histidine decarboxylase activities of the two groups.

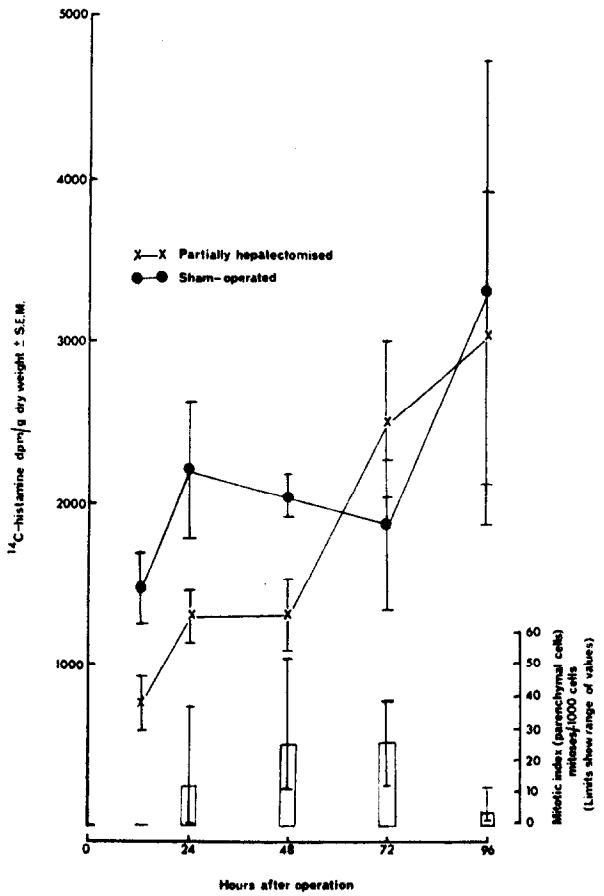


FIG. 2. Changes in the histamine-forming capacity and mitotic index during regeneration of rat liver. Each point is the mean of 6 observations.

Mitotic figures were rarely seen in livers from sham-operated rats or from rats 12 hr after partial-hepatectomy, and in this respect such livers resembled those of normal rats where only 1 in 10,000 to 20,000 parenchymal cells are in mitosis.[15] On the other hand, numerous mitoses were observed in the livers of four of 6 rats 24 hr after partial hepatectomy and in all regenerating livers removed after 48, 72 and 96 hr.

*Histidine decarboxylase in rat heptomas.* The histidine decarboxylase activity of a number of transplantable hepatomas was measured *in vitro*. Comparative determinations were also carried out on normal liver, foetal liver, and regenerating liver removed 29 hr after partial hepatectomy, all of these tissues being from Wistar rats. There is no evidence that the histidine decarboxylase activity of normal or foetal liver

varies from one strain to another; the values reported for August rats,[8] for example, are of the same order as those of Wistar rats.

Different incubation conditions were used to distinguish the specific histidine decarboxylase from the non-specific enzyme. At a high substrate concentration the specific enzyme forms histamine more rapidly at pH 6·5 than at pH 8·5; the reverse is true for the non-specific histidine decarboxylase. At a high substrate concentration and at a pH of 8·5 benzene has little or no effect on the specific enzyme, but it greatly potentiates the non-specific enzyme. At a low substrate concentration benzene slightly inhibits the specific enzyme, but potentiates the non-specific enzyme.

The nature of the tumours and other tissues examined, together with their origin and histidine decarboxylase activities, are shown in Table 1. The histamine concentration found in the blank incubation is recorded in the last column of the table; as the tumours appear to be devoid of any binding mechanism for histamine, this figure provides a representative estimate of the histamine content of the tissue.

Foetal rat liver, the tissue with the highest specific histidine decarboxylase activity, appears at the top of Table 1 while normal rat liver, with the highest level of the non-specific enzyme, comes at the foot. All the hepatomas have approximately the same rate of growth, forming masses 3–4 cm dia. in 2–3 weeks after subcutaneous transplantation. Nevertheless, their histamine-forming capacities differ widely, showing a 4000-fold variation. The ethionine-induced tumour is of particular interest, as it has a lower histamine-forming capacity than normal rat liver.

## DISCUSSION

During the first 4 days after partial hepatectomy, when the rate of regeneration is maximal, the histidine decarboxylase activity in rat liver did not exceed that in the liver of sham-operated animals. Although the measurements made at 96 hr showed that cell division of the parenchymal cells in regenerating liver was then subsiding, histidine decarboxylase activity was increasing in both partially-hepatectomised and sham-operated animals (Fig. 2). Thus the rate of division of parenchymal cells is not directly related to the histidine decarboxylase activity. The rise in histidine decarboxylase activity in both groups of rats in the 72–96 hr post-operative period may be due to an effect of the anaesthetic, ether, on the liver, or to stimulation of the adrenal cortex. Non-specific stresses produce a hypersecretion of corticosterone from the rat adrenal, and it has been shown[16] that cold-stress or injection of corticosteroids reduces the histidine decarboxylase activity of rat-liver measured *in vitro*. Thus it is possible that the changes in histidine decarboxylase activity shown in Fig. 2 represent an initial depression of activity during the first 24 hr, followed by an over-compensatory rise to an activity greater than normal. In support of this interpretation, the mean histidine decarboxylase activity of normal rat liver was found to be 1700 dpm/g *dry* weight, which is greater than the 12 hr, but less than the 96 hr, values recorded in Fig. 2 for partially-hepatectomised and sham-operated rats. Moreover, as shown in Fig. 2, the histidine decarboxylase activity in the liver of partially-hepatectomised rats, where the stress stimulus might be expected to be greater, is significantly less than that in the liver of sham-operated rats during the early post-operative period. An initial lowering in histidine decarboxylase activity in the regenerating liver of the rat was also reported by Kameswaran and West.[4]

TABLE 1. HISTIDINE DECARBOXYLASE (H-D) ACTIVITIES OF NORMAL, FOETAL, REGENERATING AND MALIGNANT LIVER TISSUE OF THE RAT

| Tissue | Tumour inducer | Source | Rat strain | H-D activity* pH 8·5 with benzene | H-D activity* pH 8·5 without benzene | H-D activity* pH 6·5 without benzene | H-D activity† pH 7·4 with benzene | H-D activity† pH 7·4 without benzene | Histamine in blank incubation μg/g |
|---|---|---|---|---|---|---|---|---|---|
| Foetal liver (19 ± 1 day gestn.) | — | — | Wistar | 31·0 | 29·5 | 176·4 | 274,500 | 1,092,000 | 24·3 |
| F-Hep | FDAB | Chester–Beatty | August | 28·7 | 35·5 | 170·5 | 183,700 | 822,000 | 24·5 |
| Primary liver tumour | DENA | This laboratory | Wistar | 10·9 | 11·7 | 40·9 | 16,400 | 111,200 | 7·3 |
| D/31 hep. | DAB | University of Nottingham | S–Dawley | 1·4 | 2·0 | 5·0 | 3300 | 9120 | 6·9 |
| D/30 hep. | DAB | University of Nottingham | S–Dawley | nil | nil | 3·5 | 4990 | 22,380 | 3·5 |
| Novikoff hep. | DAB | Inst. du Cancer de Montreal | S–Dawley | 0·2 | 1·0 | 2·3 | 580 | 7190 | 1·4 |
| P/26 hep. | DAB | Chester–Beatty | Chester–Beatty | 1·0 | nil | nil | 740 | 7080 | nil |
| U/B/P17 | Ethionine | Chester–Beatty | Chester–Beatty | 4·2 | nil | nil | 236 | 182 | nil |
| Regenerating liver (29 hr) | — | — | Wistar | 26·4 | 2·0 | 2·0 | 2016 | 380 | 2·0 |
| Normal liver | — | — | Wistar | 22·1 | 1·6 | 0·4 | 2080 | 480 | 0·7 |

DAB = 4-Dimethylaminoazobenzene; FDAB = 4′-Fluoro-4-dimethylaminoazobenzene; DENA = Diethylnitrosamine.

* Histamine formed μg/g wet wt./3 hr.

† $^{14}$C-Histamine formed dpm/g wet wt./3 hr.

Results are the mean of duplicate determinations on pooled tissues from 3 animals except for foetal liver (pooled livers from a litter of nine) and the primary tumour (one case).

With regard to the hepatomas examined, the outstanding feature was the lack of correlation between the rate of growth of the tumours and their histamine-forming capacity (Table 1). For example, the ethionine-induced tumour with a lower histamine forming capacity than normal rat liver, grew as rapidly as the tumour F-Hep in which the rate of histamine formation is comparable with that of foetal rat liver, the most potent source of histidine decarboxylase listed in Table 1. These observations indicate that a high histidine decarboxylase activity is not essential for the growth of various types of liver tissue.

The histamine content of the tissues, estimated from the amount of histamine present in the blank incubations, roughly parallels the activity of the specific enzyme. Tissues such as normal rat liver, regenerating liver and the ethionine-induced tumour, where the specific enzyme activity is low or absent have negligible amounts of histamine in the corresponding blank incubations. This suggests that the histamine contained in the tumours is formed by specific rather than by non-specific histidine decarboxylase.

In precancerous and malignant liver tissue of rats receiving DENA there was a decrease in the non-specific enzyme accompanied by the appearance of the specific enzyme.[7] The tumour F-Hep of August strain rats contained high specific histidine decarboxylase activity, but the non-specific enzyme could not be detected.[6] In the present experiments (Table 1) the measurements made at pH 8·5, with and without the addition of benzene, show that non-specific histidine decarboxylase activity in the tumours is either undetectable or is very low in comparison with that of normal rat liver. There is evidence that continued transplantation of tumours results in a gradual loss of specialised functions while the basic functions required for cell survival and reproduction are retained,[17] i.e. the tumours become progressively less differentiated. Thus the low level of the non-specific histidine decarboxylase in all the hepatomas so far examined may indicate that this enzyme is associated with a specialised function of normal liver tissue.

*Acknowledgements*—We are grateful to Dr. N. G. Waton for advice on the use of the radio-isotopic method for the determination of histidine decarboxylase, to Dr. J. T. Hemingway for guidance in the technique of partial hepatectomy and in the measurement of mitotic indices, and to Miss D. E. A. Strevens for advice on the statistical calculations. Tumour-bearing rats were kindly provided by Professor Sir Alexander Haddow, F.R.S., Dr. R. W. Baldwin and Dr. Bernard Messier. We thank the Medical Research Council for the award of a Grant.

## REFERENCES

1. W. Lovenberg, H. Weissbach and S. Udenfriend, *J. biol. Chem.* **237**, 89 (1962).
2. D. M. Shepherd and D. Mackay in *Progess in Medicinal Chemistry* (Eds. G. P. Ellis and G. B. West), vol. 5, p. 199. Butterworths, London (1967).
3. G. Kahlson, Lancet, **i**, 67 (1960).
4. L. Kameswaran and G. B. West, *Int. Archs Allergy appl. Immun.* **21**, 238 (1962).
5. D. Mackay, P. B. Marshall and J. F. Riley, *J. Physiol., Lond.* **153**, 31P (1960).
6. D. Mackay, J. F. Riley and D. M. Shepherd, *J. Pharm. Pharmac.* **13**, 257 (1961).
7. J. D. Reid, J. F. Riley and D. M. Shepherd, *Biochem. Pharmac.* **12**, 1151 (1963).
8. G. A. H. Buttle, J. Eperon, L. Kameswaran and G. B. West, *Br. J. Cancer*, **16**, 131 (1962).
9. G. M. Higgins and R. M. Anderson, *Archs Path.* **12**, 186 (1931).
10. H. M. Carleton and R. A. B. Drury, *Histological Technique*. Oxford University Press, London (1957).
11. M. Abercrombie and R. D. Harkness, *Proc. R. Soc.* B, **138**, 544 (1951).
12. G. Kahlson, E. Rosengren and R. Thunberg, *J. Physiol., Lond.* **169**, 467 (1963).

13. N. G. WATON, *J. Physiol., Lond.* **172**, 475 (1964).
14. D. S. PEARLMAN and N. G. WATON, *J. Physiol., Lond.* **183**, 257 (1966).
15. A. M. BRUES and B. B. MARBLE, *J. exp. Med.* **65**, 15 (1937).
16. J. M. TELFORD and G. B. WEST, *Br. J. Pharmac.* **16**, 360 (1961).
17. J. P. GREENSTEIN, *Biochemistry of Cancer*, p. 595. Academic Press, New York (1954).