administered in dosage of 1 mcg/kg/minute for 10 minutes to unanesthetized dogs. Similarly the veratrum derivative was administered to unanesthetized dogs premedicated with toxic dosages of digitalis preparations and to others premedicated with toxic dosages of quinidine hydrochloride. The degree of cardiac slowing from the veratrum derivative was found to be a function of pre-existing cardiac rate. No such relation was found for the bradycardia produced by digitalis. Dogs which received digitalis and veratrum showed profound bradycardia. This action appeared to be mediated by increased vagal tone since the slowing was abolished by atropine. Rhythm disturbances due to digitalis were exaggerated by veratrum. A rise in Q-T/R-R ratio above that to be expected by changing cardiac rate was associated with the administration of quinidine hydrochloride. In normal animals and in those which received digitalis, Veriloid or both, the ratio was found to vary as a straight line function of the existing heart rate. Neither quinidine alone at 15 or 25 mg/kg nor quinidine and veratrum produced appreciable incidence of arrhythmias. Cardiac slowing was observed in dogs receiving veratrum after the 15 mg/kg dose of quinidine but not after the 25 mg/kg dose.

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Action of Tri-Ethylene Melamine in Mice.* (18533)

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Cytotoxic action has been demonstrated with tri-ethylene melamine, a compound which is structurally related to the hydrolysis products of the nitrogen mustards. In a series of *in vitro* studies on lymph nodes from patients with reticulum cell sarcoma, chronic lymphatic leukemia, and lymphosarcoma, inhibition of fibroblast growth was noted(1), as well as granular disintegration of lymphocytes(2). Successful *in vivo* inhibition of certain sarcomas and leukemias in lower animals(3-7) has been obtained, as well as clinical remission in patients with malignant lymphomas(8,9) and a case of mycosis fungoides(9). Signs of toxicity were evidenced by a dramatic fall in white blood cell count(9,10), with a decrease (9,10) or an increase(9) in polymorphonuclear cells. The effect on erythrocytes was negligible, though the rat erythropoietic system was more sensitive(10). Repeated administration of effective doses at prescribed intervals was necessary in clinical application (9) and therefore the duration of toxic effect was important.

The pharmacological studies on tri-ethylene melamine were undertaken to observe the general toxic effect, as well as the latent period and duration of toxicity. The latter was of great importance since repeated administra-

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TABLE I. Mortality Rate in Mature Female Albino Mice Receiving Single Injections of Tri-Ethylene Melamine Intraperitoneally.

Dose, mg/kg	Mortality	Day of death*				
		1-2	3-4	5-6	7-8	9-31
.25	1/10					1
.5	0/10					
1	0/10					
2	0/10					
3	0/11					
4	10/17		7	2	1	
5	12/17		7	4	1	
6	7/7		2	5		
7	6/6		3	3		
8	6/6		3	3		
9	6/6		3	3		
10	11/11		5	6		
20	5/5			5		
40	5/5	3	2			
80	5/5	5				
Total	74/136	8	32	31	2	1

* Day of death represents No. of days after inj.

TABLE II. Mortality Rate in Mature Female Albino Mice Receiving 2 Inj. of Tri-Ethylene Melamine Intraperitoneally.*

Each dose	Total dose, mg/kg	Mortality	Day of death†		
			5-6	7-8	9-31
.25	.5	1/4		1	
.50	1	0/5			
1	2	0/5			
2	4	0/5			
3	6	5/6		1	4
4	8	5/6			5
5	10	4/5	4		
Total		15/36	4	2	9

* Tri-ethylene melamine administered one month interval.

† Day of death represents days after second inj.

tion of sublethal doses at intervals might cause death by cumulation.

Procedure. Saline solution of tri-ethylene melamine† was administered intraperitoneally in one or two doses to female albino mice (Swiss strain). Single injections were administered at a one month interval to surviving animals, as indicated in Table II. The median lethal doses in mg/kg were calculated on the basis of size of individual dose at various intervals according to the method of Reed and Muench(11). Peripheral blood counts were taken periodically from the tail vein, using a different mouse each time.

Effect of single injection. The effect of the administration of a single dose of tri-ethylene melamine is indicated in Tables I, IV and Fig. 1. All mice receiving 80 mg/kg and three out of five of those receiving 40 mg/kg died within 24 hours. The greatest percentage of deaths occurred between the third and sixth days, with only one mortality after the eighth day (twenty-third day). The median lethal doses calculated for the second, fourth, sixth, eighth, and twenty-third days after injection (Table III) indicated a short latent period in toxic effect. Total white blood cell counts (Table IV, Fig. 1) started to decrease within twenty-four hours, especially at the higher dosage levels. A decrease in percentage of mononuclear cells occurred at that time and continued for one month after injection, even though the total white blood cell counts had returned to normal between the sixth and tenth days. This return was slower at higher doses (Table IV). The effect on the white blood cell picture was less marked at doses lower than 3 mg/kg. No effect on the erythropoietic system was observed. The mortality rate correlated with the occurrence of severe leukopenia on the third and fourth days (Fig. 1), with the greatest number of deaths occurring between the third and sixth days.

Effect of two injections. Mortalities due to the administration of the second dose at a one month interval are presented in Table II. Deaths occurred from 6 to 31 days after the second injection, with 9 out of the 15 deaths occurring after the eighth day. The median lethal doses calculated on the eighth, tenth, seventeenth, eighteenth, twenty-seventh and thirty-first days after the administration of the second dose indicated a long delay in toxic effect (Table III). Hematological changes observed were similar to those obtained with single injections, but there was no correlation between the leukopenia and mortalities.

Comparison of latency in single and double doses. Table V represents a method of comparing latency in mortality suggested by Lees

† Furnished through the courtesy of the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

11. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

TABLE III. Median Lethal Doses in mg/kg for Tri-Ethylene Melamine on Various Days After Injection.

No. of inj.	Days after inj.									
	2	4	6	8	10	17	18	23	27	31
1	40.17	4.74	4.14	4.13	4.13	4.13	4.13	4.06	4.06	4.06
2*				4.43	4.08	3.63	3.50	3.50	2.87	2.70

* Median lethal doses calculated for second inj. only.

and Lees(12). Column 7 rpresents the ratio between the death rate fifteen to thirty-one days after injection and the death rate through the eighth day. The single figure obtained in this ratio demonstrated a longer latent period after the administration of a second dose.

Discussion. Supralethal doses of tri-ethylene melamine (40 and 80 mg/kg) produced early death, whereas a latent period of from 3 to 8 days occurred at lower doses. Severe leukopenia did not occur until the third and fourth days which might have been responsible for the high percentage of mortalities at 3 to 6 days after injection, and the sharp drop in median lethal dose on the fourth day. The toxic effect of the drug seemed extinguished when the total white blood cell counts had become normal. However, the effect of the drug was not completely eliminated and its effect on the mononuclear cell count continued for one month. At this time, when a second dose was administered, it was assumed that further deaths would not be due to cumulation. The median lethal dose for the second dose was 2.5 mg/kg which was considerably lower than the 4.06 mg/kg obtained with the first dose. Even after one month, the influence of the first dose was not eradicated and thereby reduced the tolerance to the second dose. The latent period in mortalities was much longer after the second dose. Previous administration of sublethal doses may have induced a certain amount of resistance resulting in delayed mortality or there may have been a delay in the cumulative effect.

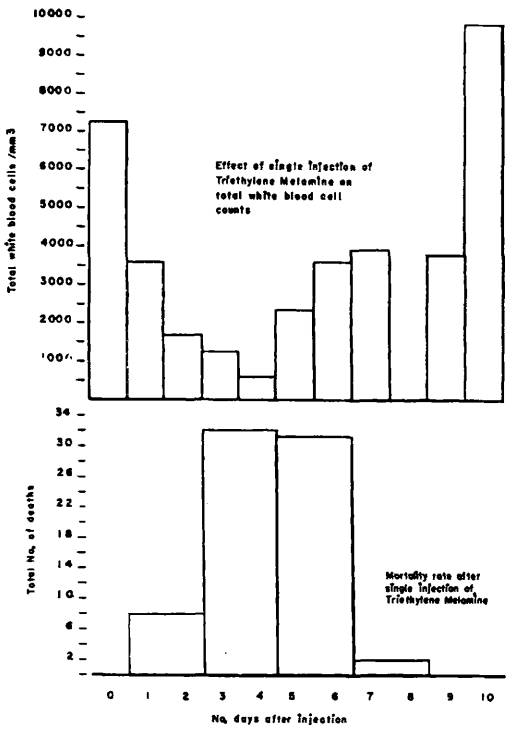

FIG. 1.

Toxic effects on mature female albino mice of single injections of 3 to 80 mg/kg of triethylene melamine.

The mortalities after single injection of a lethal dose were temporally associated with severe leukopenia whereas the mortalities after the second dose were not. This would indicate that leukopenia alone was not responsible for mortalities and the drug had a toxic effect on other tissues in addition to the hemopoietic system. This was further confirmed by the fact that a second dose was administered when the blood picture had returned to normal and animals died at a lower dosage level.

Summary and conclusions. Tri-ethylene melamine decreased the total white blood cell counts and the percentage of mononuclear cells. The total white blood cell counts returned to normal between the sixth and tenth days after injection and the mononuclear count returned to normal after one month.

12. Lees, T. W., and Lees, J. C., *Cancer*, 1950, v3, 580.

TABLE IV. Hematological Data on Mature Female Albino Mice 15 Days After Injection of Tri-Ethylene Melamine Intraperitoneally.*

Dose, mg/kg	Leukocytes†	Days after injection											
		1	2	3	4	5	6	7	9	10	11	14	15
3	WBC/mm³	5650	1600	6100	850	2350	3600	5650	5250	12800	6800		3250
	PMN %	40	27	10	33	15	15	31	51	23	37		17
	E %	6	1	0	3	0	0	0	1	2	11		1
	MNC %	54	72	90	64	85	85	69	48	75	52		82
4	WBC/mm³	4300	1650	1150	550‡			2450	5100		4500	4425	11800
	PMN %	34	57	14				35	31		65	26	25
	E %	6	0	0				0	0		0	2	1
	MNC %	60	43	86				65	69		35	72	74
5	WBC/mm³	2620	2000	650‡	350‡			1925	1200	12600		6350	15300
	PMN %	59	56					32	55	59		20	50
	E %	3	0					0	0	0		11	1
	MNC %	38	44					68	45	41		69	49
6	WBC/mm³	4100	1100		500‡								
	PMN %	50	40										
	E %	5	0										
	MNC %	45	60										
7	WBC/mm³	3750	1250	400‡									
	PMN %	37	70										
	E %	7	1										
	MNC %	56	29										
8	WBC/mm³	3050	1800										
	PMN %	37	77										
	E %	7	0										
	MNC %	56	23										
9	WBC/mm³	3200	2900										
	PMN %	83	93										
	E %	3	0										
	MNC %	14	7										
10	WBC/mm³	1600	1550	750‡									
	PMN %	67	79										
	E %	1	2										
	MNC %	32	19										
20	WBC/mm³	1750		500‡									
	PMN %	86											
	E %	0											
	MNC %	14											
40	WBC/mm³	6100		800‡									
	PMN %	81											
	E %	1											
	MNC %	18											

* Peripheral blood counts from tail vein of a different mouse each time.
† Leukocytes: WBC = Total white blood cells
PMN = Polymorphonuclear granulocytes
E = Eosinophils
MNC = Mononuclear cells—lymphocytes and monocytes.
‡ No differential count because of scarcity of leukocytes on smears.
Controls: WBC/mm³ = 3100-12700; PMN % = 9-20; E % = 0-10; MNC % = 72-90.

The highest percentage of deaths after single injections occurred between the third and sixth days when the total white blood cell counts were lowest. Surviving animals developed a resistance to a second injection, which manifested itself in a long latent period. Nine out of fifteen of the deaths in the latter group occurred between the ninth and thirty-first days, during which period the white blood cell counts were normal. The median lethal dose after a second injection was considerably lower due to the cumulative effect. The toxic effects of tri-ethylene melamine were evidenced in the dramatic fall in white blood cell

TABLE V. Mortality Rates After Injection of Tri-Ethylene Melamine.*

Drug	Each dose, mg/kg	Proportion dying, 0-8th day	Mortality rate, 0-8th day	Proportion dying, 15-31st day	Mortality rate, 15-31st day	Column 6 / Column 4
Column 1	2	3	4	5	6	7†
Tri-ethylene	.25	1/4		0/3		
melamine	3	1/6		4/5		
in 2 doses	4	0/6		2/3		
	5	4/5		0/1		
		6/21	.29	6/12	.50	+1.72
Tri-ethylene	4	10/17		0/7		
melamine	5	12/17		0/5		
in 1 dose						
		32/34	.97	0/12	0	0

* Only data satisfying the following conditions used: Dose caused 20% or more deaths; dose did not cause more than 80% deaths during first 8 days.

† Column 7 represents: Death rate for later period—15-31st (Column 6) over death rate for earlier period—0-8th days (Column 4).

count and decrease in percentage of mononuclear cells. However, the return to a normal blood picture was no indication that the effects of the drug had been extinguished.

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Aureomycin and Terramycin Treatment of *Pasteurella multocida* Infection and Neomycin's *in vitro* Effects on *P. multocida*.* (18534)

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Pasteurella multocida is primarily an animal pathogen. Recent evidence, however, indicates that it is encountered in infections of man more commonly than is generally realized(1,2). For this reason the determination of its susceptibility to various antibiotics is of interest. It was shown in a comparative study, using 8 strains, that *in vitro* aureomycin, chloromycetin, terramycin, polymyxin B and penicillin are highly effective, that streptomycin is somewhat less efficacious and bacitracin ineffective against this microorganism(3). Independently, Prier(4) reported that penicillin and streptomycin are somewhat inferior to aureomycin. Little(5) and Prier (4) found aureomycin to be effective in experimental infection of 1-day-old chicks and chick embryos. The experiments to be reported here were carried out to determine (1) the prophylactic and therapeutic efficacy of terramycin in experimental *P. multocida* infection of mice, (2) the *in vivo* effectiveness of aureomycin in an experimental animal other than previously reported, namely, the mouse and (3) the *in vitro* bacteriostatic potency of neomycin, which as yet has not been investigated.

Material and methods. Of the strains of *P. multocida* available at this laboratory and

* Aided in part by a grant of Lederle Laboratories Division, American Cyanamid Co.

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