trans(15-^{3}H)retinol (2.7 Ci/mmol, New England Nuclear, Boston, MA). For competitive binding, the mixture contained either unlabeled all-*trans*-retinol (Hoffmann-La Roche) at a molar concentration 200-fold greater than that of the labeled materials. After an incubation of 16 h in the dark at 4 °C, each sample was centrifuged at 178,000 × g in a linear (5–20%) sucrose gradient for 21 h. Radioactivity was measured in Triton X-100 Omnifluor in a scintillation spectrometer.

The observation that a retinoic acid receptor is present in the allotransplantable TA3-Ha cell, but is absent in the strain specific TA3-St cell is consistent with the finding that retinoic acid added to the culture medium of the TA3-Ha cell produces an effect (a decrease in adhesiveness), whereas under similar conditions no effect was observed in the TA3-St cell. This contrasting result appears noteworthy, since both cell lines had similar origin, i.e., both were derived from the same tumor mass[4]. Different reported effects of added retinoic acid could be explained on the basis of different cell types and tissues of origin. These included the capacity to increase[6,8], decrease[9], or have no effect on[10], adhesion; to induce[11] or inhibit[12] differentation; to enhance[13] or to inhibit[14] cell proliferation; and to alter[15] or have little or no effect upon[10] morphology. Indeed, the response of the TA3-Ha cell itself cannot be considered as unique, since retinoic acid was reported by Shapiro and Poon[9] to exert a similar effect upon adhesion and growth rate in an established cell line derived from human intestinal epithelium. The present study suggests that the adhesive properties of tumor cell lines of similar epithelial origin, when grown in culture, may be affected in quite different ways by added retinoic acid. Response was observed only in one line, TA3-Ha, the cell found to possess the retinoic acid-binding protein. Whether or not the decreased adhesion of the TA3-Ha ascites cell resulting from added retinoic acid also alters its transplantation characteristics remains to be determined.

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Different biological behavior of AKR lymphoma cells from primary and metastatic tumors

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Summary. AKR lymphoma cells derived from primary s.c. tumors (PT) and cells from their metastases (MT) were inoculated into recipient mice in order to compare their malignant behavior. A higher malignant potential of MT compared to PT cells was found. The results support the hypothesis that metastasis is a process of selection of cells possessing a potential to metastasize, which preexist in the primary tumor. In the model used, both the selection of 'variants' of malignancy and the assay of malignancy were as close as possible to natural tumor progression.
Key words. Different malignancy; primary-metastatic cells.

Studies dealing with differences between primary and metastatic tumor cells are important for understanding the nature of malignancy, as well as for their therapeutic implications.

Many studies have tried to relate the degree of malignancy of tumor cells to various structural properties: karyotypic[2], biochemical[3,4], immunological[5] and the response to therapy[6]. Many of these studies used cells of different lineage from the same tissue, like the Morris hepatoma series[3]. More recently, variants of the same tumor, prepared by different in vitro[6], in vivo[7] or combined in vivo and in vitro manipulations[8] were studied. Although much important information was gained from studies of these variants, differences between primary and metastatic tumors of the same individuals are more directly relevant to natural neoplastic development.

Investigations devoted to the comparison of primary versus metastatic tumors of the same organism[9–14] showed a number of differences, including biochemical[9,10] and immunological features[11,12] and sensitivity to drugs[13,14]. These comparative studies were recently reviewed by Weiss[15]. Studies were also performed in order to find out whether cells deriving from metastatic tumors had a higher malignant potential than those deriving from the parent primary tumor[8,16]. The assay for malignancy was often performed by i.v. inoculation[7], a procedure which does not include the phase of invasion, or by surgical excision of the primary tumor[16], a procedure which enhances metastatic spread[17]. The two procedures are obviously incomplete models of natural tumor progression. However, very recently several successful attempts have been made to perform the assay of malignancy by s.c. inoculation[18–21].

Using the AKR lymphoma, we were able to obtain real spontaneous metastasis by injecting tumor cells s.c., permitting thereby the evolution of the natural process of metastasis includ-

Table 1. Comparison of growth characteristics of tumors in mice inoculated with cells from primary and metastatic tumors of AKR lymphoma: size of local and metastatic (enlarged inguinal lymph nodes) growth and degree of cachexia

Day of observation	Mice inoculated with cells derived from 'primary' tumor					Mice inoculated with cells derived from metastatic tumor				
	No. of mice alive	Incidence of local tumor	Average size of local growth (mm) ± SD	Incidence of enlarged inguinal lymph nodes	Average weight of mice (g) ± SD*	No. of mice alive	Incidence of local tumor	Average size of local growth (mm) ± SD	Incidence of enlarged inguinal lymph nodes	Average weight of mice (g) ± SD
21	8	4/8	1.38 ± 1.68	0/8	26.00 ± 3.07	8	7/8	6.25 ± 4.30	0/8	25.62 ± 2.90
27	7	6/7	6.87 ± 6.10	0/8	26.00 ± 2.83	7	7/7	8.43 ± 4.83	5/7	20.86 ± 4.56
31	6	5/6	8.71 ± 7.23	2/6	26.57 ± 1.90	2	2/2	5.00 ± 0.00	2/2	16.50 ± 0.71
37	6	5/6	13.50 ± 9.61	2/6	25.33 ± 2.07	2	2/2	6.50 ± 0.71	2/2	16.00 ± 1.41

* The average weight of mice at the beginning of the experiment was 25.5 ± 2.45. Statistical evaluation was done by Student's t-test.

ing both the invasive and transplantation phases, as defined by Weiss[15].

We present here a comparative study of the biological behavior of AKR lymphoma cells, taken from the mass formed at the subcutaneous site of tumor inoculation (primary tumor – PT), and cells taken from metastatic growth obtained from mesenteric lymph nodes (MT) of the same mouse. The malignant potential of these two types of cells, separately inoculated in groups of recipient mice, was determined.

Materials and methods. AKR/Cu male mice, aged 6–10 weeks were purchased from the Weizmann Institute of Science, Rehovoth, Israel. Tumor cells were derived from a mouse inoculated with a first transfer of a spontaneous AKR lymphoma which developed a local mass at the s.c. site of tumor inoculation. AKR lymphoma cell suspensions from the tumor formed at the site of inoculation (PT) and from the enlarged mesenteric lymph nodes (MT) of the same animal were prepared as previously described[22]. 5×10^4, or 1×10^5 PT or MT cells were inoculated s.c. in the backs of two groups of five or eight mice each. Incidence and size of local and metastatic tumors and the weights of the mice were recorded 2–3 times a week. Incidence of primary and inguinal lymph nodes and adherence of primary tumors to the surrounding tissues were assessed by palpation in living mice and at autopsy by macro- and microscopical examination. The diameters of the primary tumors were expressed as the average between two perpendicular diameters. Mortality was recorded daily. Statistical evaluation was done by Student's t-test.

Results. Table 1 shows the average size of the local growth in mice inoculated with PT and MT cells and the weights of the mice in these groups. It can be seen that cells taken from the primary tumor form local masses which develop more slowly but reach larger maximal sizes than cells from the metastatic tumor. Moreover, local tumors in recipients of PT cells were more often movable and nonadherent to surrounding tissues, while those formed in recipients of MT cells were adherent, nonmovable, and invasive (table 2). Metastatic spread, as assessed by enlarged inguinal lymph nodes, was more rapid in mice inoculated with MT than in those injected with PT cells. This is also illustrated by the size of lung metastases on day 20 formed by the two types of cells (fig. 1).

Table 3 shows the rate of development of leukemia in mice inoculated with PT and MT cells. Recipients of MT cells de-

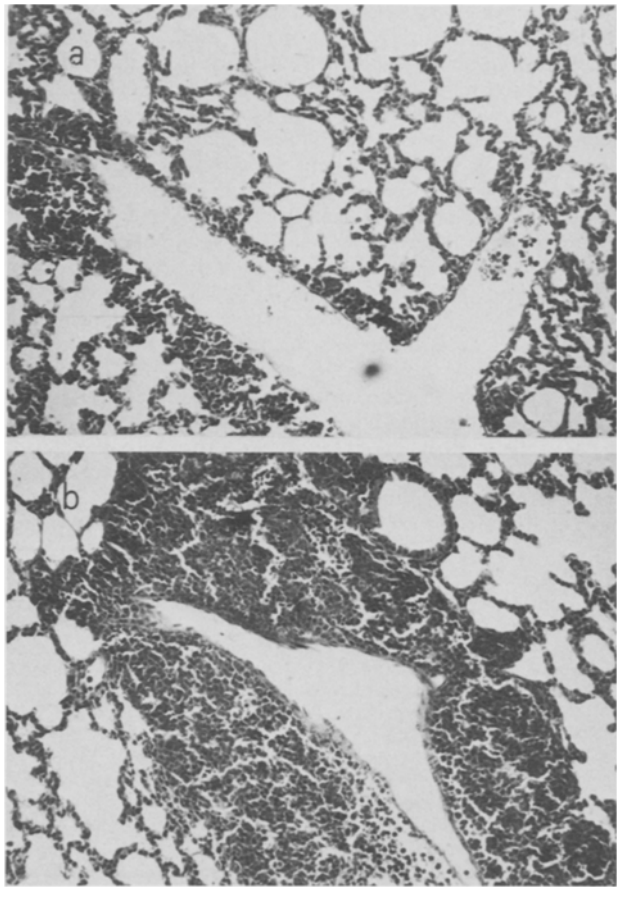

Figure 1. Rate of metastatic spread of tumors formed by cells derived from primary s.c. (PT) and metastatic (MT) growths: histological picture of metastases in lungs of PT (a) and MT (b) – bearing mice on day 20 after inoculation. Fixed in 10% formalin. H and E, × 137.

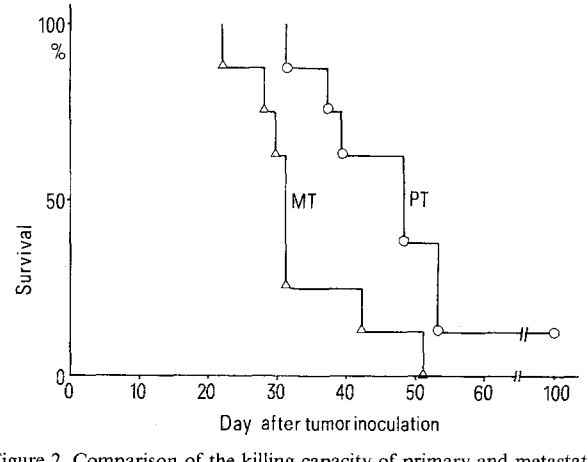

Figure 2. Comparison of the killing capacity of primary and metastatic AKR lymphoma cells. 5×10^4 AKR lymphoma cells derived from a primary s.c. tumor (PT) and from a metastatic (MT) growth from the same mouse were inoculated s.c. in the back of two groups of eight recipient mice. ○, PT; △, MT.

Table 2. Invasiveness of local s.c. tumors formed by primary compared to metastatic tumor cells: incidence of invasive tumors

Types of cells inoculated	Experiment 1 Day 14	Day 19	Experiment 2 Day 13	Day 15	Experiment 3 Day 17	Day 21
PT	1/5	4/5	1/5	1/5	0/7	0/7
MT	4/5	4/5	2/5	4/5	7/8	6/6

Invasiveness of tumors was judged by palpation according to attachment to surrounding tissues and mobility or lack of mobility.

Table 3. Rate of development of leukemia in mice inoculated with primary compared to metastatic tumor cells

Type of cells inoculated	No. of mice	No. of PBL/mm^3 on day 20 Range	Average ± SD	p
PT	5	14,600–24,400	20,200 ± 3516	
MT	6	20,450–59,400	37,613 ± 16,160	$0.025 < p < 0.05$

Table 4. Mortality rate of mice inoculated with primary compared to metastatic tumor cells

Type of cells inoculated	Experiment 1 MST (day ± SD)	p	Experiment 2 MST (day ± SD)	p	Experiment 3 MST (day ± SD)	p
PT	44.14 ± 8.51		35.20 ± 8.44		26.00 ± 6.93	
MT	33.12 ± 9.09	< 0.0005	26.40 ± 9.50	$0.05 < p < 0.1$	19.00 ± 4.10	$0.025 < p < 0.005$

The inoculum in experiment 1 was 5×10^4 and in experiments 2 and 3 1×10^5. The number of mice in experiment 1 was eight in each group, in experiments 2 and 3 five per group.

velop leukemia more rapidly than recipients of PT cells. On day 20 after tumor inoculation, the average number of peripheral blood leukocytes is almost double in MT cell-bearing mice compared to PT cell recipients.

The results in table 1 show also that the weight of mice inoculated with PT cells did not change much until day 37, while the weight of mice inoculated with MT cells already decreased markedly during this period. Comparison of the weights of mice at a point where the sizes of the tumors, and therefore their contribution to the weight of the mouse, are similar (day 31 for PT and day 27 for MT), shows that the difference is significant ($p < 0.01$).

Figure 2 shows the survival curves of mice inoculated with either PT or MT cells. MT cells are more aggressive than PT cells according to their killing capacity. The difference is highly significant ($p < 0.0005$).

The mortality data of mice inoculated with PT and MT cells in three experiments are summarized in table 4. In all experiments, the killing capacity of MT cells is higher than of PT cells.

Discussion. The data presented show that cells originating in primary and metastatic tumor cells from the same animal differ in their malignant potential. This is proven by: 1) smaller maximal size of primary tumors in mice injected with cells from metastases (size of primary tumors was previously shown by us to correlate inversely to metastatic potential[23]); 2) the local tumors formed by cells of metastatic origin are firmly attached to the surrounding tissues, while those formed by cells derived from local growth are nonadherent; 3) leukemia and 4) cachexia progressed rapidly in mice inoculated with metastatic cells and more slowly in mice injected with local tumor cells; 5) finally, mortality rate was higher in mice injected with metastatic, compared to those inoculated with primary tumor cells.

The test of malignancy in the lymphoma was done by s.c. inoculation. In fact, murine epithelial tumors seldom result in spontaneous metastases when injected s.c. This is probably the reason for the paucity of reports dealing with biological differences between local and really spontaneously metastatic tumors. An attempt to circumvent this difficulty is the widely used test of malignancy which consists in inoculating the tumor i.v. or the footpad injection of tumor cells, followed by amputation. However, these procedures hardly reflect the natural evolution of metastasis.

Very recently several reports in which the assay of malignancy was done by s.c. inoculation were published[18–21]. However the results obtained and the interpretations reached by the different groups differ: Talmadge and Fidler[19] and Neri et al.[20] have found that cells derived from metastases had a higher potential than cells derived from the primary tumors. Giavazzi et al.[18] did not find any difference in metastatic capacity and Weiss et al.[21] found differences in some of the tumor systems but not in others. Weiss et al. conclude that it cannot be generalized that metastases arise from preexisting subpopulations of tumor cells. The present study adduces evidence for a preexisting subpopulation of cells with metastatic capacity in primary tumors of a murine T cell lymphoma-leukemia.

Lymphomas in humans and animals can progress by local extension or by flooding the blood. In murine systems, lymphomas progress more easily to disseminated growth than epithelial tumors. It is therefore probably easier to show differences between primary and metastatic growths in these tumors. In the present study the selection of 'variants' of malignancy as well as the assay for malignancy were performed using methods as close as possible to natural tumor progression. This was feasible in the AKR lymphomas chosen for this study, since s.c. inoculation resulted initially in local tumor growth with subsequent metastatic spread in 100% of mice.

Metastatic growths are often much more difficult to control than primary growths, because they are less accessible to early diagnosis and therapy. Moreover, difficulty in the therapy of metastatic growth may result not only from its hidden location, but also from differences in the properties of metastatic cells compared to those of primary growths. These differences might contribute to failures following early therapeutic successes. Since a drug which acts on a local tumor may not affect metastatic growth, it is necessary to test drugs on both types of tumor growths. This system could be useful both for studies on the distinctive properties of primary and metastatic tumors and for studies on their possible difference in response to therapy[24].

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Selective effects of gonadal steroids on the response of peripheral serotonin receptors

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Summary. The maximal contraction provoked by serotonin (5-HT) in isolated stomach strips of adult rats, a functional index for peripheral 5-HT receptors, was sexually differentiated, androgen-sensitive, and estrogen refractory. This is at variance with the reported sensitivity of central 5-HT receptors to estrogen.
Key words. Rat, stomach strips, isolated; serotonin receptors, peripheral; androgen-sensitive contraction; estrogen-refractory contraction.

There is evidence that brain 5-HT receptors are modulated by ovarian hormones[1]. This finding is consistent with the existence of steroid binding sites in discrete regions of the brain[2]. There is also an apparent[3], though unlikely[4] similarity between receptor sites which bind ^{3}H-5-HT in the brain, and those[5] which putatively trigger the 5-HT induced contraction in the rat stomach fundus preparation. In fact, several compounds with high binding affinity at 5-HT central receptors would also markedly antagonize the response to 5-HT or act as serotonergic agonists on the isolated fundus strip[3,4]. Furthermore, the rank order of displacing potencies of 5-HT antagonists on ^{3}H-5-HT binding is similar in rat brain and gut membranes[6]. It was, therefore, of interest to investigate whether chronic exposure of rats to gonadal steroids would influence the isolated stomach strip response to 5-HT, as was observed with brain serotonergic binding sites[1].

Materials and methods. Newborn male (M) and female (F) albino Charles River CD rats were used. The experimental schedule of the main experiment[7] was intended to masculinize the brains of neonatal F with androgen administration, or to interrupt the masculinization process of noncastrated M with estrogen. For this purpose, M and F pups were given, respectively, a single s.c. dose of 17β-estradiol valerate (Sigma Chemical Co.; E_2; 500 µg in 0.05 ml of corn oil), or testosterone enanthate (Sigma; TS; 270 µg) within 24 h from birth. Rats then received approximately weekly injections of decreasing doses of steroids: 200 µg E_2 or 100 µg TS at days 5, 10 and 17; 100 µg E_2 or 10 µg TS on day 24; 1 µg E_2 or TS at days 34 and 44, and 0.5 µg E_2 or TS at 50 days of age. Control pups received corn oil on the same days as the steroid-treated rats. All animals were sacrificed at 21 or 60–61 days of age. Adult, control females were in late metestrus or diestrus, as assessed with vaginal smears. The stomach was dissected soon after sacrifice, and two strips were cut from the fundus according to a slight modification[8] of the classical Vane's method. The mucosa was carefully ablated, and one strip was set up in a 10-ml organ bath at 37 °C containing Tyrode solution bubbled with O_2. Composition of the Tyrode solution was (g/l): NaCl 8; KCl 0.2; $CaCl_2$ 0.2; $MgCl_2$ 0.1; $NaHCO_3$ 1; NaH_2PO_4 0.05, and glucose 1. One end of the strip was connected to a constantly loaded isotonic lever, and 5-HT-induced contractions were registered on a slowly rotating, smoked kymograph. The cumulative dose-response technique was used, and pD_2 values were calculated[9]. A pD_2 value indicates the 'affinity' of 5-HT for its receptors, and is defined as the negative logarithm of the molar concentration of 5-HT producing a contraction 50% of the maximal one in that system[10]. Duplicate to quadruplicate dose-response curves were made for each strip, and the average pD_2 was calculated for each individual experiment. Statistical significance of differences was assessed with Student's t-test.

Results and discussion. The maximum effect of 5-HT increased with age; over the same time, the dose-response curves at 21 days were shifted far to the right on the dose axis (fig., a) thus indicating neonatal hyposensitivity to 5-HT[11]. Neonatal pD_2 values were consequently 5-fold lower than those in control and hormone-manipulated adults (table). The pD_2 values at both ages were neither sexually differentiated, nor were they affected after chronic administration of E_2 to males or TS to females (table). There was, however, a consistent sex-related difference in the maximal effect of 5-HT, inasmuch as the peak

The influence of neonatal and prepubertal chronic administration of estradiol (E_2) and testosterone (TS), age and sex on the receptor affinity* for 5-HT in the isolated rat stomach fundus preparation

Sex	In vivo treatment	Days of age 21 pD_2	n_1/n_2	60 pD_2	n_1/n_2
Male	Corn oil	6.85 ± 0.07***	15/43	7.46 ± 0.08	32/102
Female	Corn oil	6.88 ± 0.08***	16/40	7.60 ± 0.06	35/110
Male	E_2	6.92 ± 0.09**	13/28	7.40 ± 0.10	27/77
Female	TS	6.77 ± 0.09***	12/28	7.55 ± 0.08	30/93

*The pD_2 values given are means ± SE of n_1 experiments on fundus strips; pD_2 values for individual experiments were calculated from 2–4 dose-response curves. n_2 = total number of cumulative curves. **$p < 0.005$; ***$p < 0.001$, compared to 60-day-old counterparts.