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A study on the survival time, sex, weight and blood content of the Ehrlich Ascites Carcinoma involved tumour

V. Nagarjuna Reddy*, Nagarathna P. K. M and M. Divya

Department of Pharmacology, Karnataka College of Pharmacy Bangalore, Karnataka, India

Corresponding author*: 9naga9@gmail.com

Abstract

Experimental tumours have great importance in modelling, and Ehrlich ascites carcinoma (EAC) is one of the commonest tumours. EAC is referred to as an undifferentiated carcinoma and is originally hyperdiploid, has high transplantable capability, no-regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumour-specific transplantation antigen (TSTA). EAC resembles human tumours which are the most sensitive to chemotherapy due to the fact that they are undifferentiated and that they have a rapid growth rate. The ideal drug being ineffective or minimally effective for normal cells have been focused on, and at this point, the usage of natural sources as an alternative cancer therapy is thought to have a great value for cancer control and programs' destruction.

Key words: Carcinoma, transplantability, Blood content and Tumour

1. Introduction

1.1 Ehrlich Ascites Carcinoma

The intensive studies on the transplantable tumors were taken into consideration in the last 2 to 3 decades. The planned goal of that research was to improve new techniques especially for experimental tumors in animals that have been underlain at the basis of recent achievements in cancer therapy .Experimental tumors have great importance for the purposes of modeling, and Ehrlich ascites carcinoma (EAC) is one of the commonest. It appeared firstly as a spontaneous breast cancer in a female mouse[1,2], and then Ehrlich[3] used it as an experimental tumor by transplanting tumor tissues subcutaneously from mouse to mouse. In 1932,[4] obtained the liquid form in the peritoneum of the mouse and named it as "Ehrlich ascites carcinoma" due to the ascites liquid, together with the carcinoma cells[5].

1.2 Role of Inducing EAC In Blood Count

It has been shown previously[6] that the adult mice used at this Institute show either a short survival time and a very haemorrhagic tumour or a long survival time and a relatively as anguinous tumour, following the intraperitoneal injection of Ehrlich's ascites carcinoma. This negative correlation between the survival time and the blood content of the tumour is not dependent on the sex or weight of the mouse. As this tumour is said to grow progressively in almost all strains of mice[7]. It was decided to investigate the cause of this difference in survival time and blood content found in our mice.

2. Material And Methods

The mice used were taken from a closed colony of previously inbred white mice. Three groups of mice (I, II and III), each containing 15 males and 15 females, were numbered 1- 15 for each sex in each group; mice of the same sex and weight (to the nearest gram) in each group receiving the same number. In this way the mean starting weight of the animals in all 3 groups was the same, being 20- 8 g. (S.D. 1-237). Each of the mice was given one intraperitoneal injection of 0-1 ml. of Ehrlich's ascites carcinoma taken from a male mouse of the 70th transplant generation. This mouse, which had received the tumour 10 days before, had 9-5 ml. of tumour ascites which was slightly blood stained, with a tumour cell count of 2,050,000/cu. mm. The cells all appeared viable, i.e. they did not take up eosin from a 1: 2,000 solution of eosin in Tyrode's solution[8], they did not clump and showed no abnormalities in films stained with haematoxalin and eosin. When the injections were complete, about one hour after the donor mouse had been killed , the tumour cells in the remaining fluid were all still viable. Some of the mice were also given subcutaneous treatment as follows Group I-the control group-received no subcutaneous treatment. Group 11-the vitally stained group-were each given 0 5 ml. of a 0 5 per cent solution

of trypan blue in sterile distilled water, subcutaneously on the back. The first injection of trypan blue was given 7 days before the mice were injected with tumour ascites. The trypan blue injections were repeated 2 and 4 days later and thereafter weekly until all the mice in the group were dead. Group III-the cortisone treated group-received a suspension of cortisone acetate as a subcutaneous injection on the back. The dose was equivalent to 25 mg. /kg. Starting weight. The first injection was given 6 days before the mice were injected with tumour ascites. The cortisone injections were repeated daily until all the mice in the group were dead. Three additional control groups were set up (1, 2 and 3). Each of these groups consisted of 5 male and 5 female mice of similar age and weight to those in the three main groups. Each of these groups was given the same subcutaneous treatment as the corresponding Roman figure group (i.e. none, trypan blue, and cortisone, respectively), but none of the mice were injected with tumour ascites. The survival time of each mouse that died in every group was recorded. When the mice in the Roman figure groups died the tumour ascites was removed and 1 ml. of this centrifuged at 2,400 r.p.m. for 45 minutes. The volume of the red blood cells at the bottom of the Wintrobe tube was taken as an estimate of the amount of blood present/ml. and recorded as a percentage of the total volume in the tube.

2.1 Comparison of the growth of The Ehrlich Ascites Tumor in Male and Female Mice

Tumor from female mice was used to inoculate 32 female and 32male recipients (FF and FM treatment groups), and tumor from male mice was similarly used to inoculate 32 female and 32male recipients (MF and MM treatment groups). Eight mice from each treatment group were killed by cervical dislocation on each of the 5th, 7th, 9th, and 12th days after inoculation, and the total number of cells present, the volume of tumor, and the cell concentration were determined as follows (12, 17): After killing, the abdomen of each mouse was cleaned of fur and incised over a clean vessel. The volume of the fluid obtained (Vi) was immediately measured and the fluid diluted to a volume (F2) with isotonic saline in order to prevent coagulation. The Peritoneal cavity of the mouse was then irrigated thoroughly with isotonic saline until the return was clear, and the volume of the washing plus tumor obtained (V3) was recorded. Cell counts were then performed in duplicate, using a standard hemocytometer, on each of the fluids contained in the volumes Vi and T'3; these counts were designated C2 and C3, respectively.

Although for each mouse Inoculants of 10^4,10^5,10^6, and 10^7cells per mouse were used. An attempt was made to determine whether intraperitoneal tumor growth was inhibited in mice previously inoculated subcutaneously with tumor from donors of the same sex. Twenty male mice were divided into 2 groups of 10; one group was inoculated, using the subcutaneous tissue of the tail, with 10^8 tumor cells from the "male" tumor subline, while the other group was similarly inoculated with the same number of cells from the "female" tumor subline. Five mice from each group were then inoculated intraperitoneally 7 days later with 10^7 cells from the"male" subline, and the other 5 were similarly inoculated with10^7 cells from the "female" subline. Twenty female mice, divided into similar groups, were treated in the same way. The 8 combinations of sex of first donor, sex of second donor, and sex of recipient are best seen in Table 5. Control animals in this experiment received intraperitoneal but not subcutaneous tumor inoculation.

3. Results

Table I shows the sex difference in the survival time and blood content of the tumour ascites in the mice in groups I, I1 and III, all of which died. The table gives the values for the total, male and female series, with the number of mice, the mean value with S.D., the S.E. of the actual difference between the male and female means, and the t and P values for this difference.

Table I.- The Sex Differences in Survival Time and Blood Content of the Tumour Ascites in the Three Groups

	Survival Time			Blood Content		
Group	**S.E. Difference**	**T**	**P**	**S.E. Difference**	**T**	**P**
I	1.013	0.4862	0.7>P>0.6	1.736	0.0806	P>0.9
II	1.33	0.9773	0.4>P>0.3	1.702	0.2974	0.8>P>0.7
II	1.233	5.077	0.001>P	0.9806	0.6424	0.6>P>0.5

The number of mice in each group and series is shown, the mean value for the factor under consideration(x) with its S.D., the S.E. of the actual difference between male and female means, and the t and P values for this difference

The mean survival time for the total series was shortest in the control group I(11-03 days, S.D. 2-683), and longest in the cortisone treated group III (14-93 days, S.D. 4-528). The vitally stained group II fell between these two extremes (12.24days, S.D. 3-332). The sex difference within the groups was not statistically significant in groups I and II (0-5 and 1-3 days respectively), while it was highly significant in group III (6.26 days, 0-001 > P). The mean blood content of the tumour ascites was highest in the control group 1 (4.3 per cent, S.D. 4-638), and lowest in the cortisone treated group III (1-/*216 per cent, S.D. 2-616). Once again the vitally stained group II showed an intermediate value (1.717 percent, S.D. 4-286). The sex differences were not significant.

Table IIa.-Comparison of the Differences in Survival Time Between the Three Groups

Comparison between groups x and y	series	n_x	n_y	Difference between x^- and y^-	S.E difference between x^- and y^-	T	P
I and II	Male + female	30	29	1021	0.7891	0.1533	0.9>p>0.8
I and III	Male	15	15	1.0	4.187	0.2388	0.9>p>0.8
I and III	Female	15	15	6.76	1.172	5.768	0.001>p
II and III	Male	14	15	1.1	1.204	0.9137	0.4>p>0.3
II and III	female	15	15	6.46	1.356	4.764	0.001>p

The difference in survival time between the groups for the total series, or for the male and female series separately when their difference is significant, is shown, with the number of mice used and the S.E., t and P values for the difference.

Table IIb.-Comparison of the Differences in Blood Content of the Tumour Ascites Between the Three Groups

Comparison between groups x and y	series	n_x	n_y	Difference between x^- and y^-	S.E difference between x^- and y^-	T	P
I and II	Male+female	30	29	2.583	1.161	2.224	0.05>p>0.02
I and III	Male+female	30	30	3.184	0.9705	3.281	0.01>p>0.001
II and III	Male+female	29	30	0.501	0.7416	0.675	0.5>p>0.4

The difference in blood content between the groups is shown for the total series, with the number of mice used, the S.E., t and P values for the difference.

Table IIb compares the differences in blood content of the tumour ascites between the three main groups for the total series, giving the number of mice used, the S.E., t and P values for the difference. The difference between groups I and 11 (2-583 per cent) is significant (0-05 > P > 0-02), while that between groups I and III (3-184 per cent) is highly so (0.01 > P > 0-001).

Table 1. Total Number of Cells per Mouse after Inoculation of Cells from Male or Female Donors into Male or Female Recipients

Donor	Recipient	Mean total number of cells ($1x10^6$ per mouse)			
		5^a	7^a	9^a	12^a
Female	Female	315	642	1119	1904
Female	Male	252	421	831	1656
Male	Male	166	208	604	1189
Male	Female	310	450	616	1468

Analysis of variance: Treatment t ime interact ion vs. error :F, 9,112 = 1.41; 0.2 > P > 0.1; Time vs. error: F, 3,112 = 126.74; P < 0.001: Treatment vs. error: F, 3,112 = 13.59; P < 0.001; [S.D. for each mean (8 mice) A ±102]; [a] Days after inoculation.

Table 2 Tumor Volume and Cell Concentration after Inoculation of Cells from Male or Female Donors into Male or Female Mice

Donor	Recipient	Mean Tumor Volume				Mean cell concentration ($1x10^6$ per ml)			
		Days after inoculation							
		5	7	9	12	5	7	9	12
Female	Female	1.1	3.2	4.3	9.4	180	203	272	226
Female	Male	1.0	1.4	4.1	6.4	282	258	217	274
Male	Male	0.5	0.9	0.9	5.0	80	147	237	250
Male	female	1.2	2.0	2.0	9.3	229	234	189	164

Analysis of variance

Tumor volume: Treatment time interaction vs. error; F, 9,112 = 2.580; 0.05 > P > 0.01; Treatment vs. interaction: F, 3,9 = 4.681; 0.05 > P > 0.01; Time vs. interaction: F, 3,9 = 40.745; P < 0.001; [S.u. for each mean (8 mice) A±0.6]

Cell concentration: Treatment time interaction rs. Error : F, 9,112 = 3.1105;0.01 > P > 0.001; Treatment vs. interaction: F, 3,9 = 1.500; P > 0.2; Interaction vs. time: F, 9,3 = 2.440; P > 0.2; [S.D. for each mean (8 mice) A±31]

Table 3 Effects on Total Cell Number Due to the Sex of Donor and to the Sex of Recipient: Analysis by Partitioned Sums of Square

	Total cells (1x10^6 per mouse)		
	Female recipient	**Male recipient**	**Donor effect**
Donor female	**(a)**	**(b)**	**(a+b)**
Sum	31,838		
No.	32		
Mean	995		
Donor male	(c)	(d)	(c+d)
Sum	22,747	17,331	57,115
No.	32	32	64
Mean	711	995	892
Recipient effect	(a+c)	(b+d)	
Sum	54,585	42,608	
No.	64	64	
Mean	853	666	
Total			(a+b+c+d)
sum			97,193
no			128
mean			759

Donor effect: F, 1,112:27.205; P < 0.001
Recipient effect: F, 1,112:13.445; P < 0.001
Interaction: F, 112,1:8.138; P > 0.2
[S.D. of each mean (32 mice) A ±51]

Table 4 Effects on Tumor Volume and Cell Concentration Due to the Sex of Donor and to the Sex of Recipient: Analysis by Partitioned Sums of Squares

	Tumour volume (ml)			Cell concentration(1x106 cells per ml)		
	Female recipient	**Male recipient**	**Donor effect**	**Female recipient**	**Male recipient**	**Donor effect**
Donor female	a	b	a+b	A	b	a+b
sum	143.5	102.6	246.1	7049	8244	15293
No.	32	32	64	32	32	64
mean	4.5	3.2	3.8	220	258	239
Donor male	c	d	c+d	C	d	C+d
sum	130.8		203.5	6528		12242
No.	32		64	32		64
Mean	4.1		3.2	204		191
Recipient effect	a + c	b +d		a+c	b+d	
Sum	274.3	175.3		13577	13598	
No.	64	64		64	64	
Mean	4.3	2.7		212	218	
Total			a+ b+ c+ d			a+ b+ c+ d
Sum			449.6			27535
No.			128			128
Mean			3.5			215

Tumor volume: Donor vs. error: F, 1,112 = 5.544; 0.05 > P > 0.01; Recipient vs. error: F, 1,112 = 29.790; P < 0.001; Error vs. interaction: f, 112,1 = 1.112; P > 0.2; [S.D. of each mean (32 mice) A±0.3]

Cell concentration: Donor vs. interaction: F, 1,1 = 2.306; P > 0.2; Interaction vs. recipient: F, 1,1 = 27.806; I' > 0.1; Interaction vs. error: F, 1,112 = 4.189; 0.05 > P > 0.01; [S.D. of each mean (32 mice) A±15]

Chromosome studies were carried out after the 59th and 60th consecutive transplants and were compared with observations previously made on the parent tumor. Mice used in these studies were given intraperitoneal desacetylmethylcolchicine in a dose of 7.5 µg per gm body weight 90 minutes before being killed. Metaphase plates were prepared by a hypotonic saline-air-drying technique similar to that used for peripheral blood (23) and were stained with Leishman. At least 50 well-spread metaphases were counted and analyzed from each of 4 mice from each subline and from each of 7 mice carrying the original tumor.

Table 5 Effect of Prior Subcutaneous Tumor Inoculation on Survival of Mice Given Intraperitoneal Tumor 7 Days Later

Sex of donor of subcutaneous tumour	Sex of donor of intra peritoneal tumour	Sex of recipient	Number of mice	Survival	
				Median	Range
Female	Female	Female	5	53	37-92
		Male	5	19	17-37
	Male	Female	5	17	12-92
		Male	5	21	17-37
Male	Female	Female	5	21	17-73
		Male	5	33	19-40
	Male	Female	5	19	17-91
		Male	5	37	17-88
All pre-treated mice			40	33	12-92
No prior inoculation	Female	Female	10	18	10-22
		Male	9	18	17-22
	Male	Female	10	17	17-21
		male	10	19	17-21
Control mice(not pre-treated)			39	18	10-22

Significance of difference in survival between pretreated and control animals (distribution-free method): x2 = 16.506; p < 0.001.

Survival times were not amenable to analyses based on the normal distribution, even after logarithmic transformation of the data, and for this reason they were analyzed by a distribution free method[11]. (Survival times after prior subcutaneous inoculation (Table 5) were amenable to analysis of variance within the pretreated group, and within the control group, after logarithmic transformation[12], but a distribution-free method was necessary to compare results between the 2 groups

3.1 Maintenance of Tumor in Mice of One Sex.

The subline maintained in male mice Appeared to be growing less vigorously at each transplant than the subline maintained in females. Tumor growth in male mice slowed to such a degree after the 40th transplant that sub inoculation often had to be delayed, and as a result the number of consecutive transplants in male mice was 1, and later 2, less than the number in female mice during the second year in which separate sublines were maintained.

3.2 Differences in Tumor Growth.

The total number of cells per mouse increased with time in each of the treatment groups studied (Table 1). Table 3 shows that both the sex of the mice in which the tumor had been maintained and the sex of the mouse inoculated contributed significantly and independently to the observed effects. Thus, when compared in recipient mice of one sex, tumor which had been maintained in females produced more growth than did tumor which had been maintained in males. Conversely, however, both tumor sublines grew better when tested in females. Further analysis of the effect of treatment on tumor volume (Table 4) showed results similar to those found with the total cell count, namely, Significant and independent effects due both to the sex of the donor and to the sex of the recipient. Analysis of cell concentration also showed significant interaction between treatment and time, and in this case neither the effect of time nor the effect of

treatment was significant. The reason for the interaction effect is obvious in Table 2, where it will be seen that the mean cell concentration tended to increase with time in the FF and MM groups, to decrease with time in the MF group, and to remain approximately the same in the FM group.

The treatment sums of squares were partitioned, but not surprisingly, no significant differences were found (Table 4).

Survival. Females inoculated with 10^5cells from female donors succumbed slightly sooner than did males inoculated with the same number of cells from male donors (median survival for females, 24 days; for males, 28 days; $0.05 > P > 0.02$). However, there was no significant difference in survival between males and females inoculated with 10^4, 10^6,or 10^7cells.

3.3 Effect of Prior Subcutaneous Inoculation on Intraperitoneal Tumor Growth.

The local effects of subcutaneous inoculation of tumor cells into the tail were variable. Slow tumor growth occurred in all animals, but in some the tail underwent necrosis and separated spontaneously; in others the tumor reached a certain size (approximately 5-8 mm in diameter) and then failed to grow any further

4. Discussion

In this experiment there are three factors to be considered: the tumor, the relationship of the mice to the tumour and the interrelationship of the mice themselves. As regards the tumour[14], have shown that provided a sufficient tumour cell dose is used Ehrlich's ascites carcinoma can be expected to grow progressively, and the mouse to die within a time limit dependent on that tumour cell dose. The tumour cell dose used in the present experiment is comparable to that used in the author's previous experiment[6]. The adult mice were taken from the same closed colony. The mice were, of necessity, of different genetic make-up to the tumour, as Ehrlich's ascites carcinoma came originally from a heterozygous mouse[15] .Therefore, one would expect that the mice would not accept transplants. But Ehrlich's ascites carcinoma is one of the so-called " non-specific " tumours that is said to contain fewer antigens than usual and so is less demanding than most in its choice of host[16]. However, a perfect fit between host and tumour cannot be expected. While the fit may be close enough to allow the tumour to grow the difference may show up in other ways-for example in the stromal reaction. Thus, although the mice were not, as in Barrett's experiment referred to earlier[16], sensitized before hand with a foreign antigen, the tumour may possess tissue antigens that the mice lack. If they then react against this foreign protein their immunological response might be expected, i.e. by a change in the stromal reaction. This hypothesis is based on the assumption that resistance to homotransplants is genetically determined. This has been shown to be true in the case of red blood cells[17] and normal tissues[18].

The Mendelian nature of the genetic influences determining susceptibility to transplanted tumours has also been established[19]. When a tumour is transplanted the tumour cells reproduce, the stroma is supplied by the host[20]. In the case of Ehrlich's ascites carcinoma the stroma is represented by the ascitic fluid, which contains a variable number of white blood cells .In addition, in some cases, this stroma contains large number of red blood cells[21]. These red blood cells have appeared in the stroma either in direct response to the tumour cells, or in response to the mouse's reaction to these cells. If the first is the case we would expect that the amount of blood in the stroma would be constant for a given tumour cell dose. But the author has previously shown[6] that the amount of blood varies inversely with the survival time in mice receiving the same tumour cell dose. If the second is then true, the amount of blood should vary in accordance with the genetic dissimilarity between the mouse and the tumour. All the mice receiving Ehrlich's ascites carcinoma in the present experiment were given the same dose of viable tumour cells. The dose used was chosen as it was known to give an ascitic tumour and a relatively short survival time. The dosage of trypan blue was based on that used by[22], and the cortisone dosage[23] on that used by Hobson (1960).

It may be that this haemorrhagic reaction is a Shwartzman-like phenomenon. It has been suggested that the Shwartzman phenomenon may be a manifestation of an immune response[24]. Stetson[25] has shown that injection of bacterial endotoxin can elicit hemorrhage in skin areas previously prepared by the intradermal injection of homologous or heterologous bacterial products, and compares the reaction to that following the injection of tuberculin in specifically sensitized rabbits. It [26] has drawn analogies between this type of delayed sensitivity and homograft rejection. In the present experiment it has been shown that the hemorrhagic response occurring in some of the mice following transplantation of the tumor is dependent on their natural resistance to the transplant.

4.1 Ehrlich Ascites in Male and Female

In the present studies the effect on tumor growth due to the sex of the mice in which the tumor had been maintained (the "donor effect") was analyzed separately from the effect due to the sex of the mouse inoculated (the "recipient effect"). In each case significantly less tumor growth, as measured by the total number of cells and by tumor volume, was found with male mice. The total cell number found in female mice agreed closely with that reported by[27] in

their studies of the growth of the hyperdiploid Ehrlich ascites tumor in female mice. The significant donor effect is particularly interesting, since so far as is known there have been no previous reports of such a finding. Two possible explanations for the donor effect could be considered: (a) the presence of a substance in the sujwrnatant fluid of the transplanted tumor which either enhanced growth of tumor from female donors or impaired growth of tumor from male donors; (b) a change in the tumor cells which either enhanced or impaired tumor growth. The first possibility seems unlikely since cells were thoroughly washed before inoculation. This leaves the possibility of a change in the tumor cells, which could have been due to the selection, either of a line of cells in female mice with a greater growth potential or of a line of cells in male mice in which this potential was less. The observed changes in chromosome number suggest that a cell line with 45 chromosomes was being selected in both male and female mice to replace the original cell line (44 chromosomes).

In tumor maintained in males, the new cell line had almost replaced the original, while in the female tumor the 2 were equally represented. If the cells with 45 chromosomes produced less tumor growth than did the cells with 44 chromosomes, their predominance in the male sub line could explain the observed donor effect; the sex difference would then have been due to impaired growth of tumor maintained in males rather than to enhanced growth of tumor maintained in females. The difficulties frequently experienced in maintaining the sub line in male mice, due to delayed tumor growth and small tumor volumes, would be consistent with this interpretation. One such condition could have been a lower ratio of tumor-cell antigens to host-defensive cells in the case of male mice, since these tended to be between 2 and 4 gm heavier than females. Small tumor doses are known to cause the immune selection of tetraploid cell lines from hyperdiploid populations due to a greater ratio of host defense to tumor-cell antigens [27].

However, it can be calculated that females of the same weight as males in the present study would have received only 1.1 X 10^6,instead of 1.0 X 10^6cells, and it seems unlikely that this difference could have produced the observed effects. Differences in tumor behavior related to the sex of the animal inoculated have been reported [28-35], although in some cases more growth has been found in females whereas in others the opposite has been the case Thus, the Ehrlich ascites tumor was reported to show greater initial growth in female mice [28] and better growth at all stages after hetero transplantation into female hamsters [29]. More pulmonary metastases were found in female than in male mice after intravenous injection of ehrlich ascites cells[30], although this was probably partly due to the trapping of a greater number of tumor cells in the lungs of female mice[36]. Bittner [31] found more "takes" in females when F2 mice were inoculated with a tumor which had arisen spontaneously in an FI hybrid, and Klein [34] found more "takes" in female rats inoculated with a sarcoma which had arisen in a different strain. By contrast, a mammary adeno carcinoma was shown to grow more vigorously in male mice [35] and a mouse sarcoma took in more males than females when small inocula were used [33]. Hormonal imbalance induced in male mice by treatment with androgens or with alternating androgens and estrogens was found to favor growth of both the S-180 and Krebs-2 carcinoma, compared with growth in untreated males or in males treated with estrogen alone [32]. Hartveit[37] found survival to be the same in male and female mice inoculated with the Ehrlich tumor. Despite the sex differences in the amount of tumor found, no significant difference could be found in cell concentration; this agrees with reports that cell concentration remains constant while tumor growth continues by expansion of the volume of ascetic fluid[28, 13]. However, the susceptibility of the host, as well as the rate of tumor growth, could determine the duration of survival; a similar survival in each group of mice could have been due either to a tolerance to greater tumor growth in females or to a susceptibility to a smaller tumor mass in males.

The prolonged survival of mice pretreated with subcutaneous tumor suggested that they may have become immunized against antigens on the tumor cells. Viable tumor cells are probably better antigens than killed cells [38], and adverse effects of local tumor growth were largely avoided in the present study by using the tail for subcutaneous inoculation. Immunity to transplantable tumors, such as Ehrlich ascites, can be induced by prior exposure of the potential host to the same tissue as the subsequent graft [39], even though these tumors have weak histo-compatibility antigens[40,41]. There have been many reports of changes in the chromosome number of transplantable tumors, occurring either spontaneously[42], after immunoselection [27, 43, 44], or during in vitro conditions [45, 46]. The changes reported have been gross, usually from a hyperdiploid to a polyploid mode or vice versa, and not small changes of chromosome number as found here. It is nonetheless possible that the addition of only one chromosome could modify cell behavior.

5. Conclusion

EAC has a resemblance with human tumors which are the most sensitive to chemotherapy due to the fact that it is undifferentiated and that it has a rapid growth rate. Due to the resemblance, some researchers reported that some plant extracts were effective against EAC[47.] Nonetheless, the search of new toxic agents from natural sources has been conducted in collaboration with scientists, world wide[48]. Three groups of 30 heterozygous mice were injected with Ehrlich's Ascites carcinoma. Two of the groups of animals were treated with trypan blue and cortisone, respectively, in an

attempt to abrogate their natural resistance to the tumor. The amount of blood in the tumour ascites was found to be less following these treatments. (Average per cent: Control-4 3, trypan blue- 17, cortisone-1-2). Thus the blood content of the tumor ascites can be regarded as an expression of the animal's reaction against, i.e. natural resistance to, the tumour.

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