

## THE ROLE OF MAST CELLS IN HUMAN LIVER CIRRHOSIS

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### ABSTRACT

Mast cells appear to play significant roles in physiological and pathological conditions. Some functions of mast cells may relate to liver cirrhosis, which is characterized by degeneration, necrosis and regeneration of parenchyma as well as fibrosis, but little information regarding this association has been obtained. This study was carried out in order to clarify the pathogenesis of liver cirrhosis as related to the distribution and the role of mast cells. Fifty-five cases of liver cirrhosis were divided into five groups, according to Kim's morphological classification and the mean values with the standard errors in each group were determined with regard to the number of mast cells, the relative volume of the trabecula and the liver weight. A statistical analysis of these determinations was also performed. The results of the present investigation revealed that a greater number of mast cells were found in the I-B and the II-B groups in which a more marked regeneration was histopathologically recognized, and that in these groups a statistically significant correlation existed between the number of mast cells and the liver weight which mostly increased with regeneration. These findings strongly suggested that mast cells in human liver cirrhosis had a close relationship to regeneration of hepatic parenchyma followed by occasional incidence of malignant hepatomas.

### INTRODUCTION

Since the original description of the mast cells in the connective tissue by Ehrlich<sup>1)</sup>, there have been a great deal of investigations along diverse directions and a large amount of information has accumulated about the mast cells along with much speculation as to their functions. Many studies indicate that these cells, in relation to their contents, namely heparin, histamine, serotonin, and various enzymes, etc., may have important functions in growth and regeneration physiologically, and on the other hand play a role in the dynamics of organization of edema, wound healing, inflammation, tumors, endocrine disorders, and other pathologic states. The details are referred to some excellent reviews<sup>2) 3) 4) 5)</sup>.

The functions of mast cells are considered to have a close relation to human liver cirrhosis which in most cases arises from degeneration and necrosis

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of liver cells, followed by successive fibrosis and regeneration of hepatic parenchyma, occasionally accompanied with malignant hepatomas. Although some attention has been paid to the possible relationship between mast cells and liver cirrhosis, only a few findings<sup>(6) (7) (8) (9)</sup> concerning the occurrence and the distribution of mast cells in this disease have been obtained. In addition, neither table nor figure has been presented and a statistical analysis has not yet been attempted with regard to the number of mast cells.

Because of the paucity of exact information regarding the distribution of mast cells in human liver cirrhosis and much less on the types of liver cirrhosis according to morphological classification, this study was undertaken. The object of this work is to count mast cells in each type of human liver cirrhosis systemically and to compare the values in each type statistically.

#### MATERIALS AND METHODS

This study was made on 55 cases of liver cirrhosis, and 5 cases of chronic hepatitis, from 1,300 necropsies carried out at the First Department of Pathology, Nagoya University School of Medicine, from April 1, 1964 to June 30, 1969. Other types of cirrhosis, the cause of which has been explained, *e.g.* biliary cirrhosis, cardiac cirrhosis, hemochromatosis, and Wilson's disease were excluded.

The specimens were collected from the cirrhotic livers of these cases, fixed with 10% formalin and embedded in paraffin. The paraffin blocks were cut by a Jung's microtome adjusted to 10  $\mu$  in thickness for mast cell staining, and 5  $\mu$  for general staining. The former sections were stained with 0.01%

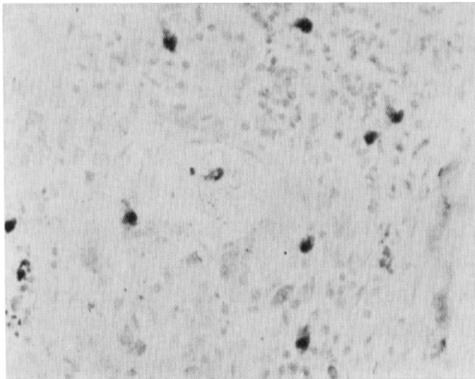


PHOTO. 1

PHOTO. 1. The granules of mast cells are very distinctly stained with toluidine blue. Mast cells are distributed exclusively in the hepatic trabeculae (Toluidine blue,  $\times 100$ ).

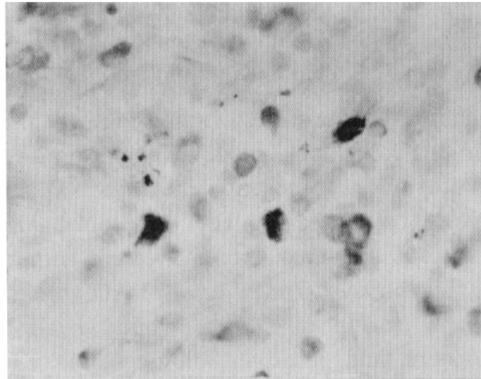


PHOTO. 2

PHOTO. 2. Three mast cells with metachromatically stained granules are very distinct (Toluidine blue,  $\times 400$ ).

toluidine blue aqueous solution as recommended by Lison<sup>10</sup> (Photo. 1 and Photo. 2). The latter sections were stained with haematoxylin-eosin staining, Van-Gieson elastica staining, Azan-Mallory staining, silver impregnation staining for reticulin and periodic acid-Schiff staining.

The determinations of the number of mast cells per mm<sup>3</sup> of whole liver and the relative volume of the trabeculae in all cases were carried out at a magnification of  $\times 400$  with a biocular Nikon microscope. Nucleated mast cells in 250 fields were counted by random selection of the fields throughout the tissue sections, and the size of one visual field was 0.152 mm<sup>2</sup>. In each case the number of mast cells per mm<sup>2</sup> of liver section was determined and finally the number of mast cells per mm<sup>3</sup> of the section ( $N$ ) was calculated according to Floderus' formula<sup>11</sup>):

$$N = \frac{n \times 1.000}{a + d - 2h}$$

where  $n$ =number of nucleated mast cells per mm<sup>2</sup>,  $a$ =thickness of section in  $\mu$  (10),  $d$ =diameter of nucleus in  $\mu$  (5), and  $h$ =height in  $\mu$  of the thinnest portion of nuclei taken into consideration (0.5). The values for  $d$  and  $h$  are the same as those used earlier<sup>12</sup>).

The relative volume of the trabeculae was determined with the aid of a cross ruled micrometer. Four corners and a crossed center point of the micrometer were regarded as points. The number of hits by these points on trabeculae was counted in each visual field simultaneously with the counting of mast cells. The volume of trabeculae as a percentage of the whole liver (Trab.) was obtained from

$$\text{Trab.} = \frac{h \times 100}{v \times 5}$$

where  $h$ =number of hits on trabeculae and  $v$ =number of visual fields (250). This method of determining the relative volume of the trabeculae was suggested by a review of point sampling methods<sup>13</sup>).

Since the values obtained in the manner described above are somewhat too high because of shrinkage of the specimens by fixation and paraffin embedding, they should be corrected. The shrinkage in length of the specimens was close to 10 per cent and was thought to be the same in all directions. Therefore, the shrinkage in volume was 27.1 per cent, by which the values obtained for mast cells were reduced.

Fifty-five cases of liver cirrhosis were divided into five groups according to the morphological classification proposed by Kim Tu Jung *et al.* (D.P.R. Korea) in 1964<sup>14</sup>). Kim's classification was compared with the representative classifications which have been generally accepted. As shown in Table 1, Kim's I type, II type and III type nearly corresponded to Nakamura's<sup>15</sup> I type,

TABLE 1. Comparison of Kim's Classification with Other's

Other's ↓	Kim's →	I type	I type	II type	II type	III type
		A phase	B phase	A phase	B phase	
Nakamura, T. (1961)		I type		II type		III type
Miyake, M. (1960)		A	A'	B'	B	F
Gall, E. A. (1960)		Post-necrotic		Post-hepatic		Nutritional

II type and III type, or Gall's<sup>16)</sup> postnecrotic type, posthepatic type and nutritional type, respectively: Further, as Kim's A phase and B phase in each type meant the initial stage and the developed stage respectively, it was concluded that Kim's I-A, I-B, II-A, II-B and III type coincided with Miyake's<sup>17)</sup> A, A', B', B and F type in this order.

## RESULTS

Table 2 summarizes the frequency of cases in each group and its association with hepatocellular carcinoma. The I-B and the II-B group, belonging to relatively or well advanced cirrhosis, comprise 14 and 20 cases respectively and together they account for approximately 60 per cent of the entire cases. Hepatocellular carcinomas are found exclusively in the I-B, II-A and II-B groups, the respective incidence being 36, 63 and 50 per cent. There was seen no hepatocellular carcinoma in the I-A and III groups.

TABLE 2. Numbers of Cases in Each Group of Liver Cirrhosis and Those associated with Malignant Hepatomas

	I-A	I-B	II-A	II-B	III	Total
No. of cases	7 (13)	14 (25)	8 (15)	20 (36)	6 (11)	55 (100)
No. of cases with malignant hepatomas	0 (0)	5 (36)	5 (63)	10 (53)	0 (0)	20 (36)

Numbers in parentheses represent percentage.

Table 3 lists the mean values and the standard errors of the determinations with regard to the number of mast cells per mm<sup>3</sup> of whole liver and of the trabeculae, the relative volume of the trabeculae, and weight of the liver in each group.

Fig. 1 shows the mean value and the standard error of the number of mast cells per mm<sup>3</sup> of whole liver in each group. The mean values of the number of these cells per mm<sup>3</sup> of whole liver in the II-A group and the I-B

TABLE 3. Mean Values and Standard Errors of Determinations in Each Group

	No. of cases	Mast cells per mm <sup>3</sup> of whole liver	Mast cells per mm <sup>3</sup> of trabeculae	No. of cases	relative volume of the trabeculae	No. of cases	Weight of the liver
I type A phase	4	289±110	587±189	5	48.4±3.9	6	901±118
I type B phase	10	587± 86	1580±238	11	38.0±1.0	11	1026± 76
II type A phase	5	628±150	3110±928	4	18.3±1.3	4	1383±118
II type B phase	11	334± 65	1507±325	12	24.2±1.9	10	1021± 63
III type	4	174± 60	530±146	4	33.6±5.3	6	1710±167
Chronic Hepatitis	5	56± 32	747±200	5	12.5±1.3	5	1100±144

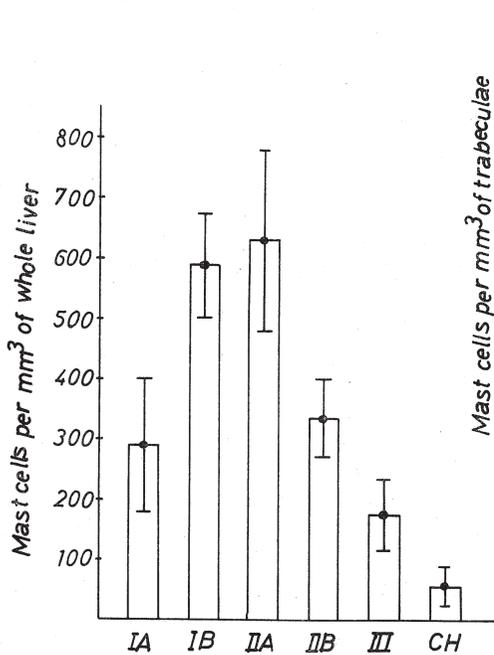


FIG. 1. Mast cells per mm<sup>3</sup> of whole liver in each group.

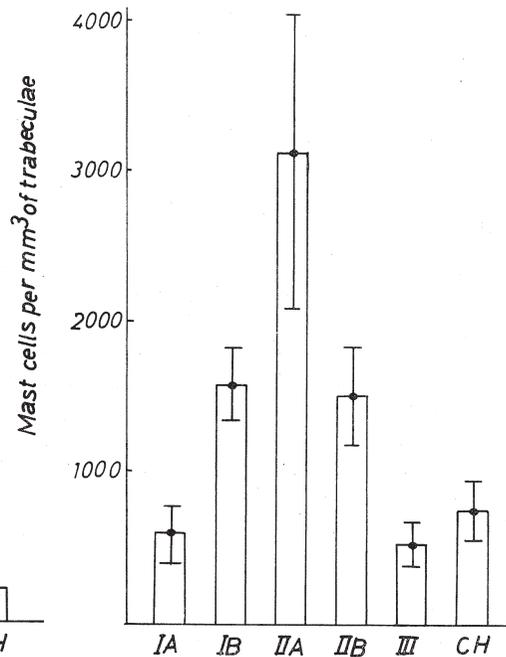


FIG. 2. Mast cells per mm<sup>3</sup> of the hepatic trabeculae in each group.

group are 628 and 587 respectively, approximately twice as numerous as in other groups, but this fact has not been statistically significant.

Fig. 2 shows the mean value and the standard error of the number of mast cells per mm<sup>3</sup> of the trabeculae in each group. The mean in the II-A group is 3,110, the highest, with a very wide range, and the means of the I-B and the II-B groups are almost the same, 1,580 and 1,509 respectively, and they are more than twice as numerous as those of the I-A group and the III

group. These findings, however, are not statistically significant. Additional statistical analysis has revealed a significantly less number of mast cells per  $\text{mm}^3$  of the trabeculae in the III group than in the I-B and II-B groups, namely advanced cirrhosis.

There appears to be no possible correlation between the relative volume of the trabeculae and the number of mast cells per  $\text{mm}^3$  of whole liver or of the trabeculae. A statistical analysis has not been performed and a diagram

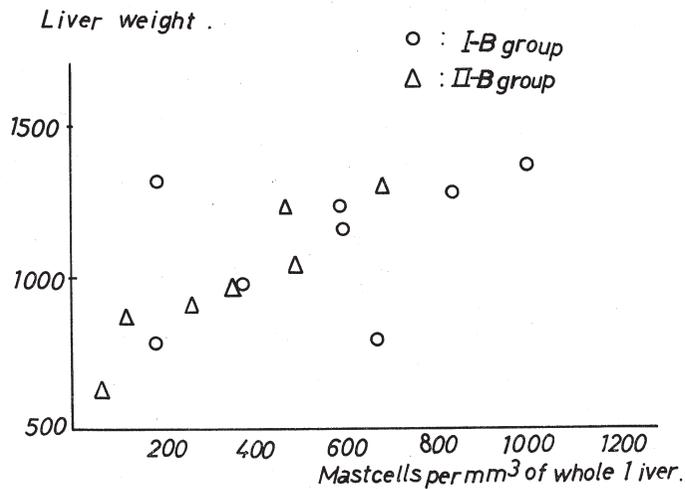


FIG. 3. Diagram showing the relationship between the liver weight and the number of mast cells per  $\text{mm}^3$  of whole liver in groups I-B and II-B.

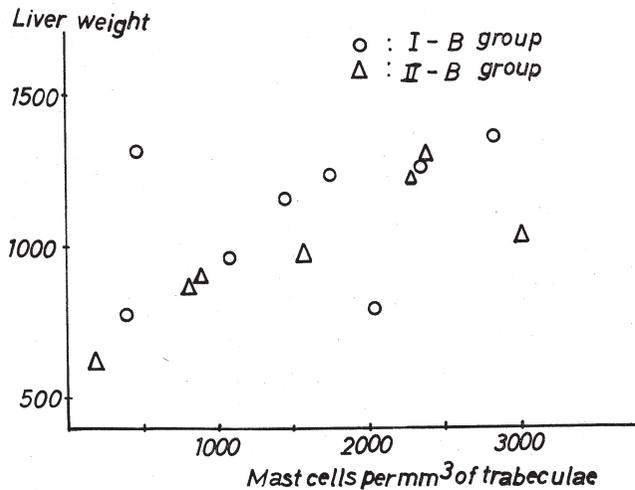


FIG. 4. Diagram showing the relationship between the liver weight and the number of mast cells per  $\text{mm}^3$  of hepatic trabeculae in groups I-B and II-B.

of this relationship is not presented.

In the group lumping together the I-B group and the II-B group, there is a positive correlation between weight of the liver and the number of mast cells per mm<sup>3</sup> of whole liver (Fig. 3) and of the trabeculae (Fig. 4). Their coefficients of correlation are +0.658 in the former and +0.567 in the latter, and their P-values are 1 per cent and 5 per cent, respectively.

#### DISCUSSION

A few observations on mast cells in human liver cirrhosis have been made. In three of four cases of atrophic cirrhosis increased numbers of mast cells were found in the connective tissue<sup>6)</sup>. Also by other investigators<sup>7)9)</sup> large numbers of mast cells have been observed in human cirrhosis. Though Ellis<sup>9)</sup> counted mast cells in ten cases of portal cirrhosis of the liver, an increase in these cells was not found. In the present study, mast cells in liver cirrhosis were generally more numerous than those in the chronic-hepatitis group, except for a slightly less number of mast cells in the I-A group and the III group than in the chronic-hepatitis group as regards mast cells per mm<sup>3</sup> of the trabeculae.

The increase of mast cells in liver cirrhosis remains to be explained. A close relationship between mast cells and fibroplasia has been pointed out by some workers<sup>2)9)18)19)</sup>. Morrione<sup>9)</sup> has suggested that the mast cells take part in the formation of collagen by secreting heparin, on the other hand it has been maintained that mast cells may participate in the production of connective tissue ground substances<sup>2)18)</sup>. In experimental cirrhosis, Ahlqvist<sup>20)</sup>, and Wahi and Bansal<sup>21)</sup> separately obtained results that the mast cell count did not run parallel to the trabecular volume, but both stated quite different opinions. No significant correlation between the number of mast cells and the relative volume of the trabeculae was also noted in this study, and no information on the role of mast cells in fibrogenesis could be obtained.

As to the relationship between mast cells and tumorigenesis, Ehrlich<sup>22)</sup> commented upon the abundance of these cells in neoplasms, and this association has been subsequently studied by many authors<sup>6)23)24)25)26)27)28)29)30)</sup> in a variety of neoplasms, benign and malignant, human and experimental.

Higuchi<sup>24)</sup> observed a small number of mast cells in fibroadenomas as well as in carcinomas of the breast. On the other hand these cells were found in large numbers along the borderline between normal tissue and carcinoma. Similarly, Jane and McDonald<sup>26)</sup> reported that mast cells tended to accumulate in the adjacent normal tissue and in the regional lymph nodes with no invasion of tumor, while they were very rare in tumors. The results of this study, which revealed the occurrence of more numerous mast cells in the groups frequently associated with hepatocellular carcinoma than in those without hepatocellular carcinoma, are apparently consistent with these observations concerning the increase of mast cells in neoplastic growths.

The abundance of mast cells has been observed also in the so-called precancerous conditions<sup>25) 28) 29) 32)</sup>. Cramer and Simpson<sup>25)</sup>, from their study of mast cell reaction in mouse skin undergoing methylcholanthrene-induced carcinogenesis, found the mast cell response, unrelated to the carcinogen *per se*, significantly greater in precancerous epidermal hyperplasia than in overt carcinoma. In humans, Dunn and Montgomery<sup>28)</sup> also noted a greater number of mast cells in the stroma immediately beneath precancerous hyperplasia and carcinoma in situ of the uterine cervix than in invasive carcinoma.

Many workers<sup>23) 25) 28) 33)</sup> have regarded this mast cell reaction as an expression of the host's defence against neoplastic growth although there are still unsettled opinions concerning the functional significance of this relationship. They have considered this defensive action to be the antimitotic activity and the growth-inhibiting effect of heparin<sup>34) 35) 36) 37)</sup>. Csaba and his associates<sup>38)</sup> also regarded the mast cell reaction encountered in neoplastic growth as a defence mechanism. They have, however, held that these cells are the competitors with the tumor cells for heparin or its polysaccharide precursors found in the connective tissues.

On the contrary, it was presented by Sylvén<sup>18)</sup> that the mast cell actually promoted tumor growth as well as hyperplasia. He observed that diffuse metachromasia, which perhaps originated from heparin, was distributed in various types of growing tissues, *e.g.* embryonic tissues, granuloma, and regenerating epithelia etc., and maintained that the mast cells discharged their granules into those tissues where growth actively proceeded. In addition, another finding<sup>39)</sup>—the appearance of metachromatic high molecular polysaccharide ester sulfates in the stroma beneath the growing normal or cancerous epithelia—strongly suggested the existence of a very close functional interaction between tissue mast cells and growing epithelia, aiming at the supply of raw-material for epithelial growth.

These findings and the interpretations of Sylvén are not incompatible not only with the observations reported by Cramer and Simpson<sup>25)</sup>, Dunn and Montgomery<sup>28)</sup>, Stolk<sup>32)</sup>, and Nozaka and Simpson<sup>29)</sup>, but also with the results obtained from this investigation: Firstly, that a greater number of mast cells were found in the stroma of the liver in I-B and II-B groups, where more marked regeneration of the hepatic parenchyma has been histopathologically recognized throughout the sections. Secondly, that there is a statistically significant correlation between the number of mast cells and weight of the livers in these two groups taken together. Since the weight of the liver may be largely influenced by hyperplasia and hypertrophy of liver cells except for deposition of the definite substances, *e.g.* lipids, glycogen, or other materials not directly related to the proliferated response<sup>40)</sup>, this finding is considered to represent a close relationship between mast cells and regeneration of the hepatic parenchyma. Ahlquist's observations<sup>20)</sup> in rats on a low protein, high fat diet

are somehow closely pertinent: In advanced fatty cirrhosis more mast cells tended to occur with an increasing degree of nodular regeneration inspite of a negative correlation with weight of the liver.

The greater number of mast cells in I-B and II-B groups should be considered to be related to rather regeneration *per se* than to hepatoma, and the high incidence of hepatomas in these groups seems to support the significance of parenchymal regeneration as precancerous condition<sup>41)42)</sup>.

For the time being, it does not seem possible to draw a definite conclusion with regard to the functional significance of mast cells in neoplastic growths. Although some facts including the results obtained in this study appear to favour a promoting effect, this requires more confirmation and accumulation of evidence to support it.

It is known that watery fixing fluids are not suitable for the study of mast cells in most species of animals including man, because the metachromatic granules in the cells are incompletely precipitated by the watery fixative. Therefore, 10 per cent basic lead acetate, which was recommended by Holmgren and Wilander<sup>43)</sup> as a fixative that render the metachromatic substance of the mast cell insoluble, has been used in most of the numerical study of mast cells.

On the other hand Westphal<sup>44)</sup> stated that in man, guinea pig and rat aqueous formalin solution was sufficient to fix the mast cell granules. In practice there have been unexpectedly many works<sup>24)27)28)31)45)46)</sup> where the materials were fixed with aqueous formalin solution. This investigation has also been carried out on specimens of the liver fixed with 10 per cent aqueous formalin solution owing to circumstances beyond control, and mast cells with metachromatic granules were stained distinctly enough for counting. The report<sup>47)</sup> which showed no significant effects of time, temperature and fixatives on mast cell counts in postmortem materials, stands security for the validity of the present results.

#### CONCLUSION

1) Fifty five cases of liver cirrhosis in human were divided into five groups according to Kim's classification and compared with representative classifications. Kim's I-A, I-B, II-A, II-B and III types correspond to Miyake's A, A', B', B and F types in this order.

2) The number of mast cells per mm<sup>3</sup> of whole liver and of trabeculae in each group was determined and subjected to statistical analysis. A quantitative study for mast cells in human postmortem materials fixed with aqueous formalin solution might be relatively valid.

3) No significant result was obtained on the participation of mast cells in fibroplasia.

4) Mast cells per mm<sup>3</sup> of the trabeculae in the III group were significantly less than in the I-B and the II-B groups, namely advanced cirrhosis.

5) There was found a statistically significant correlation between the number of mast cells and weight of the liver in I-B, II-B groups, and this suggested a close relation of mast cells to regeneration of hepatic parenchyma.

6) A greater number of mast cells were found in the II-A, I-B and II-B groups frequently associated with hepatocellular carcinoma. In the latter two groups, more marked regeneration was noted histopathologically.

7) The abundance of mast cells seemed to be related to rather regeneration *per se* than to hepatocellular carcinoma, and it was concluded that mast cells might have a promoting effect on regeneration of the epithelium, as related to neoplastic growths.

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