# Pre-Clinical Studying of Antitumor Ramon Preparation: Specific Activity

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**Abstract**—In article the data of pre-clinical researches of Ramon preparation is described. Antitumor activity of Ramon has been studied on 19 strains of transplantated tumors of different hystogenesis.

*Keywords*—Cancer, antitumor activity, pre-clinical testing, anthraquinones, phytopreparation, Ramon.

#### I. INTRODUCTION

NATURALproducts remain an important source of new drugs, new drug leads and new chemical entities. The plant based drug discovery resulted mainly in the development of antitumor agents including plants, marine and microorganisms. Beside this there is numerous agents identified from plants can used in anticancer therapy.

Anthraquinones are natural compounds that are ubiquitously in plants. They have been shown to possess a variety of biological activities at nontoxic concentrations in organisms. Compelling data from human clinical trials indicate that natural anthraquinones have important effects on cancer chemoprevention and chemotherapy. Many mechanisms of action have been identified, including carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance or a combination of these mechanisms. Based on these results, natural anthraquinones may be promising anticancer agents [1].

#### II. RESULTS AND DISCUSSION

Antitumor therapeutic course has been developed for animals with transplantated tumors and has provided tumor growth retarding to 83 to 89%.Ramon appeared to be rather active against 4of 7 studied transplantated substrains and 1.3 to 2.1 times more active than the best of control preparations for each of three substrains resistant to these preparations therewith four of such substrains have been studied. Ramon has as well been proved to be highly active against cells of bladder tumors and ones of human gastrointestinal tract being transplantated to animals in special camera [2].

Lethal dozes for mice in conditions of single intraperitoneal injection of Ramon 2% water solution were:  $LD_{10}=345$  mg/ml;  $LD_{50}=610$  mg/ml;  $LD_{99}=916$  mg/ml. Toxicity of the preparation didn't depend on sex of animals. Under the same conditions lethal dozes for rats came to:  $LD_{10}=320$  mg/ml;  $LD_{50}=530$  mg/ml;  $LD_{99}=940$  mg/ml.

Suggested materials present clinical picture of acute and chronic poisoning with Ramon and resulting methabolic disorder, pathomorphological picture of viscera, changes in hormone status of organism, preparation influence over diuresis, cardiovascular system and detoxicizing function of liver.

Tolerable and toxic single and coarse dozes of 5 to 10%Ramon solutions has been proved to be established for rats, rabbits and dogs therewith the preparation was being injected intravesically. Character and dynamics of pathomorphological changes in bladder mucosa, ureters, testicles and uterus has been elicited [2].

Three medicinal Ramon forms and seven therapeutic courses were tested against Plisslymphosarcoma model (4 to 5 day of injection after the tumor transplantation; the tumor diameter -8 to 12 mm; interval between injections -24 to 96 hours) (see Table I).

TABLE I GROWTH RETARDING OF PLISSLYMPHOSARCOMA OF RATS WITH VARIOUS MEDICINAL FORMS OF RAMON

WITH VARIOUS MEDICINAL FORMS OF RAMON					
Medicinal	The doze mg/kg		Tumor growth retarding		
form			Mean weigh of tumors at		Inhibi-
			the end of t	rial, g (M±m)	tion
	single	total	control	preparation	
2% water	35	350	20.9±1.6	5.7±0.9	72.7
solution of	40	400	26.4±1.8	2.70±0.30	89.9
Ramon-	45	450	26.4±1.8	2.00±0.15	92.4
substance	50	500	31.7±4.6	1.20±0.06	96.2
	90	450	32.4±4.3	6.9±1.4	78.7
	120	460	38.8±7.4	14.6±1.8	62.4
	150	450	44.3±4.9	28.9±3.1	34.7
2% solution	40	400	17.1±2.3	3.9±1.2	77.2
of Ramon-	50	500	17.1±2.3	3.1±1.0	81.9
lyo in 5%					
glucose					
10% solution	40	400	15.9±2.1	2.7±1.0	83.4
of Ramon-	50	500	15.9±2.1	1.30±0.04	90.7
lyo in 5%					
glucose					

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## International Journal of Medical, Medicine and Health Sciences ISSN: 2517-9969 Vol:7, No:8, 2013

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All the medicinal forms appeared to show high therapeutic effect equally optimal for them in the following therapeutic course: one-time doze -40 mg/kg, course doze -400 mg/kg daily for 10 days. In the case when treatment started 4 to 5 days after tumor transplantation its growth was retarded to 83 to 89% and none of animals were lost. Time of treatment starting has definite meaning because in the foregoing case the results were 20 to 30% higher than when treatment started 7 to 8 days after transplantation [2].

Histological study showed Plisslymphosarcoma of rats even after Ramon injection exhibiting morphological changes of tumor tissue: cells became shallow, rounded with hyperchromaticnuclea, narrowly neighboring each other. One could watch dystrophy centers in cells, rhexic and pycnoticnuclea, vacuolised cytoplasm, necrotic centers in comparison with control, strip-like eosinophyllycoloured structures with extended nuclea, slightly marked stroma. After six injections necrotic and dystrophic centers increased, cells became fine, polymorphic, incompactly situated. Fine reticular network was seen in necrotic centers. Stroma was slightly developed, its circulatory vessels dilated and polyhemic, walls thickened. After treatment course completing one could watch wide centers of necrosis and dystrophy in tumor tissues with fine, rarely situated cells and hyperchromicnuclea; nuclea discomplexation; a lot of pycnotic cells. DNA was revealed according to Felgen therewith its concentration was observed in nuclea of numerous cells. Nucleoproteids reducing in nuclea and their concentration at the edge of nuclear shell was observed. When nuclear membrane was broken nucleoproteids poured out and situated in intermediate substance as chromatinic blocks. Histological picture of tumors in the control case was peculiar for Plisslymphosarcoma strain [3]-[4].

Next table represents the results of comparative investigations of Ramon effect to the growth of mice transplanted tumors. Ramon was injected intraperitoneally. Study of tumor growth retarding reveals that the preparation exhibits statistically reliable antitumor activity against all of eight tried strains of tumors therewith maximal activity was marked against Sarcoma-180 (to 89%), solid Erlich tumor (to 84%) and asciteErlich tumor (to 82%) (see Table II).

TABLE II
AMON INFLUENCE TO THE GROWTH OF TUMORS TRANSPLANTATED TO
MOUNTHING

MOUNTHES						
Substrains	Doze, mg/kg		The result of the tumor		Tumor	
			measurement at the end		growth	
			of the tria	al (M±m)	retar-	
	sing.	total	control	preparation	ding, %	
Sarcoma 180	35	350	5.40±0.70	3.30±0.40	38.8	
(Croker)	45	450	2.44±0.55	0.46±0.05	88.8	
Erlich solid	35	350	0.83±0.01	0.36±0.003	56.6	
tumor	45	450	0.99±0.19	0.16±0.001	83.8	
AsciteErlich	35	260	8.10±1.20	5.80±1.00	28.4	
tumor	45	360	7.60±0.80	3.20±0.30	57.8	
Tumor cells	35	280	57.8±7.2	20.2±1.1	65.0	
	45	360	62.3±11.3	11.0±1.8	82.3	
Ascite sarcoma	45	360	10.6±1.4	5.30±0.80	50.0	
37	45	360	9.9±1.4	$5.80 \pm 0.80$	41.4	
Tumor cells	45	360	97.1±8.5	53.3±3.3	45.1	
Carcinoma NK	45	450	3.33±0.29	2.92±0.25	12.3	
Harding-Passi	45	450	$1.48\pm0.14$	0.90±0.12	39.2	
melanoma						
Hepatoma 22a	45	450	1.37±0.14	1.20±0.11	12.4	
Leucosis La	45	450	X=2.66	$X_{H} = 1.52$	X=1.22	

Histological study of the strains under Ramon therapy confirmed its antitumor effect. Sarcoma-180 standard animals consists of continuous block of undifferentiated, irregularly situated polymorphic cells. Nuclea are of various forms with clumply located chromatine. Mitosises are numerous, stroma is extremely slight. Sarcoma-180 cells treated mice are dystrophically changed and incompactly located. There are zones of marked pycnosis and lysis, large necrotic centers and a lot of connective tissue interlayers.

Solid Erlich tumor of control mice consists of slightly differentiated polymorphic cells therewith ones of rounded form prevail, they are compactly located with wide, locally vacuolized cytoplasm, rounded and longitudinal nuclear with visible chromatine granules. Tumor stroma is slightly developed. Tumor tissue of Ramon treated mice is preserved in small region as sharply dystrophically changed tumor cells many of which are in condition of pycnosis and lysis, necrotic zones are visible in the centre [4].

The results of comparative study of Ramon influence to the growth of tumors transplantated to rats are represented in next table. The preparation is showed to reveal statistically proved antitumor activity against four of seven-studied tumor strains according to the test of tumor growth retarding. The highest effect is against alveolar liver cancer PC-1 (to 70%),Herene carcinoma (to 63%),Woker carcinoma (to 65%)[see Table III].

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Substrains	Doze, mg/kg		Results measure end of tr	Tumor growth retarding,	
	single	total	control	preparation	%
Alveolaric	30	300	29.9±2.1	14.3±0.96	52.2
cancer of liver	40	400	51.2±9.4	15.2±3.2	70.3
Herene	30	300	45.1±7.7	36.0±5.3	20.0
carcinoma	40	400	35.9±4.2	13.4±1.9	62.7
Sarcoma 45	35	350	21.9±3.8	11.6±0.11	47.2
	40	400	19.5±4.7	9.2±2.1	52.8
Wokercarcinos arcoma	40	400	16.6±3.0	5.8±0.8	65.0
Mammary gland cancer	40	400	21.6±4.9	18.0±3.2	16.7
Ovary tumor	500	5000	39.2±4.7	29.6±0.5	24.5
Zaidelascitehep atoma	500	5000	28.7±4.9	25.1±2.6	16.0

 TABLE III

 RAMON INFLUENCE TO THE GROWTH OF TUMORS TRANSPLANTATED TO RATS

After three injections (36.4% of tumor growth retarding) tumor tissue of PC-1 consisted of rounded, polymorphic, incompactly located cells; there appeared cells with signs of dystrophy where nuclear substance was changed, rough and irregularly dispersed; cells with light nuclea and small amount of DNA; picnotic cells and small amount of cells subjected to cariorhexis. Fat filled out cytoplasm of numerous cells with shallow drops. RNA was preserved in cytoplasm of the most part of cells in considerable concentration. Being silvered argyrophylicfabers became disfibrous, thickened therewith intensity of black colour reduced [4].

After six Ramon injections (52.4% of tumor growth retarding) dystrophic changes enlarged. Numerous cells with pycnoticnuclea cytoplasm as thin rim as well as hyperchromic ones containing large amount of DNA. When nuclear shell was broken nucleoproteids situated out of cells. Connective stroma locally thickened and mainly consisted of fuchsilecoloured hyperemic blood vessels.

The day after ten Ramon injections (70.3% of PC-1 growth retarding) wide necrotic centers were observed, tumor capsule consisted of not wide fuchsilecoloured faber layer with fibroblasts and fibrocytes between them. Blood vessels were dilated and hyperemic. Stroma strands extended from the capsule formed barriers. Cellules were enlarged, stroma barriers thin and broken here and there. Local thickening of stroma barriers developing from thickened collagen fabers was observed. When impregnated with silver fabers lost colour intensity, fragmentized and disappeared at some places at all. There remained few tumor cells being dystrophic, extremely pycnotic, hyperchromic and intensively coloured according to Felgen. Cells-"shadows" could have been met with lysicnuclea. Large areas of tumor were necrotic; therewith cytoplasm was fattily depressed and fatty drops normal [2].

Ramon antitumor activity against Plisslymphosarcoma and sarcoma-45 of rats and then sarcoma-180 and Erlich solid tumor of mice was compared with one of sarcolysine, cyclophosphane, thiophosphamide, 6-mercaptopurine, methathrexate (see Table IV).

	TADL					
OR GROWTH BY RAMON AND COMPARATIVE PREPARATIONS (IN %)						
paration; single	Plisslymp	Sarcoma	Sarcoma	Erlich		
loze mg/kg	ho-	45	180	solid		

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doze, mg/kg	ho-	45	180	solid
	sarcoma			tumor
Ramon; 40	92.3	52.5	80.3	84.6
Sarcolysine; 3	38.1	98.2	31.4	25.8
Cyclophosphane; 20	59.4	88.4	51.6	16.3
Thiophosphamide; 20	42.0	85.6	42.2	40.6
6-Mercaptopurine; 35	21.2	7.2	59.5	35.2
Methathrexate; 4	57.6	49.6	32.7	-
Vinblastine; 0.2	48.5	62.1	59.1	27.2

Preparations were being injected intraperitoneally in maximal tolerable dozes daily in 10 days. The table presents mean results of two-three series of trials. Ramon effect against three of four investigated strains appeared to be 1.3 to 2.1 times as more as one of the best comparative preparation against the corresponding strain.

The next tables presents the results of comparative study of Ramon and five other preparations activity against substrains of Plisslymphosarcoma and sarcoma-45 of rats resistant to rubomycine (one of two investigated substrains appeared to be suppressed by Ramon twice as actively as by each of five comparative preparations); the results of comparative study of Ramon and sarcolysine activity against Wokercarcinosarcoma substrain resistant to sarcolysine (Ramon 78.8% effect seemed to be four times as more as one of sarcolysine); the results of study of Ramon activity against of rats ovary tumor resistant to antitumor preparations and transplantated to abdominal cavity (two of three tested substrains appeared to be 2.8 and 2.3 times as less resistant to Ramon as to the respective preparation; way of injection: Ramon - through the mouth, 6sarcolysine or mercaptopurine, thiophosphamide subcutaneously) (see Tables V-VII).

 TABLE V

 GROWTH RETARDING OF SUBSTRAINS RESISTANT TO RUBOMYCINE

 (PLISSLYMPHOSARCOMA AND SARCOMA-45 OF RATS) WITH RAMON AND COMPARATIVE PREPARATION (IN %)

 Preparation
 Mean tumor weigh at the end of trial, g; tumor growth retarding, %

 Plisslymphosarcoma
 Sarcoma-45

	retarding, %					
	Plisslym	ohosarcoma	Sarcoma-45			
	Initial Substrain		Initial	Substrain		
	strain	resistant to	strain	resistant to		
		rubomycine		rubomycine		
Control	38.90±7.20	42.30±5.69	17.80±2.63	19.80±4.24		
Ramon (45 to	8.90±1.26	12.60±1.28	11.0±1.67	12.0±2.03		
450 mg/kg)	77.1%	70.2%	38.2%	39.3%		
Rubomycine	7.50±0.56	33.40±3.66	4.60±0.48	15.60±3.44		
(4 to 40	80.7%	21.0%	74.1%	21.2%		
mg/kg)						
Sarcolysine	26.70±3.41	31.10±4.66	3.50±0.50	4.20±1.03		
(2 to 20	31.3%	26.4%	80.4%	78.7%		
mg/kg)						
5-Fluoro-	13.70±2.04	30.70±4.91	4.30±0.67	14.20±0.56		
uracil (25 to	62.7%	27.4%	75.8%	28.2%		
250  mg/kg						

TABLE VI GROWTH RETARDING OF SUBSTRAINS RESISTANT TO SARCOLYSINE (WOKER

	CARCINOMA OF KAIS) WITH KAMON					
Preparation	Group	Doze		Mean tum the end of tr growth re	or weigh at rial, g; tumor etarding, %	
		single	total	initial	resistant to sarcolysine	
Woker	Control	-	-	21.2±2.4	16.9±1.9	
carcinoma	Ramon	45	450	0.2±0.08	3.6±0.8 78.8%	
	Sarcolysine	2	20	0.95±0.03 95.5	13.4±1.2 20.7%	

TABLE VII GROWTH RETARDING OF SUBSTRAINS RESISTANT TO 6-MERCAPTOPURINE, SARCOLYSINE OR THIOPHOSPHANIDE (TUMOR OF RATS OVARY) WITH

		RAMON	
Substrain	Group	Mean number of tumor	Diminishing
resistant to:		cells in the ascite	of tumor cells
		liquid, millions	number, %
Initial	control	4837.8±407.8	-
strain of	Ramon	3998.0±430.1	17.3
ovary	(500 to 5000		
tumor	mg/kg)		
6-	control	348.4±14.8	-
mercapto-	6-mercapto-	297.7±15.1	14.5
purine	purine		
(22 to 220	Ramon	231.6±22.6	33.5
mg/kg)			
Sarcolysine	control	308.8±7.9	-
(2 to 20	sarcolysine	277.4±9.3	10.1
mg/kg)	Ramon	221.9±19.4	28.1
Thiophosph	control	1331.6±150.4	-
amide	thiophosp-	710.8±91.1	46.6
(1 to 10	amide		
mg/kg)	Ramon	701.2±83.4	47.3

Ramon effect against human tumors transplants was studied in diffusive cameras. Millicelled filters Synpor with cell diameter 0.1 to 0.3 mmc were used after three-fold boiling in distilled water, 30 minutes sterilization in ethanol heated to 70% and 15 minutes UV-exposure. The tumor tissue was put into sterile vial and crushed by scissors for 3-5 minutes.

Then vile was filled with medium 199 and tissue cellular was isolated from none-crushed residue by its filtration throughout several-layered sterile gauze. Millicelled filters 2x3 cm were put to the bottom of sterile Petri dish filled then with cellular suspension. Cells subsided to the filter for 30 minutes and attached the base. Slightly wetted filters were glued by acetonic glue and sewed in white mongrel rats intraperitoneally (three cameras per animal). Preparation efficiency was evaluated by mytotoxic index growth, degenerating cells amount and explantates survival appraised in 5-ball scale [3].

Effect of Ramon against human bladder cancer was studied in 5 experiments (75 rats, 225 diffusive cameras). The preparation was injected in one-time doze 330 mg/kg intraperitoneally on the sixth day of tissue cultivating. The second and the third injections of Ramon were made in a day one after another (the summary doze 990 mg/kg).

Complete tissues necrosis is observed in 60% of diffusal cameras while in control experiments this happened in none of cameras.

In control survival in 15% of cameras was evaluated as 5 balls with none of cameras with such a survival in the experiment. When treated by Ramon cellular nuclea "swelled", chromatin fragmentated, cytoplasm vacuolised and amount of fissionable cells fell. Mitotic activity therewith was 1.8 times lower and amount of degenerating cells – 4.4 times more.

Effect of Ramon against esophagus cancer was evaluated with respect of 7 patients. Number of diffusal cameras with survival value 4 and 5 balls appeared to be 53.7% in control and 51% in the experiment.

Number of preparations valued with 2 balls is somewhat more and with 3 balls less in the experiment than in control. Mitotic index is 1.3 times lower (so as number of degenerating cells).

When Ramon was tested against human uterus body cancer number of degenerating cells was 1.4 times as more, mitotic index – 1.2 times as lower and number of diffusal cameras with survival value of explantants 1 and 2 balls – increased from 15.3% in control to 21.3% in experiment.

Antitumor activity of Ramon against cancer of human rectum was evaluated as follows: mitotic index 1.6 times as more, number of degenerating cells -1.8 times, number of diffusal cameras with survival value of cells 1 and 2 balls - increased from 15.1to 45.9% and number of completely necrotic explanators -3 times increased.

Data on Ramon activity against stomach cancer were the following: mitotic activity of cells was 2 times diminished, number of degenerating cells -1.4 times increased, number of diffusal cameras with complete necrosis -2.4 times increased and with survival value 1 and 2 balls -1.4 times increased.

So, human tumors localizations may be disposited according to their sensitivity to Ramon as follows: a) highly sensitive – bladder cancer; b) moderately sensitive – cancer of rectum and stomach; c) slightly sensitive – cancer of esophagus and uterus body.

### **III.** CONCLUSIONS

Ramon when injected intraperitoneally displays marked activity against a number of transplantated tumors of animals: growth retarding for Plisslymphosarcoma appeared to be 92%, for Erlich tumor and sarcoma-180 - 80 to 84%, for alveolaric slimy cancer of rats liver - 70%.

The identical results of high antitumor activity were obtained when aqueous solution of substantial Ramon and its lyophilized medicinal form in 5% glucose solution were used [5]-[6].

Histologically there were observed: developing of broad necrosis; dystrophy of tumor cells; lysis and pycnosis; blood vessels dilation in tumor tissue and their polyhemia, break and hemorrhage into stroma of neoplasms. Ramon efficiency depends on sensitivity of tumors to it, doze and interval between injections. Thus 10-multiple injection with 24-hours interval produces greater specific effect than 5-, 4-and 3-multiple injection (with 48-, 72- and 96-hours interval between intraperitoneal injections) [5]. The highest activity was observed when dozes were equal to 3/4 MPD.

## International Journal of Medical, Medicine and Health Sciences ISSN: 2517-9969 Vol:7, No:8, 2013

The growth retarding effect of Ramon in case of Plisslymphosarcoma and Erlich tumor was watched even when the start of treatment was postponed (to 7-th – 8-th day correspondingly). Growth inhibiting of Woker carcinoma, Herene carcinoma and P-388 came 65%. Ramon produced depressive effect against La-leucosis ( $k_s = 2.66$ ;  $k_H = 1.58$ ;  $k_L = 1.22$ ) while carcinoma NK, hepatoma 22a, cancer of mammary gland (RMC-1), ovary tumor, melanoma B-16, Garding-Passi melanoma, sarcoma-45, sarcoma-37, leukemia L-1210, adenocarcinoma-755 were only slightly sensitive to Ramon.

Spectrum of Ramon antitumor effect differs in experiments from one of alkylating agents (sarcolysine, cyclophosphane, thiophosphamide), antimetabolites (6-mercaptopurine, methathrexate) and alkaloids (vinblastine).

Ramon prevents lymphogenic metastasizes for Plisslymphosarcoma. Tumor substrain of sarcoma 45 resistant to sarcolysine was collaterally sensitive to Ramon. Distinct antitumor effect (70 to 85% of retardation) was revealed in experiments with rats against Plisslymphosarcoma resistant to rubomycine.

Ramon produced distinct antitumor effect against human bladder cancer when cultivated in diffusal cameras. The preparation caused complete tissue necrosis in 60% of cameras, depression of cells prolifirative activity, fragmentation or granular dystrophy of nuclear chromatine, cytoplasm vacuolization and marked decrease of fissionable cells amount. When comparatively studied effect of Ramon against cancer of bladder exceeds one of thiophosamide. Cancer of rectum and human stomach were moderatively sensitive to Ramon in conditions of diffusal cameras; cancer of endometry and oesophagus was slightly sensitive.

### IV. EXPERIMENTAL

Study of Ramon specific antitumor activity has been carried out with 980 mongrel white rats, 750 mongrel white mice, 125 mice of C<sub>3</sub>HA line, 190 mice of C<sub>57</sub>BI line and 260 hybrid mice BDP<sub>1</sub> to whom 19 strains of transplantated tumors of different hystogenesis and 10 substrains of these tumors resistant to antitumor preparations had been transplantated: sarcoma-45, Plisslymphosarcoma, Herene carcinoma. Wokercarcinosarcoma, alveolar mucous cancer of liver PC-1, rat mammary cancer CMR-1, Zaidelascitehepatoma (AHZ), rat ovary ascite tumor, sarcoma-180, ascite and solid Erlich tumors, lymphocytic leukemia P-388, lymphoid leukemia L-1210, lungs Lewis carcinoma (LLC), adenocarcinoma Ca-755, melanoma B-16, carcinoma HK, Harding-Passi melanoma, hemacytoblastos La, mouse hepatoma 22a.

Ramon was tried against various lymphogenioushematogen metastasizing transplantated tumors of white mongrel rats and mice: Plisslymphosarcoma, Wokercarcinosarcoma, Pliss carcinoma, Wokercarcinosarcoma, Herene carcinoma, Erlich tumor and lungs Lewis carcinoma being transplantated to animals by various methods.

Following tumors were used as medicinally resistant: Woker carcinoma resistant to sarcolysine, sarcoma-45 – to rubomycine, Plisslymphosarcoma – to rubomycine, 5fluorouracil and sarcolysine, three substrains of rat ovary ascite tumor – to 6-mercaptopurine, thiophosphamide and sarcolysine as well.

*Treatment of animals* was started when measurable tumor ganglions appeared and lasted for 10 days, in case of mice with ascite tumors – for 8 days. 2%Ramon solution was used, the preparation being dissolved in distilled water, lyophilized form (Ramon-lyo) – in 5% glucose solution apart from the particularly mentioned cases. All the trials were reproduced again to reveal results recurrence. Histological investigations were carried out by classic methods [3].

*Cutted pieces of tumor tissue* were fixed in neutral formalin, Karnua and Buen liquid and poured over paraffin. Sheares were coloured by hematoxiline-eosine, collagen fabers – according to van-Gizone, impregnation of argirophilicfabers – by the Foot method [4].

*Histochemical methods* used: revealing of DNA-granules according to Felgene therewith preparations without preliminary hydrolysis worked as reaction control; RNA revealing – by the Brasche method therewith sheares were treated by ribonuclease to control the reaction; neutral lipoids revealing – by colouring with sudan III [2].

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