

## CHANGES IN THE QUANTITY OF MITOSIS AND BINUCLEATE CELLS IN THE LIVER OF PARTIALLY HEPATECTOMIZED RATS WITH EXPERIMENTAL CHAGAS' DISEASE

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### SUMMARY

Wistar rats with partial hepatectomy and experimental infection with *Trypanosoma cruzi* show greater number of mitosis and binucleate cells in the liver than the animals only hepatectomized.

### INTRODUCTION

The influence of the *Trypanosoma cruzi* on induced experimental tumors has been the subject of great and controversial investigation (ENGEL<sup>3</sup>; MALISOFF<sup>10</sup>; HAUSCHKA & GOODWIN<sup>7</sup>; HAUSCHKA et al.<sup>6</sup>; HAUSCHKA<sup>8</sup>; SPAIN et al.<sup>16</sup>) since the pionner work by ROSKIN & EXEMPLIARSKAIA<sup>15</sup>, ROSKIN & ROMANOVA<sup>14</sup> and KLYUEVA<sup>9</sup>.

The Russians intended to demonstrate that some products isolated from the parasites and the experimental infection with the *T. cruzi* were able to inhibit the growth and sometimes to produce complete regression of neoplasias, in animal and man.

These actions were supposed to be due to "cancerolytic" activity of the lysates or to "tumortrophic" property of the parasites.

HAUSCHKA & GOODWIN<sup>7</sup> demonstrated that neither of these properties exist and if there is some tumor inhibition it is due to the general weakness of the animals.

No reference is made to the possible action of the parasites and/or its products upon the cellular division.

In the present work we have tried to investigate, quantitatively, the probable in-

fluence of the trypanosomiasis *cruzi* on the regeneration of the hepatic cells of rats submitted to a partial hepatectomy.

### MATERIAL AND METHODS

Forty four females Wistar-albino strain rats, 22 days old and with a mean weight of 28 g were selected and maintained on the same standard diet; 12 were inoculated intraperitoneally with 0.1 ml of rat's blood containing 210,000 *T. cruzi* of the Y strain isolated by PEDREIRA DE FREITAS and maintained in our Department through successive reinoculations; 12 days latter, parasitemia was present in all animals and they were then submitted to partial hepatectomy.

Under ether anesthesia and without strict asepsis half of the right lobe of every animal was resected. The surgical incision was closed with cotton thread in a continuous suture; 12 others animals not inoculated, were submitted to the same operation, a third group of 10 rats was only inoculated with the same strain and the same quantity of parasites and 10 others animals were kept as controls.

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The animals of each of the first two groups were beheaded after the hepatectomy on the following order: 2 after 1 day, 1 after 2 days, 3 after 3 days, 4 after 4 days and 2 after 5 days. Five hours before the sacrifice, the animals received intraperitoneally an injection of 0.1 ml of colchicine.

All the animals were killed at identical time, i.e., between 1 P.M. and 2 P.M.

The animals that received only inoculation were sacrificed after the 9<sup>th</sup> and 14<sup>th</sup> day.

The rats kept as controls were killed all at the same day.

The animals of this two latter groups were beheaded at 2 P.M. five hours after receiving an intraperitoneal injection of 0.1 ml of colchicine.

The organs were fixed in 10% buffered Formaline solution.

From the livers two sections were taken, one from the right and another from the left lobe.

The sections, 3  $\mu$  thick, were cut in a cryostat and stained by H. & E.

The mitosis and binucleate cells were counted in 1,000 cells in every section of

every lobe. For the analysis of the results of this work the mean between the two lobes was considered.

## RESULTS

The results of our experience are summarized on the Tables I and II.

Through the application of the *t* test we could conclude that the difference between the mean number of binucleate cells in normals (A) and inoculated rats (B) is significant ( $t = 3.05$ ;  $\alpha = 5\%$ ).

The same difference between the groups of hepatectomized (C) and hepatectomized + inoculated animals (D) is not significant ( $t = 1.56$ ;  $\alpha = 5\%$ ) (Table I). It seems that the infection with *T. cruzi* stimulates the amitotic division of the liver cells of normal rats and perhaps that of the hepatectomized too.

The mean number of mitosis presented a very great variation in all groups and we must use a non parametric test for two independent populations (Wilcoxon test) to test the differences between two groups.

TABLE I  
Number of binucleate cells/1,000 hepatic cells

A) Normals	B) Inoculated	C) Hepatectomized	D) Hepatectomized + inoculated
58.5	102.0	90.5	108.0
59.5	57.5	50.0	89.0
75.5	118.5	76.0	128.0
62.5	76.5	158.0	128.0
54.0	98.5	111.5	125.0
70.5	102.5	65.0	99.0
50.5	78.0	126.0	176.0
66.5	85.0	89.5	122.5
78.5	76.5	84.0	82.5
88.5	76.5	63.5	56.5
		83.0	155.0
		79.5	143.0
66.5 $\pm$ 3.7 *	87.2 $\pm$ 5.7 *	89.7 $\pm$ 7.1 *	117.7 $\pm$ 6.4 *

\* Standard error of the mean

TABLE II

Number of mitosis/1,000 hepatic cells

A) Normals	B) Inoculated	C) Hepatectomized	D) Hepatectomized + inoculated
25.5	1.0	0.0	18.0
7.0	9.5	0.5	0.5
6.0	2.0	23.0	18.0
2.5	4.5	2.0	12.0
1.0	3.5	2.0	11.0
41.0	0.5	0.5	2.5
13.0	5.0	16.0	10.0
14.5	2.5	1.5	4.5
6.5	1.5	1.0	3.5
14.0	2.0	0.5	1.5
		2.5	18.0
		2.5	5.5
M* = 10.0 Q** = 5.00	M* = 2.25 Q** = 1.375	M* = 1.75 Q** = 1.00	M* = 7.75 Q** = 4.75

\* Median

\*\* Semi interquartil range

The comparison between the group *a* and *b* (Table II) is significant (value observed = 71;  $\alpha = 5\%$ ).

The same significance is observed between the groups *c* and *d* (Table II), (value observed = 112;  $\alpha = 5\%$ ). It seems that the infection with *T. cruzi* reduces the mitotic division of the hepatic cells of intact rats and increases that of hepatectomized animals.

In general what we have found has been a stimulation of the parenchymal cells reproduction in the animals with infection and partial hepatectomy.

#### DISCUSSION

The increase in the number of mitosis and binucleate cells is accepted by the majority (FISHER & SAFFER<sup>4</sup>; FUCHS et al.<sup>5</sup>; McDONALD et al.<sup>11</sup>; PASCHKIS et al.<sup>12</sup>; POPPER & SCHAFFNER<sup>13</sup>; BRUES & MARBLE<sup>1</sup>) as a good index of hepatic cellular regeneration. Discordant opinions have appeared more recently.

The discussion of this subject is beyond of the scope of this work, but we can con-

clude with FISHER et al.<sup>4</sup> that the great variation in the number of mitosis decreases the value of this parameter in the appreciation of the hepatic parenchyma regeneration.

#### RESUMO

*Modificações na quantidade de mitoses e células binucleadas no fígado de ratos, parcialmente hepatectomizados, com doença de Chagas experimental*

Ratos Wistar parcialmente hepatectomizados e infetados com *Trypanosoma cruzi* apresentam maior número de células binucleadas e de figuras de mitose no parênquima hepático do que ratos unicamente hepatectomizados.

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