

**TIME RELATED RETICULOENDOTHELIAL FUNCTION
IN NORMAL AND TUMOR BEARING RATS**

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Abstract — Authors measured the activity of the reticuloendothelial system (RES) in healthy and tumor bearing rats with subcutaneous fibrosarcoma by the clearance of the radioactive colloid from the abdominal cavity. After the seventh day of incubation the relative activity of the colloid in the liver of the control group was $35\% \pm 8\%$, in the spleen $2.8\% \pm 1.3\%$ and in the mediastinal nodes $1.6\% \pm 1.9\%$ of the total activity in the animal. Time related accumulation exerted constant accumulation of the colloid in the liver and mediastinal nodes till the seventh day of incubation, while in the spleen the activity remained unchanged. Significantly elevated activity was found in the liver ($55\% \pm 5.7\%$ versus $35\% \pm 8\%$, $p = 0.01$) and in the mediastinal nodes ($4.7\% \pm 1.2\%$ versus $1.6\% \pm 1.9\%$, $p = 0.01$) in tumor bearing rats when compared with the control group, while the activity in the spleen was not significantly changed ($2.9\% \pm 0.4\%$ versus $2.8\% \pm 1.3\%$, $p = 0.5$). The activity per gramme of the tissue (A/g) of the tumor bearing rats in comparison to the control group was elevated in the liver ($4.4\%/g \pm 0.7\%/g$ versus $2.6\%/g \pm 1.4\%/g$, $p = 0.01$), while in the spleen the A/g was decreased ($1.5\%/g \pm 0.3\%/g$ versus $2.6\%/g \pm 0.8\%/g$, $p = 0.01$).

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Introduction — Reticuloendothelial system (RES) has many important functions in both physiological and pathological conditions (2, 3, 6). The function of this system has been evaluated mostly by the clearance of the injected colloid from the blood and uptake of the colloid in various organs after intravenous injection (4, 5).

Tumor transplantation in experimental animals results in a rapid increase in the weight of the spleen, and changes in RES activity (7, 8, 9, 10). Recent data also indicate that the reticuloendothelial phagocytic capacity in rats with different degrees of intra and extrahepatic tumor load is altered (6).

In our study we tried to evaluate the RES function by measuring the colloid accumulation of different organs in experimental animals after intraperitoneal injection of the colloid. The incubation time was prolonged to seven days in order to evaluate the time related uptake of the colloid and possible differences in the colloid uptake between the normal and fibrosarcoma bearing rats.

Material and methods — In the experiment four months old inbred Wistar rats were used. Animals were kept in standard laboratory conditions and fed ad libitum on rat pellets and water.

The control group consisted of twenty healthy rats and the tumor bearing group of fifteen rats with fibrosarcoma. The tumor was transplanted subcutaneously dorsolaterally by a subcutaneous passage of the tumor which was originally 3-methylcholantrene induced fibrosarcoma. RES activity measurements were performed 30 to 36 days after the tumor transplantation, when the mean tumor weight was $31\text{ g} \pm 19\text{ g}$ (range 5.7 g to 69 g).

The test substance for the RES function was commercially prepared ^{199}Au colloid (The Institute Boris Kidrič, Vinča). Animals in the experiment were injected intraperitoneally with 0,5 ml colloid solution (activity from 7,4 MBq to 18,5 MBq, depending on the incubation time). The minimal incubation time was 60 minutes and subsequently the animals were killed by cervical dislocation after the first, second, fourth and seventh day.

Incubation time	Relative activity in different organs (A. M. \pm 1 S. D. in %)					
	liver		spleen		mediastinal nodes	
	control	tumor	control	tumor	control	tumor
60 min.	15 \pm 9	10.4 \pm 2.7	0.5 \pm 0.2	1.3 \pm 0.5	0.7 \pm 1.3	0.8 \pm 0.6
1st day	21 \pm 5	28 \pm 3.5	2.6 \pm 1	3.2 \pm 1.8	0.6 \pm 0.1	1.5 \pm 0.6
2nd day	29 \pm 8	27 \pm 6	3 \pm 0.6	2.9 \pm 1.4	1.1 \pm 0.6	2.6 \pm 1.8
4th day	34 \pm 11	41 \pm 2	2.8 \pm 1.7	3.2 \pm 0.6	2.1 \pm 0.9	3.1 \pm 1.1
7th day	35 \pm 8	55 \pm 6	2.8 \pm 1.3	2.9 \pm 0.4	1.6 \pm 1.9	4.7 \pm 1.2

Table 1 — Relative activity of different organs (% of the total animal activity in the organ) in control group (Control) and in the tumor bearing rats (Tumor) related to the length of incubation

Tabela 1 — Relativna aktivnost različnih organov (% celotne aktivnosti živali v organu) kontrolne skupine (Control) in podgan s tumorji (Tumor) v odvisnosti od časa inkubacije

Group	No. of animals	Relative weight (A. M. \pm 1 S. D. in %)	
		liver	spleen
Controls	20	4.8 \pm 0.7	0.36 \pm 0.05
Tumor bearing animals	15	4.6 \pm 0.6	0.9 \pm 0.3

Table 2 — Relative weights of the liver and the spleen of the tumor bearing rats (as % of the total body weight) in comparison to the control group

Tabela 2 — Relativna teža jeter in vranice (% celotne teže živali) kontrolne skupine in skupine podgan s tumorji

The radioactivity of various organs and total activity of the animals were measured on the scintillation counter (EKCO Electronics, type N 559 D). Dissected organs were previously washed by rinse solution in order to remove the colloid that was stuck to the organ, washed, and the activity per gramme (A/g) of the tissue was calculated.

Results — The distribution of the colloid injected into the abdominal cavity of the animal was measured in different organs after 60 minutes, one, two, four, and seven days of incubation. Colloid injected into the abdominal cavity remained at first stuck to the abdominal fat and further it was distributed into other organs of the rat. The highest radioactivity was detected in the liver, spleen and mediastinal nodes. In the remaining organs and in the tumor the activity was low, and it was presumed that the activity was caused by contamination and not by uptake (Table 1).

Relative weights of various organs were calculated. The tumor bearing rats had

significantly elevated relative spleen weight ($p = 0.01$), while the relative liver weight in this group was not changed in comparison to the control animals (Table 2).

The difference between both groups of animals in uptake of the colloid in the liver, which became evident after two days of incubation, was further increasing till the seventh day of incubation ($p = 0.01$). No difference in colloid uptake in the spleen could be detected between control and tumor bearing rats, $p = 0.5$ (Fig. 1).

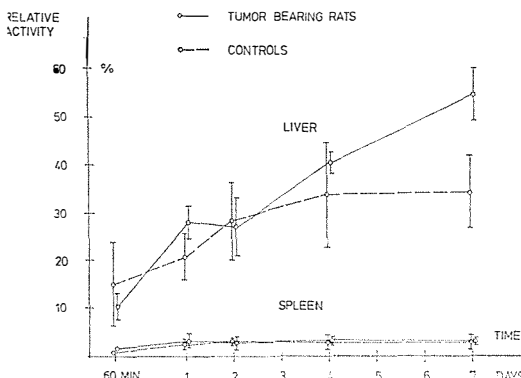


Fig. 1 — The relative uptake of the colloid in liver and spleen (as % of the total body activity) of both experimental groups of animals

Slika 1 — Relativna aktivnost jeter in vranice (% celotne aktivnosti) obeh skupin poskusnih živali

The A/g of the liver was elevated in the tumor bearing rats compared to the normal rats because of the higher relative liver activity and unchanged relative weight. The difference was significant after the seventh day of incubation ($p = 0.01$). The tumor bearing rats had significantly decreased

A/g of spleen tissue, when compared with control animals, due to elevated relative spleen weight and unchanged colloid uptake, $p = 0.01$ (Fig. 2).

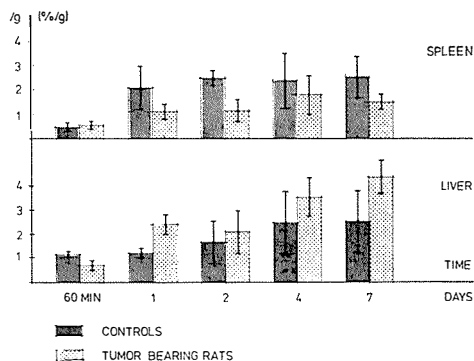


Fig. 2 — Time related uptake in the liver and spleen per gramme of the tissue (A/g) in normal and tumor bearing rats

Slika 2 — Aktivnost na gram tkiva v jetrih in vranici (A/g) v obeh poskusnih skupinah živali

The colloid was accumulated also in the mediastinal nodes and the uptake was time related in both groups of animals. Tumor bearing rats had significantly elevated values of colloid uptake when compared with the control group, $p = 0.01$ (Table 1).

Discussion and conclusion — According to our results liver colloid uptake in normal rats is completed after two days incubation. In comparison to the control group the tumor bearing rats accumulated colloid in the liver without saturation through seven days of incubation. It is presumed that the colloid is accumulated in lysosomes of Kupffer and endothelial cells of the liver (1). These data were confirmed also by our electron micrographs. Therefore, it could be predicted that altered activity in the liver of the tumor bearing rats is due to changed activity in previously mentioned cells. Different histological types of tumors influence the RES activity in different ways. According to our results, subcutaneously growing fibrosarcoma is influencing the liver RES activity by increasing its capacity to accumulate the colloid.

Decreased A/g spleen tissue uptake is the result of unchanged relative spleen acti-

vity and elevated relative spleen weight in tumor bearing rats, compared with control group. In the process of the neoplastic growth spleens of tumor bearing rats enlarge due to hyperplasia of the white pulp and increased number of lymphocytes and big mononuclear cells (10). We assume that subcutaneously growing fibrosarcoma exerts in these experimental conditions a suppressive effect on the activity of these cells. With this mode of action the tumor exhibits its complex influence on the activity of RES system in the spleen and in the liver.

In future it would be necessary to investigate the high uptake of the colloid in mediastinal nodes. Probably the colloid is accumulated by lymph influx from the abdominal cavity. Higher relative lymph node activity in tumor bearing rats may be a consequence of accelerated inflow and scavenging of the abdominal fluid.

Izveleček

AKTIVNOST RETIKULOENDOTELIALNEGA SISTEMA ZDRAVIH PODGAN IN PODGAN S TUMORJEM

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Avtorji so v raziskavah merili aktivnost retikuloendotelialnega sistema (RES) v zdravih podganah in podganah s podkožnim fibrosarkomskim tumorjem z merjenjem razporejanja vbrizganega koloida v trebušno votlino živali. Po sedmih dneh inkubacije je bilo v jetrih kontrolne skupine izmerjeno $35\% \pm 8\%$, v vranici $2.8\% \pm 1.3\%$ in v mediastinalnih bezgavkah $1.6\% \pm 1.9\%$ relativne aktivnosti. V jetrih in mediastinalnih bezgavkah je relativna aktivnost od prvega do sedmega dne inkubacije stalno naraščala, medtem ko se relativna aktivnost v vranici ni spreminjala. Skupina podgan s podkožnim fibrosarkomom je imela po sedmih dneh inkubacije v primerjavi s kontrolno skupino značilno povišano relativno aktivnost jeter ($55\% \pm 5.7\%$ proti $35\% \pm 8\%$, $p = 0.01$) in mediastinalnih bezgavk ($4.7\% \pm 1.2\%$ proti $1.6\% \pm 1.9\%$, $p = 0.01$), medtem ko v vranici ni bilo značilnih sprememb ($2.9\% \pm 0.4\%$ proti $2.8\% \pm 1.3\%$, $p = 0.5$). Zaradi povišane relativne aktivnosti jeter skupine podgan s fibrosarkomom je povišana tudi aktivnost, preračunana na gram tkiva organa (A/g), v primerjavi s kontrolno skupino ($4.4\%/\text{g} \pm 0.7\%/\text{g}$ proti $2.6\%/\text{g} \pm 1.4\%/\text{g}$, $p = 0.01$). Pri podganah s fibrosarkomom je bila A/g vranice $1.5\%/\text{g} \pm 0.3\%/\text{g}$ in je bila značilno nižja od A/g kontrolne skupine ($2.6\%/\text{g} \pm 0.8\%/\text{g}$, $p = 0.01$).

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