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HISTOLOGICAL AND ENZYMATIC CHANGES IN THE LIVERS OF RATS FED THE HEPATIC CARCINOGEN DIETHYLNITROSAMINE

JOAN D. REID, J. F. RILEY and D. M. SHEPHERD

University of St. Andrews,
Department of Pharmacology and Therapeutics, Queen's College, Dundee

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Abstract—When the liver carcinogen diethylnitrosamine (DENA) was fed to Wistar rats in their drinking water over a period of 5 months, two main changes in liver structure were observed to develop in sequence: first the gradual formation of hepatomas (circumscribed nodules of highly abnormal parenchymal cells); later cholangiomas (progressive overgrowth of bile duct epithelium) which eventually dominated the histological picture. Specific and non-specific histidine decarboxylase activities were measured in livers of treated and control animals at regular intervals, the two enzymes being characterized by their Michaelis constants, pH optima, effect of benzene, and effect of inhibitors. The results indicate that the specific decarboxylase (pH 6·5) is associated with the period of hepatoma formation (maximal in excised hepatoma tissue) and not with proliferation of bile duct epithelium (cholangioma). The enzyme is thus associated with the growth of a particular cell rather than with growth in general. In the livers of both control and DENA-treated rats a highly significant positive correlation was observed between the histidine decarboxylase activity at pH 8 and the dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP) decarboxylase activities; this suggests that these three activities are due to a single enzyme, a non-specific aromatic amino acid decarboxylase.

DURING the last third of pregnancy in the rat the output of histamine in the urine is considerably raised,[1] and the livers of the growing foetuses have high histidine decarboxylase activity.[2] Regenerating livers of partially hepatectomised rats also possess high histidine decarboxylase activity, and, during the regenerating phase only, large amounts of histamine are excreted in the urine.[3] When a transplantable hepatoma, F-Hep, is implanted subcutaneously into adult rats of the August strain, the amount of histamine excreted in the urine increases enormously as the tumour grows, returning to normal when the tumour is excised; the tumour itself possesses high histidine decarboxylase activity.[4, 5] Collectively the above observations support the view that there may be a functional relationship between histidine decarboxylase activity and tissue growth,[6] and there is evidence[7, 8] that the decarboxylase in question is specific for the decarboxylation of histidine. In this, and in certain other respects, this enzyme differs from the histidine decarboxylase of, for example, such static tissues as rat liver and guinea pig kidney, which is non-specific and for which the term aromatic L-amino acid decarboxylase has been suggested.[9]

By following the process of tumour *formation* in the liver of rats it was hoped to test the hypothesis that a functional relationship exists between histidine decarboxylase

activity and tissue growth, and also to ascertain which of the two histidine decarboxylases is involved. As a carcinogen we selected the simple organic compound diethylnitrosamine (DENA) which is known to produce tumours in the livers of rats over a relatively short period of administration.[10] Changes in the activity of DOPA, 5-HTP, and histidine decarboxylases were followed throughout the induction period, and have been correlated with the corresponding histological changes. A preliminary account of these studies has been published.[11]

METHODS

Diethylnitrosamine

This was prepared from commercial diethylamine,[12] and was twice distilled before use (b.p. 174°).

Feeding experiment

Female rats of the Wistar strain, fed on the standard laboratory diet 41b, were divided into two groups, one of which received diethylnitrosamine (1·5 mg per rat per day) in the drinking water. At intervals one treated rat and one control rat were killed, and the livers were removed for histological and enzymatic study.

Urine collection

Every 14 days two groups of three permanently marked rats were placed in separate metabolism cages and urine was collected daily for 5 successive days for the estimation of histamine; the three treated rats continued to receive DENA during this period.

Measurement of enzyme activities

Liver extracts were prepared in distilled water (3 ml/g tissue) and divided into three aliquots. DOPA decarboxylase and 5-HTP decarboxylase activities were determined using the method of Dietrich[13] the substrate concentration in the incubation mixture being 2·5 μmoles per ml of the L-form of the appropriate amino acid and the pyridoxal 5′-phosphate concentration 40 μg/ml. DOPA decarboxylase activity was measured at pH 7·0 and 5-HTP decarboxylase activity at pH 8·0; internal standards were used, and the duration of the incubation was 20 min. Histidine decarboxylase activity was determined by the method of Mackay *et al.*,[8] benzene being added to incubations at pH 8·0 but not at pH 6·5.

Histology

Fresh biopsy specimens were taken from the edge of the liver and were placed at once in formol-corrosive fixative. Paraffin sections were later stained with haematoxylin–eosin (general topography), toluidine blue (metachromasia), silver impregnation (reticulin) and a modified Masson trichrome method (collagen and connective tissue).

RESULTS

Histological

In DENA-treated animals the toxic effects of the drug become apparent from 2 weeks onwards, the hepatic cells showing cloudy swelling, vacuolation and patchy

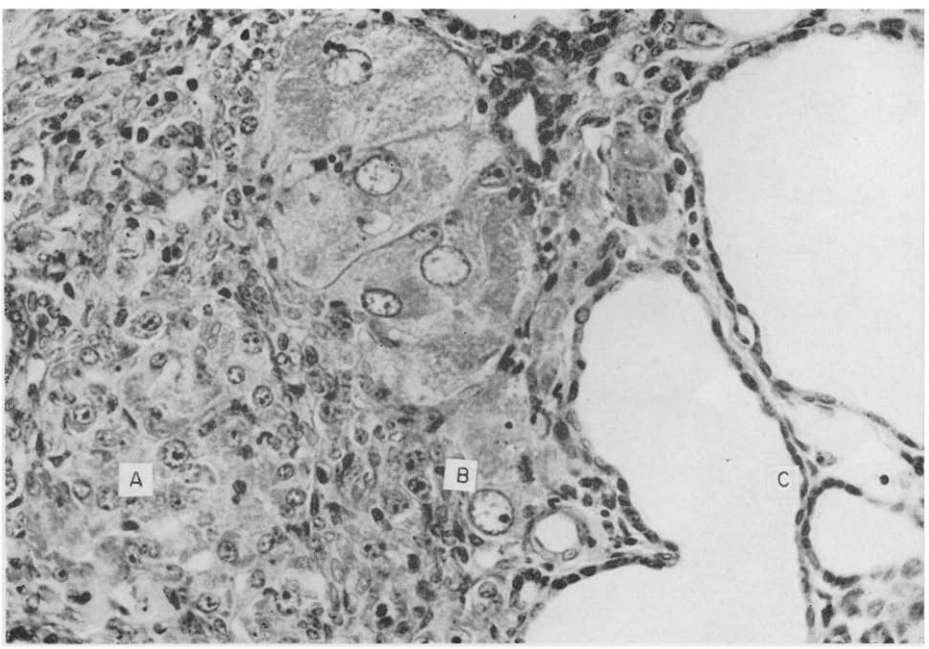

FIG. 1. Examples of the three successive effects of feeding the hepatic carcinogen, DENA, to rats are shown in this single paraffin section of liver, taken at 4 months, stained modified Masson method (×310). To the left is toxic, but still functioning liver tissue (A); in the centre (B) is a vertical row of grossly abnormal, hypertrophied cells, a small hepatoma; to the right (C) is cystic hyperplasia of bile duct epithelium. Specific histidine decarboxylase activity is associated only with the central hepatoma cells (B).

necrosis (Fig. 1A). Compensatory regeneration of adjacent healthy cells occurs around these areas until they in turn come under the influence of the carcinogen. At first the portal tracts remain normal. However, around 3 months there is seen a gradual increase in the delicate connective tissue of the portal tracts which spreads outwards to subdivide the substance of the liver into a number of discrete lobules. This would suggest a conventional multilobular cirrhosis of toxic origin were it not that a remarkable change now begins to show itself in certain of the parenchymal cells that have survived the initial toxicity. These cells are scattered throughout the lobules in small groups, apparently at random, and are at once obvious by their relatively large size and aberrant staining properties. In volume both cytoplasm and nucleus are many times that of the surrounding cells. Occasionally such a cell is found in mitosis; some are binucleate. An aggregate of such cells can now be described as a specific type of adenoma, a hepatoma, that is a tumour derived from cells of the liver parenchyma.[14] The larger nodules can be recognized with the naked eye. This stage, which is reached after $3\frac{1}{2}$ to 4 months of DENA-treatment, is shown in Fig. 1B.

As the hepatomas increase in size and number there now occurs a second pathological process which gradually comes to dominate the histological picture. This is a progressive overgrowth of bile-duct elements which increase to form irregular cysts and loculi lined with small darkly-staining cells. So great is this cholangiomatous overgrowth during the later months of feeding DENA that some of the histological sections appear to the naked eye more like lung than liver, Fig. 1C. The final picture of the liver of the DENA-fed rat is thus one of secondary cholangiomatous overgrowth of bile-duct elements, between which lie compressed nodules of hepatoma tissue and the few remaining patches of more normal liver cells. Sometimes a liver at this stage undergoes total, acute necrosis; haemorrhage may occur. More usually the animal succumbs to a more gradual suppression of hepatic function. In general, the later changes described above correspond to those described by Magee and Barnes[14] who used dimethylnitrosamine as the carcinogen.

Pharmacological

Urinary histamine excretion in DENA-fed rats showed only a small, though significant, increase over control values after about 4 months of treatment. This is in contrast to the enormous increase in histamine excretion in August-strain rats implanted with the fast-growing transplantable hepatoma, F-Hep.[4] In a second experiment the histamine excretion of the treated rats barely exceeded the control levels.

Enzymological

The DOPA, 5-HTP and histidine decarboxylase activities in the livers of control and treated rats are shown in Table 1. No histidine decarboxylase activity was detected at pH 6·5 in the livers of control animals whether or not benzene was added. However, at pH 8 histidine decarboxylase activity was readily detectable provided that benzene was added.

In contrast to the above, histidine decarboxylase activity at pH 6·5 was almost invariably found in the livers of DENA-fed rats from 3 months onwards, and in a few animals the activity was higher at pH 6·5 than at pH 8·0 (animals 5, 28 and 29 in Table 1). In a separate piece of recognisable hepatoma tissue from animal 17, activity

was much higher at pH 6·5 than at pH 8·0 although in the remainder of the same liver the activity at pH 6·5 was low. The mean activities of DOPA decarboxylase, 5-HTP decarboxylase and histidine decarboxylase (measured at pH 8·0) in the livers of DENA-treated animals from 3 months onwards were significantly lower than the corresponding mean values in the livers of control rats ($P < 0.001$ in each case). A positive

TABLE 1. ENZYME ACTIVITIES IN RAT LIVER THROUGHOUT THE COURSE OF DIETHYLNITROSAMINE TREATMENT

Animal	Day	DOPA decarboxylase μmoles dopamine/g/hr	5-HTP decarboxylase μmoles 5-HT/g/hr	Histidine decarboxylase μg histamine/g/3 hr	
				pH 8·0	pH 6·5
	0*	56 ± 3·8	11·2 ± 0·83	44 ± 2·9	nil
1	14	34	8	41	nil
2	28	36	8	30	nil
3	56	17	6	17	nil
4	70	20	6	—	nil
5	84	10	3	15	35
6	98	34	7	25	nil
7	106	27	5·5	24	1
8	111	34	5	24	3
9	111	46	10	43	1
10	112	33	7·5	35	1·5
11	112	31	6·5	32	1·5
12	113	17	2·5	6	1
13	116	30	4	17	1·5
14	116	49	6·5	26	1
15	118	46	8·5	59	1·5
16	118	47	6	40	1·5
17	118	6	1·5	5	1
17†	118	9	—	9	32
18	118	6	2	4	nil
19	118	18	4	12	1
20	119	39	8	46	1·5
21	119	43	10	38	1·5
22	123	21	4	16	1·5
23	123	37	5·5	26	1
24	123	22	2	6	1
25	125	37	10	32	1·5
26	125	44	8	36	3
27	130	40	7·5	49	nil
28	130	17	1	7	13
29	132	19	4	10	18
30	132	27	7	9	3
31	132	20	3	6	2
32	133	24	4	19	2·5
33	138	31	6	33	nil
34	138	36	7	41	4
35	156	17	4	20	1

* Enzyme activities are the means of 13 control animals with standard errors.

† Animal 17 had a large hepatoma nodule in which the enzyme activities were studied separately.

correlation was found between the activities of histidine decarboxylase measured at pH 8·0, DOPA decarboxylase and 5-HTP decarboxylase ($P < 0.01$ in each case). Inspection of Table 1 clearly indicates that there is no correlation between histidine decarboxylase measured at pH 6·5 and DOPA decarboxylase or 5-HTP decarboxylase.

Table 2 summarises the properties of the two histidine decarboxylases.

DISCUSSION

Pathological changes

As stated above, the feeding of DENA to the female Wistar rat results in a series of pathological changes, only one of which (the hepatoma) is associated with an increase in specific histidine decarboxylase activity.

The initial toxic changes and the mild cirrhosis require no special comment. Present interest centres upon the hepatoma which differs from the established, transplantable hepatoma (F-Hep) ,previously studied, in that it arises as scattered foci of grossly abnormal liver cells which at first grow comparatively slowly. It would seem that the poorly-differentiated, rapidly growing transplantable tumour is the result of selection,

TABLE 2. COMPARISON OF THE HISTIDINE DECARBOXYLASES IN THE LIVERS OF CONTROL AND DIETHYLNITROSAMINE-TREATED RATS

	Present in control and DENA-treated livers	Present in DENA-treated livers only
pH optimum	8·0	6·0–6·5
K_m	$5{\cdot}3 \times 10^{-1}$ mole l^{-1}	$1{\cdot}2 \times 10^{-3}$ mole l^{-1}
Substrates	DOPA, 5-HTP, histidine	histidine
Effect of pyridoxal phosphate	potentiation	potentiation
C_{50} value* of α-methyl-DOPA	$9{\cdot}0 \times 10^{-6}$ M	$2{\cdot}4 \times 10^{-2}$ M
C_{50} value* of α-methylhistidine	$1{\cdot}1 \times 10^{-2}$ M	$3{\cdot}6 \times 10^{-3}$ M
Effect of benzene	potentiation	none

* Concentration of inhibitor required to reduce the initial velocity of the uninhibited reaction by 50 per cent.

by passage, from such a nodule. Neither tumour tends to metastasize; but whereas the transplantable tumour, growing subcutaneously, is soon heavier than the entire liver, the nodules which arise within the liver from feeding DENA seldom reach an appreciable size. The largest nodule seen by us (rat No. 17) was not more than 1·5 cm in diameter. Nevertheless in enzymic activity it closely resembled the transplantable tumour in that it was rich in specific histidine decarboxylase and poor in the non-specific enzyme. The absolute amounts of tumour in the two types of hepatoma-induced and transplanted, are likewise reflected in differences in urinary histamine: in contrast to the transplant, growing in skin, muscle or peritoneal cavity, there was seldom sufficient of the induced hepatoma present at any one time to influence significantly the output of histamine in the urine.

The later, cholangiomatous overgrowth is presumably also due to the continued feeding of the carcinogen, since the diet contained adequate protein and no signs of cholangioma were observed in the controls. The microscopic appearances suggest that islets of hepatoma, as well as remnants of more normal liver parenchyma, are gradually overwhelmed by this secondary hyperplasia of bile-duct elements. As this occurred, the level of the specific histidine decarboxylase in the liver was observed to fall.

The two decarboxylases

The highly significant positive correlation in both control and DENA-treated rats between the histidine decarboxylase activity at pH 8·0 and the DOPA decarboxylase

and 5-HTP decarboxylase activities indicates that this decarboxylation of histidine is due to a non-specific aromatic amino acid decarboxylase.

However, when the histidine decarboxylase activity at pH 6·5 was high (for example, in rats 5 and 29 in Table 1), the DOPA decarboxylase and 5-HTP decarboxylase levels were not correspondingly raised. Hence the histidine decarboxylase which develops during the DENA treatment is a separate enzyme. The presence of two distinct histidine decarboxylases in the livers of DENA-treated rats is further confirmed by their comparative properties as set out in Table 2.

The above results thus indicate that the specific histidine decarboxylase of rat liver is restricted to the period of hepatoma formation, its activity being maximal in excised hepatoma tissue. Once the hepatoma tissue has been overgrown by a yet more vigorous proliferation of bile duct cells, the activity of the specific histidine decarboxylase falls. It is therefore concluded that, under these circumstances, specific histidine decarboxylase is associated with the growth of a particular type of cell, the hepatoma, but not with growth in general.

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