Numerous fields chosen for electron examination showed many virus particles with tail structure. Fig. 1 shows broad tail forms from the intranasal shrew passage after virus cultivation in embryonated eggs. Virus particles from the intracerebral series appeared similar to those from the intranasal series. Fig. 2 shows an electron micrograph of the egg-adapted California strain carried only in embryonated eggs.

Summary. (1) NDV from short-tailed shrews infected by intracerebral inoculation and intranasal instillation was 100% pathogenic for embryonated eggs in the first embryo passage. Shrews infected by these two methods of exposure showed typical Newcastle disease symptoms. The virus in the allantoic fluid of eggs inoculated with the shrew brain material from both intracerebral and intranasal series was neutralized by positive Newcastle disease serum but was not affected by normal chicken serum. (2) Electron micrographs of Newcastle disease virus preparations from chick embryos following infection by the virus propagated in the short-tailed shrew showed a predominance of tailed forms. (3) Intracerebral and intranasal serial passages of NDV in the short-tailed shrew are being continued at the present time and will be reported in a later paper.

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Resistance in Leukemic Cells to a Guanine Analog, 8-Azaguanine. (19118)

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The development of resistance in mouse leukemic cells, lymphocytic in type, to folic acid antagonists has been reported(1,2). The resistant variants, in certain cases, proved to be partially dependent upon folic acid antagonists for optimal growth(3,4). Experimental evidence favors the assumption that specific changes in the hereditary mechanism of the cell have occurred, independent of the action of antagonists. The transformations produced were found to be stable, irreversible and heritable(4) and occurred generally in large populations of leukemic cells(5). The triazolopyrimidine analog of guanine, 5-amino-7-hydroxy-1H-v-triazolo (d) pyrimidine (8-azaguanine) has been shown to be moderately inhibitory to certain bacteria(6) and to *Tetrahymena*(7,8) and a definite inhibitory action toward certain experimental tumors has been observed(9) and confirmed (10-12). It is quite clear, however, that this metabolic inhibitor is entirely inactive against other tumors in mice and rats(10,11,13,14).

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TABLE I. Influence of 8-Azaguanine on Survival Time of Mice Bearing Transplants of Sensitive (Control) Leukemia L 1210.*

Exp.	Treatment	Strain of mice	No. mice	Dosage (mg/kg) Daily	Dosage (mg/kg) Total	Mean survival time (range) in days	% increase in survival time
1	Control	dba	10	0		10.1 (9-12)	
	8-Azaguanine		10	75	900	17.3 (16-19)	71.3
2	Control	dba	10	0		10.1 (9-12)	
	8-Azaguanine		10	75	900	17.1 (15-19)	69.4
3	Control	dba	10	0		8.8 (8-9)	
	8-Azaguanine		10	75	750	14.1 (13-16)	60.2
4	Control	BDF_1	8	0		10.6 (8-14)	
	8-Azaguanine		8	75	975	17.1 (11-25)	61.3

* Inoculations of a standard dose of leukemic cells done intraperitoneally. Injections of 8-azaguanine begun 24 hr post-inoculation. BDF_1 mice = ♀ C_{57} black × ♂ dba.

In certain acute lymphocytic leukemias of the mouse(12), particularly the transplantable leukemia, L 1210, a definite, regular and reproducible inhibition of growth results from parenteral administration of the guanine analog at near maximum tolerable doses (Table 1). It was of interest, therefore, to determine whether resistance to this anti-leukemic compound similar to resistance to anti-folics could be developed.

This report offers evidence for the development of resistance to 8-azaguanine and partial dependence for growth on this guanine analog in leukemia L 1210 and presents some of the pertinent characteristics of these resistant and dependent leukemic cells.

Transplant generations 100 to 124 of the sensitive subline of leukemia L 1210(15) have been used as control material for the present study. Beginning with the 100th transfer, a standard dose of leukemic cells (8×10^5 cells) in Lockes' solution was inoculated subcutaneously in the right axillary region, into dba strain mice which subsequently received daily injections of 75 mg/kg of 8-azaguanine. The guanine analog was dissolved in 0.01 N NaOH, and was injected subcutaneously on the left side, 0.05 cc per gram body weight. Injections were begun 24 hours following transfer of leukemic cells and were continued until the next transfer. As can be seen from Table II, during the first 7 serial transfers, subcutaneous growths were obtained for further transfer at from 13 to 18 days. At the 8th transfer, however, palpable growths were obtained at the 4th post-inoculation day and at 9 days the mean weight of the lymphomatous mass was 573.9 mg. Subsequently through the next 16 transfers sizable tumor growths were obtained in mice receiving a total dose of 600 mg/kg of 8-azaguanine, indicating the development of resistance. In contrast, identical transfer generations of the control subline, carried in untreated mice, remained sensitive to 8-azaguanine, showing nearly complete inhibition of growth of lymphomatous tissue at 9 days (Table II).

Partial dependence for growth of these resistant leukemic cells on the guanine analog is shown at transfers 18, 19, 21 and 23 in Table II. Significantly greater mean tumor weights were obtained in mice supplied with a total dose of 600 mg/kg of 8-azaguanine in contrast to mean weights of dba strain mice growing the resistant cells but deprived of the analog. Differences in survival time of mice growing the resistant line of leukemia L 1210, and supplied with or deprived of analog, also reflect a degree of dependence (Table III).

The possibility was considered that resistance to 8-azaguanine might extend to other agents exerting specific anti-leukemic effects. It can be seen by reference to Table II, transfers 19 and 21, that resistance to 8-azaguanine

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TABLE II. Response of 8-Azaguanine-Resistant and Sensitive (Control) Lines of Leukemia L 1210 to 8-Azaguanine and Other Anti-Leukemic Compounds.

Transfer generations	Strain of mice	No. mice	Time transplanted, days	Compound	Dosage (mg/kg) Daily	Dosage (mg/kg) Total	Tumor wt (mg) at 9 days	
				8-Azaguanine-resistant				
G–1 to 7	dba	3 each	13–18	8-Azaguanine	75	825–1500	0	
8	”	5	9	”	75	600	573.9	
9	”	10	”	”	”	”	173.6	
10	”	5	”	”	”	”	623.3	
11	”	10	”	”	”	”	287.3	
12	”	10	”	”	”	”	164.4	
G–13 to 15	”	3 each	”	”	”	”	Carried only	
16	”	5	”	”	”	”	725	
17	”	9	”	”	”	”	632.8	
18	”	5	”	0	0		545.6 ± 48.6	2.7*
		5		8-Azaguanine	75	600	204.6 ± 26.8	
19	BDF_1	10	”	”	”	”	382.1 ± 46.8	1.4
		10		0	0		274.5 ± 32.1	
		10		A-Methopterin†	3	12	8.8	
20	dba	5	”	8-Azaguanine	75	600	735	
21	”	10	”	”	”	”	462.6 ± 39.4	2.3
		10		0	0		205.2 ± 21.2	
		10		TEM	.5	2	129.4	
		10		A-Methopterin	3	12	5	
22	”	5	”	8-Azaguanine	75	600	700	
23	”	10	”	”	”	”	776.9 ± 27.5	2.4
		10		0	0		318.1 ± 40.2	
				8-Azaguanine-sensitive (control)				
8	dba	10	”	8-Azaguanine	75	600	43.5	
		10		0	0		682.8	
16	”	10	”	8-Azaguanine	75	600	26.7	
		10		0	0		1190	
19	BDF_1	10	”	8-Azaguanine	75	600	10	
		10		0	0		528.3	

* Dependency ratio: $\frac{\text{Mean tumor wt in mice given analog}}{\text{Mean tumor wt in antagonist-free mice}}$.

† A-methopterin and TEM given intraperitoneally every other day beginning at 24 hr post-inoculation.

TABLE III. Comparison in Response of 8-Azaguanine-Resistant and Sensitive Leukemic Lines to 8-Azaguanine.*

	Line of leukemia	Dosage (mg/kg) Daily	Dosage (mg/kg) Total	Survival time, days	Peripheral blood counts Hb	wbc	agran.	gran.	blasts	Organ infiltrations Lymph nodes	Spleen	Liver
							(16%)	(43%)	(41%)			
1	8-Azaguanine resistant	75	675	9.8 ± .16†	10.3	127150	20344	54675	52131	3+	3+	3+
							(53%)	(44.7%)	(2.3%)			
2	sensitive	75	675	See table I	15	18080	9582	8082	416	—	—	—
	”	None			9.2	91610	(30%) 27483	(48.5%) 44430	(22.5%) 19697	3+	3+	2+

* Inoculations of a standard dose of leukemic cells (8×10^5) done intraperitoneally. All determinations are at 9 days post-inoculation. Blood and histologic studies represent means of 5 mice each and survival times were determined on 20 mice in each group.

† In contrast, dba mice growing 8-azaguanine-resistant cells and deprived of analog had a mean survival time of 11.6 ± .31 days.

was not extended to 4-amino-N^{10}-methyl PGA (A-methopterin) nor to 2,4,6-triethylene-imino-s-triazine (TEM), these resistant cells being sensitive to the two compounds.

The morphology and biologic behavior of the 8-azaguanine-resistant subline grown in dba strain mice receiving 8-azaguanine resembles the sensitive control line grown in antagonist-free mice. At 9 days, in addition to growth of lymphomatous tissue subcutaneously, there results a florid leukemia with severe infiltration into lymph nodes, spleen and liver, leukocytosis with escape of lymphoblasts into the blood and a severe secondary anemia. The mean survival time of mice bearing the resistant and dependent cells and given 8-azaguanine is strikingly similar to the control line. Table III gives the comparative blood picture of dba mice growing the sensitive and resistant leukemic cells, the histologic pattern of infiltration and a comparison of the mean survival times of these groups.

It has been assumed by Kidder and coworkers(9) that neoplasms, unlike normal tissues, require guanine for growth and that 8-azaguanine acts as a competitive inhibitor of this changed metabolism of guanine by cancerous tissues. Results have been equivocal, however, relating to the question as to whether or not free guanine is metabolized by cancerous tissues in contradistinction to normal tissues(16-18). It is hoped that the variant leukemic cells described here, which are resistant to and partially dependent on 8-azaguanine for growth, will offer more suitable material for studies on the role of guanine in cancer.

Summary. (1) The development of resistance in a transplantable acute lymphocytic leukemia of the mouse to a guanine analog, 8-azaguanine, is reported. (2) Optimal growth of the resistant cells is attained in mice receiving near maximum tolerable doses of 8-azaguanine, indicating partial dependence of the variant line on the analog used in developing resistance. (3) The sensitive (control) line of leukemia has shown nearly complete inhibition of tumor growth at comparable transfer generations. (4) A-methopterin and TEM inhibit growth of the resistant line. (5) The biologic behavior of resistant leukemic cells, grown in mice receiving 8-azaguanine, strikingly resembles sensitive cells grown in antagonist-free mice: A florid leukemia develops with leukocytosis and escape of lymphoblasts into the blood, severe infiltration into lymph nodes, spleen and liver, resulting in death at approximately the same time as controls.

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Protein Metabolism of Amphibian Embryo. III. Incorporation of Methionine into Protein of Gastrulae.* (19119)

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An increasing number of studies is being made of regional differences in the physiology of the amphibian gastrula which may eventually elucidate the general problem of embryonic induction and, specifically, the mechanism whereby dorsal mesoderm (organizer of Spemann) evokes the differentiation of neural tissue from ectoderm. Friedberg and Eakin(1) showed that C^{14} of glycine is more rapidly incorporated into protein or intermediary compounds by the dorsal halves of gastrulae and neurulae than by the ventral

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