Modifications of Phagocitary Activity by Splenic Cells of Rat Carriers of Sarcoma, Isolated *in vitro*

Following upon observations made during previous work[1-3], I have observed that rat carriers of big Galliera sarcoma show a considerable increase of spleen, reaching sometimes up to five times the bulk of the spleen of normal rats.

Analogous observations have been made by others[4-6] on animal carriers of tumors, and WINN demonstrated that spleen of animal carriers of tumors produces a cellular antibody that is specific for the tumor. Histological modifications of the spleen of rat carriers of Galliera sarcoma consist in hyperplasia and hypertrophy of the reticulo-endothelium, whose cells sometimes show phagocitated granules and sometimes vacuoles in cytoplasm; thickening of the connective tissue of the spleen was also observed.

On the basis of such observations, possible functional modifications of the cells of the spleen of rat carriers of Galliera sarcoma were investigated studying the phagocitary activity of the splenic cells isolated *in vitro*, according to the technique described by SHEPARD[7-9] and duly modified.

Material and Method. I used for this research 30 rats having an average weight of about 230 g each, divided into two groups. The first (control) group consisted of 15 normal rats; the second group of 15 rats, carriers of big non-ulcered sarcoma. After having killed each animal by beheading and further bleeding, I took away the spleen, whose total weight was controlled. Furthermore I homogenized 0.5 g of splenic tissue in 10 ml of sterile physiological solution of NaCl; I then put the homogenates in sterile test-tubes, for a spontaneous sedimentation of 5 min, in order to remove the non-homogenated tissue. The supernatant was centrifugated for 90 sec at 300 *g*, in order to collect only splenic cells, without erythrocytes; the cells were suspended in 10 ml of sterile physiological solution and centrifugated a second time at the same speed for 90 sec. The pureness of the cells isolated *in vitro* was controlled by microscope. The splenic cells were then suspended in 2 ml of sterile physiological solution in order to study their phagocitic activity. In a sterile test-tube I put 0.5 ml of suspension of splenic cells with 0.5 ml of fresh guinea pig complement and with 7 ml of a fresh suspension of *Staphylococcus pyogenes aureus*, Oxford stock; after mixing, I put the test-tube in a thermostat at + 37° C for 3 h, having found in the preliminary tests that such period of incubation was the optimal.

Furthermore I made slides of the cells, which were fixed by heat and stained by Gram's method. For each experience I counted the number of cells having phagocited one or more staphylococchi, on a total of 400 cells, and calculated the average percentage for each experiment.

	Controls	Rat carriers of Galliera sarcoma
	mg	mg
Average weight of spleen	926 ± 45	3369 ± 1316
Phagocitic activity	4.1% ± 0.9	11.2% ± 1.8

The Table refers to the average data of the total weights of the spleens and of the phagocitic activity of the splenic cells of controls and of rat carriers of sarcoma. As regards the dry weights of spleens, I found no difference between the normal rats and the rat carriers of sarcoma.

Conclusion. The results of this research permit the conclusion that the phagocitic activity of the splenic cells of rat carriers of Galliera sarcoma, isolated *in vitro*, is much greater than the phagocitic activity of the splenic cells of normal rat, as observed histologically.

The hypothesis that appears the most probable to explain the increase of the spleen and the increase of phagocitic activity of splenic cells, is that the tumor produce one or more substances (probably also antigens) stimulating the reticulo-endothelial tissue and connective tissue, of which the considerable increase of bulk may be considered responsible.

Riassunto. L'autore ha osservato che l'attività fagocitaria delle cellule spleniche di ratto portatore di sarcoma Galliera, isolate *in vitro*, risulta sensibilmente aumentata rispetto a quella delle cellule di milza di ratto sano. La significatività dei risultati viene accertata calcolando la '*t*' di Fisher.

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Macromolecular Hypertension: Hypertensive Cardiovascular Disease from Subcutaneously Administered Polyvinyl Alcohol[1]

It has recently been reported from this laboratory that rats, treated subcutaneously with methyl cellulose, develop ascites, edema, hypertension, foam cells within glomeruli, glomerulonephritis, and a variety of arterial and arteriolar lesions[2,3]. The syndrome may be dependent upon impairment of glomerular circulation caused by accumulation of methyl cellulose within glomerular endothelial and epithelial cells. Such intraglomerular sequestration of methyl cellulose has been described by HUEPER[4,5] and others[6,7], although these investigators did not report the induction of hypertensive cardiovascular disease thereby.

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