interchanged one. Its small size was probably the result of a highly unequal exchange of segments (i.e., a large segment of a chromosome was exchanged with a very small segment of an other nonhomologous chromosome). Unequal exchanges in heterozygous conditions led to the formation of 2 heteromorphic bivalents, as noted in the interchange heterozygote in M_1, as well as in the mutant in M_2. In these heteromorphic bivalents, tail-like structures in both closed and open bivalents represent the unpaired nonhomologous segments (fig. 4). Observations on low frequency of quadrivalents (12%) in the interchange heterozygote (M_1) and absence of higher association in the mutant are parallel and in conformity with the other reports[13,14] concerning similar situations, that is interchanges involving highly unequal exchange of segments between nonhomologous chromosomes. In addition, the extra chromosome (interchange type) in the mutant might be also partly responsible for the absence of higher association.

Thus, the chromosome constitution of the mutant (tertiary trisomic interchange heterozygote) could be presented as 5^{II} (normal) + 2^{II} (heteromorphic) + 1^{I} (interchanged chromosome with reduced size). Studies with regard to the chromosomes involved, the transmission rate of the extra chromosome and the breeding behavior of the mutant (selfed and F_1 seeds produced by crossing with normal including reciprocal) are being made.

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Teratogenic effects of azaserine in the Syrian golden hamster[1]

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Summary. Azaserine is a potent pancreatic carcinogen in the rat, but not in the Syrian golden hamster. The present study shows, however, that the hamster shares with the rat susceptibility to the embryotoxic and teratogenic effects of this drug.

Azaserine (O-diazoacetyl-L-serine), a structural analog of glutamine, was originally synthesized and used as an antitumor agent in the early 1950's[2-5]. Although the teratogenic properties of this drug on chick and rat embryos were soon demonstrated[6-8], only recently has it been shown to also have potent carcinogenic effects on the exocrine pancreas of the rat and other laboratory rodents[9]. Interestingly, the Syrian golden hamster was found to be resistant to the carcinogenic effects of azaserine[10]. No explanation for this resistance to azaserine carcinogenicity has yet been found, but in vivo studies indicate that the damaging effect of this drug on DNA of pancreas is similar in the rat and hamster[11,12]. The differential refractoriness of the hamster to the carcinogenic effects of azaserine encouraged us to investigate if this rodent was also resistant to the teratogenic properties of this drug.

Methods. Timed-pregnant Syrian golden hamsters of a standard, commercially available outbred line (Lak:LVG, Charles River Breeding Laboratories, Wilmington, Massachusetts, USA) were used. These were caged individually, given free access to food and water, and housed in a controlled environment room (14 light/10 dark h cycle) until used. In the morning (08.00 h) of the 8th gestational day (the beginning of the teratogenic 'critical period' in this species) hamsters of the experimental group were anesthetized with pentobarbital sodium (50 mg/kg) and given a single i.v. (lingual vein) or i.p. injection of a sterile solution of azaserine (Calbiochem-Behring, LaJolla, California, USA) at dose levels ranging from 2.0 to 3.0 mg/kg b.wt. Matching control hamsters were anesthetized and given i.v. or i.p. injections of sterile saline in volumes equivalent to those administered to the experimental animals ($\simeq$ 0.5 ml). All experimental and control hamsters were sacrificed under deep anesthesia on the 12th gestational day (beginning of the fetal period). Gravid uteri were removed from the animals and the number of gestation sacs (implantation sites) in each recorded. The uteri were then incised and counts made of the number of viable and dead fetuses recovered. Fetuses delivered live were examined, both in the fresh state and after preservation in Bouin's fixative, for gross external abnormalities only.

Results and discussion. Azaserine-treated pregnant hamsters displayed no outwardly obvious signs of toxicity. Their reproductive performance compared to controls is summarized in table 1. In azaserine-treated dams, the fetal death rate (resorptions and stillbirths) increased steeply over the narrow dose range of 2.0 to 3.0 mg/kg. However, differences in fetal death rates between dams injected by the i.p. vs the i.v. route were not statistically significant. The incidence of abnormalities in live fetuses delivered from the different groups of azaserine-treated dams ranged from 9 to 100%. No consistent dose or administration route

Table 1. Reproductive performance of azaserine-treated and control hamster dams[a]

Azaserine treatment mg/kg (route)	No. of dams	Gestation sacs		Live fetuses		Dead fetuses (stillborns and resorptions)			Abnormal fetuses (malformed, runted)		
		Total	Per dam	Total	Per dam	Total	Per dam	Mortality (%)	Total	Per dam	Percent of live fetuses
2.0 (i.p.)	3	34	11.3	26	8.7	8	2.7	24	6	2.0	23
(i.v.)	5	70	14.0	47	9.3	19	3.8	27	4	0.8	9
2.5 (i.p.)	8	113	14.1	49	6.1	64	8.0	57	7	0.9	14
(i.v.)	6	74	12.3	21	3.5	53	8.8	72	8	1.6	38
3.0 (i.p.)	2	24	12.0	4	2.0	20	10.0	83	4	2.0	100
(i.v.)	3	40	13.3	2	0.7	38	12.7	95	1	0.3	50
Saline control (i.p. or i.v.)	13	176	13.5	168	12.9	7	0.5	5	5	0.4	3

[a]Dams were treated on day 8 and fetuses were recovered on day 12 of gestation.

related differences in the incidence or type of fetal abnormality were observed in the litters delivered from these animals.

For comparison, Murphy and Karnofsky[7] in an earlier study on rats found that i.p. injection of dams with 2.5 mg/kg of azaserine on day 9 of gestation (comparable to day 8 in the hamster) resulted in a 100% fetal mortality rate in litters delivered near term. Similar administrations of the drug to dams on later days of gestation (10–13) resulted in fetal mortality rates of over 50%, while the incidence of abnormalities (primarily skeletal defects) among recovered fetuses ranged from 83% (day 10 injection) to 60% (day 13 injection). Injection of dams with azaserine after day 13 caused no fetal deaths or abnormalities. Table 2 is a tabulation of the specific gross abnormalities encountered in azaserine-treated and control fetuses. (Because the route of azaserine administration, i.p. or i.v., had no apparent influence on the incidence or type of fetal abnormalities produced, the data for these 2 treatment groups were combined in table 2.) Two types of fetal abnormalities predominated: runting (general growth retardation) and limb malformations.

In litters from saline-treated dams the only fetal abnormality observed was a low incidence of runting. Runting was much more common among the fetuses delivered from the azaserine-treated dams. The difference is the incidence of runts found among all fetuses from azaserine-treated dams (13/149) vs that in fetuses from control dams (5/168) was statistically significant ($p < 0.03$; χ^2 test).

In rodent species, fetal growth retardation has been noted after treatment with a variety of teratogens[13,14] and especially with antimetabolites of DNA synthesis. Azaserine, a structural analog of glutamine, blocks in incorporation of the amide group in purine biosynthesis, thus inhibiting de novo DNA synthesis[15]. This inhibitory effect has been demonstrated under both in vivo and in vitro conditions[15,16]. It is thus likely that the increased runting observed in fetuses treated with azaserine was the result of a generalized inhibition of DNA synthesis during embryonic development.

Approximately 15% of the fetuses recovered from azaserine-treated dams had malformations of one or more limbs (see footnotes to table 2). Most frequently the distal portion of a limb was malformed and there was an apparent predilection for the left side. In a few other cases, the entire limb was missing (amelia) or the limb was retarded in its normal developmental sequence (meromelia).

Similar types of limb malformations have been observed in azaserine-treated rat fetuses[8] and chick embryos[6,7]. These limb malformations were probably due to the previously mentioned inhibitory action of azaserine on DNA synthesis, affecting more specifically here the rapidly dividing cells of the apical ectodermal ridges in developing limb buds. Other inhibitors of DNA synthesis, for example, 5-mercaptopurine, have been shown to have a similar teratogenic effect on the limbs of rodent fetuses[14].

Table 2. Specific gross abnormalities in azaserine-treated and control fetuses[a]

Azaserine dose (mg/kg)	2.0	2.5	3.0	0 (Saline control)
No. dams	8	14	5	13
Abnormal/live fetuses	10/73	15/70	5/6	5/168
Limb defects	8[b]	10[c]	4[d]	0
Eye defects	0	1	0	0
Tail malformation	0	1	1	0
Edema (subcutaneous)	0	1	0	0
Hydropericardium	0	2	2	0
Runting	2	10	1	5

[a]Dams were treated on day 8 and fetuses recovered on day 12 of gestation. [b]4, retarded digital development (left fore and hind limbs); 1, amelia (left fore limb); 3, meromelia (distal left fore limb). [c]1, cleft paws (left fore and hind limbs); 1, cleft paw (right fore limb); 2, cleft paw (left fore limb); 5, retarded digital development (left fore and hind limbs); 1, retarded digital development (left hind limb). [d]2, amelia (left fore limb); 1, retarded digital development (left fore and hind limbs); 1, retarded digital development (left fore limb).

In conclusion, the results of this study show that the Syrian golden hamster is susceptible to the teratogenic properties of azaserine. However, on the basis of the limited data now available, it appears that the teratogenic response of the hamster to this drug is less pronounced than that of the rat. Additional comparative studies of the carcinogenic and teratogenic susceptibility of rats and hamsters are warranted. Especially intriguing is the observation that several related nitrosamines are potent pancreatic carcinogens for the Syrian golden hamster[17] but not for the rat[18]. The teratorgenic properties of these nitrosamines have not been reported.

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Spontaneous AKR lymphomas differ in their degree of malignancy and sensitivity to the polysaccharide levan

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Summary. Spontaneous AKR lymphomas differ in their biological behavior as judged by formation of local tumors at the site of inoculation, latency in the appearance of both local and distant tumors and mean survival time of the mice. Spontaneous AKR lymphomas differ markedly in their sensitivity to levan (polyfructose). An inverse correlation was observed between the degree of malignancy and sensitivity to levan.

AKR lymphoma has been shown to respond to therapy by the polysaccharide levan[2,3]. We have also observed that this tumor loses its sensitivity to levan following serial passages[3,4]. Serial passages also caused an increase in the malignant behavior of the tumor, a feature which was also found by another group[5]. The loss of sensitivity to levan seemed to be related to the degree of malignancy.

In the present study we investigated the malignant behavior and sensitivity to levan treatment of various spontaneous AKR tumors.

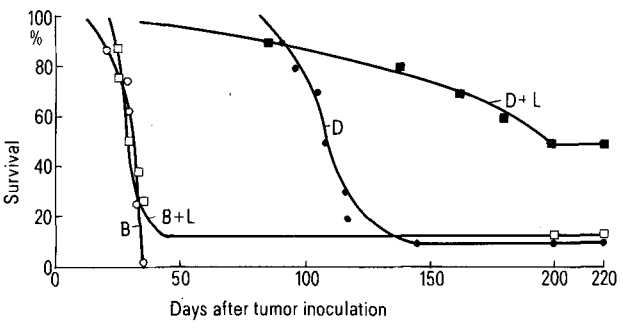
Materials and methods. Experiments were performed with AKR/Cu male mice, 6–10 weeks old, obtained from the Weizmann Institute, Rehovoth. Spontaneous tumors were obtained from aged AKR mice. Tumor cell suspensions were separately prepared from 4 spontaneous tumors. The cell suspensions were prepared from mesenteric lymph nodes as previously described[2]. 5×10^4 tumor cells from each spontaneous tumor were inoculated s.c. in the back of groups of 20 mice. Ten mice of each group were treated locally with levan obtained from the Department of Biological Chemistry, Technical Unit, the Hebrew University, Jerusalem. Levan was prepared from *Aerobacter levanicum* according to Hestrin et al.[6] and solutions were prepared according to Shilo et al.[7]. Daily injections of 10 mg in 0.2 ml saline per mouse, were begun on the day of tumor inoculation and continued throughout the experiment.

Local and distant (inguinal lymph nodes) growth was assessed by palpation every 2–3 days. Local growth, when present, was measured. Mortality of mice was recorded daily.

Results. The table shows the growth characteristics and sensitivity to levan of 4 spontaneous tumors, D, E, C and B, in the 1st transfer. The survival curves of untreated and levan-treated mice of tumors B and D are shown in the figure. The various spontaneous tumors differed from one another in the degree of malignancy as gauged by formation of local tumors at the site of inoculation, latency of local and distant tumor appearance, and mean survival time (MST).

The less malignant tumor was D; the most malignant were B and C. The latency of tumor appearance and the MST were positively correlated. Tumors D and E formed local growths at the site of inoculation, while tumors B and C did not. Although the capacity to form local tumors denotes a lesser degree of malignancy, no strict correlation was observed between the size of local tumors and MST.

The B tumor was much more aggressive than the D tumor; 100% of mice were already dead on day 35 in tumor B, while tumor D killed 9/10 mice only on day 145. Sensitivity to levan treatment was inversely proportional to the degree of malignancy of the tumor. While tumor D was effectively inhibited, tumor B was practically insensitive to the polysaccharide; in animals bearing the less malignant tumor D, 5/10 treated mice survived for 199 days, whereas in those injected with the malignant tumor B, only 1/10 remained alive by the 43rd day.



Survival of AKR mice inoculated with B and D tumors, treated or untreated by levan. 5×10^4 AKR lymphoma cells of the 2 spontaneous tumors B and D were inoculated s.c. in the back of 18 or 20 AKR mice. Eight animals inoculated with tumor B and 10 inoculated with D were not further treated. Similar numbers of mice were treated with levan. Tumor B: untreated, ○; treated with levan, □; tumor D: untreated, ●; treated with levan, ■.