dose of virus, makes this highly improbable.

Summary and conclusions. The effectiveness of the autolyzed normal mouse brain method for isolation and adaptation of poliomyelitis virus was investigated in 19 experiments with mice and 4 experiments with cotton rats. Three monkey adapted strains (Tennessee, Mahoney and Buffalo) and 12 stool specimens from poliomyelitis patients were used. The Lansing strain was also included in the experiments.

In the experiments with mice, 2 strains of neurotropic viruses were isolated. However, the evidence presented suggests that the strains isolated were of mouse origin. The cotton rat experiments were entirely negative. A shortening of the incubation period in mice inoculated with the Lansing strain was not observed.

The data presented do not confirm the effectiveness of this method for the isolation or adaptation of poliomyelitis virus.

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Actions of 2,6-Diaminopurine in Mice, Rats, and Dogs.* (17448)

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Recent studies have shown 4-aminopteroylglutamic acid (4-amino-PGA) to cause changes in hematopoietic tissues and intestinal mucosa which closely resemble lesions found in folic acid-deficient manmals.[1,2] These findings and the fact that 4-amino-PGA and its congeners act as competitive antagonists of folic acid (PGA) in certain microorganisms[3-6] have led to the conception that the agents damage tissues in mammals by interference with metabolic functions of PGA.[1,2,5,6]

Studies of derivatives of naturally occurring purines and pyrimidines have uncovered another antagonist of PGA in microorganisms,[7] namely, 2,6-diaminopurine, which also appears active in higher organisms. Thus 2,6-diaminopurine delays development of an experimental leukemia in mice[8] and inhibits estrogen-induced growth in chick oviduct.[9] The purine is related structurally to 4-amino-PGA insofar as both compounds are condensed ring-systems containing 2,4-diamino-pyrimidine. These observations suggested that the purine might act as an antagonist of PGA in mammals. The following studies were undertaken to determine in detail, sites of action of 2,6-diaminopurine in mice, rats and dogs and to compare the lesions obtained with those seen after administration of 4-amino-PGA.

Procedure. Mice, rats, and dogs of the same strains and corresponding in sex, weight, age and maintenance to animals used in previous studies with 4-amino-PGA[1,2] were sub-

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TABLE I.
Toxicity of 2,6-Diaminopurine Following Intraperitoneal Administration.

Species	No. of inj.	Dose, mg/kg/day	Mortality	Day of death					Approx. LD50† mg/kg/day
				1	2	3	4	5–14	
Mouse	1	395	12/12	10	2				170
		198	11/12		9	2			
		99	0/12						
Mouse	5*	158	12/12		5	3	4		90
		79	2/12			2			
		40	0/12						
Rat	1	316	12/12	12					100
		158	11/12	7	2	2			
		79	3/12	1		1		1	
		40	0/12						
Rat	5*	158	12/12	6	6				45
		79	12/12		7	4	1		
		40	2/12					2	

* Administered on 5 successive days.
† Estimated on log-probability paper.

jects of the present investigation. Procedures followed herein were also similar to those described previously. The purine‡ was used as the lactate or hydrochloride which is more soluble than the free base and was injected at physiologically tolerable temperatures after heating to dissolve the salts.

Course of intoxication in mice and rats. Animals receiving large intraperitoneal doses of 2,6-diaminopurine (≧ 500 mg/kg) evidenced ataxia, weakness and dyspnea within 5 to 15 minutes. The initial signs of intoxication increased progressively in severity and animals succumbed with respiratory failure within 1 to 6 hours. Single lethal doses, less than 500 mg/kg caused death usually within 24 to 48 hours (Table I). Such animals lost approximately 20% of initial weight and exhibited severe diarrhea prior to death. Rats and mice which survived single, large doses lost less than 10% of initial weight during the first 24 to 48 hours and were fully recovered before the end of the first week. Repeated doses of diaminopurine had similar, though delayed, effects (Table I). On the basis of toxicological data the response of mice and rats to diaminopurine may be differentiated from that following 4-amino-PGA. Regardless of the dose employed, animals receiving 4-amino-PGA did not respond with early manifestations of intoxication and survived for at least 3 days after poisoning. Furthermore, repeated administration of the PGA-analog proved cumulative in effect and more toxic than injection of single doses.[1] Table I suggests that repeated doses of diaminopurine equivalent to large fractions of the acute LD_{50} were tolerated without fatality.

Lesions in rats. Animals receiving 100 mg/kg/day, and sacrificed after 24 and 48 hours showed at autopsy distended gastrointestinal canals filled with yellow fluid. Femoral bone marrow contained purple fluid instead of a gelatinous, greyish red substance. Findings in peripheral blood and smears of femoral marrows obtained at time of sacrifice are found in Table II. The findings in peripheral blood indicated no significant change in leucocytes or reticulocytes. Hemoglobin concentrations were elevated indicating hemoconcentration. Sections of sternal and femoral marrow showed progressive depletion of total nucleated elements estimated to be about 50% after 24 hours and 80% after 48 hours. Studies of

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TABLE II.
Hematological Data from Rats Treated with 2,6-Diaminopurine. (No. of animals used shown in parentheses).

Group	Dosage, mg/kg/day	No. of inj.	Day of sacrifice		Peripheral blood				Femoral marrow				
					GRCYT, $10^{-3}/mm^3$	LYCYT, $10^{-3}/mm^3$	HB, g/100 cc	RETIC, %	N-GRCYT, %	EOS-GRCYT, %	MYEL, %	ERYTH, %	LYCYT, %
Control*				Mean	1.2(11)	5.8(11)	13.4(11)	11(6)	33(12)	6(12)	19(12)	35(12)	9(12)
				range	0.4-2.7	1.9-12.4	12.1-15.5	7-17	10-57	2-20	10-30	16-70	3-21
Acute	100	1	1		2.4	0.4	17.6	8.0	21	3	12	16	48
					3.2	1.7	18.0	15.3	21	3	12	20	44
					4.6	3.0	16.5	6.3	14	2	8	26	50
					3.4	2.6	17.6	7.2	37	3	5	16	39
		2	2		1.0	1.2	—	3.0	7	6	15	40	32
					0.1	2.9	15.8	3.2	17	1	19	28	35
					0.6	2.1	13.8	8.2	6	3	9	33	49
I	20,40	12-17	17-25	Mean (8)	3.0	8.7	13.2	1.3	29	15	24	23	9
				range	0.6-4.7	2.8-19.8	7.0-15.4	0-4.9	8-54	3-26	14-40	7-35	2-30
II	20,40,79	15-21	22-30	Mean (8)	1.4	1.9	13.7	0	10	8	23	17	42
				range	0.4-2.8	0.4-4.4	10.7-18.1	0	2-25	3-14	6-31	3-35	26-65

* From data published previously.[1]
EOS-GRCYT, eosinophilic granulocytes.
GRCYT, granulocytes.
LYCYT, lymphocytes.
MYEL, metamyelocytes, myelocytes, and myeloblasts.
ERYTH, nucleated erythroid cells.
HB, hemoglobin.
N-GRCYT, neutrophilic granulocytes.
RETIC, reticulocytes.

sections and differential counts of nucleated elements in smears of femoral contents (Table II) revealed increased proportions of lymphocytes. These were located in the blood-filled, dilated sinuses of the marrow. No foci of necrosis or degeneration were found.

Histological examination of gastrointestinal tissues from the above animals revealed a general hyperemia. Actual lesions were confined to ileum and colon. Tips of villi were distended by enlarged vessels and plasma transudate under epithelial linings. Dilated capillaries in sub-mucosa, adjacent to muscular coats and surrounding crypts, contained increased numbers of leukocytes. Migration of leukocytes into the lumen of the crypts was evident, forming plugs with desquamated epithelial cells. In crypt-epithelium mitosis was almost absent and enlargement of nucleus and cytoplasm as well as desquamation with pyknosis was observed.

Animals receiving repeated injections for 17 to 30 days are listed in Table II (group I and II). Group I received either 20 mg/kg/day during the first three weeks followed by injections of 40 mg/kg/day for the remainder of the period, or 40 mg/kg/day throughout. Group II was treated in similar fashion but, in addition, was given prior to sacrifice 2 or 3 final daily doses of 79 mg/kg. Significant weight loss was observed only in rats of group II after receiving 79 mg/kg/day.

In spite of prolonged treatment animals of Group I and II were less affected than "acute" rats. However, the effect of diaminopurine on erythropoiesis was readily discernible in animals receiving the extended treatment. Depletion of nucleated red cells in marrow and reticulocytopenia were significant (I and II, Table II). Anemia also occurred in 2 of each group. No significant alteration occurred in erythrocyte-volume or content of hemoglobin. Only 6 of 8 animals in group I revealed depletion of nucleated cells in bone marrow, approximating 50%, while reduction in group II was no greater than that found in "acute" rats. Increased proportions of lymphocytes appeared only in marrows of group II (Table II). A predominance of basophilia in nucleated red cells and hypersegmentation of polymorphonuclears were evident in marrows of both groups. Gastrointestinal lesions were not found microscopically in group I and were confined to minimal epithelial changes in crypts of colon and ileum in 3 of 8 rats in Group II.

Histological examination of other organs of animals in the 3 groups described above, revealed no significant lesions but for a moderate reduction of lymphoid tissue in thymus, spleen, mesenteric nodes, and intestinal wall. Reduction of myeloid tissues in spleen was also noted in animals with depleted marrows.

Course of intoxication in dogs. The administration of 40 or 50 mg/kg in dogs 1 to 4 (Table III) produced an immediate, transient hyperpnea. Vomiting occurred at 3 hours and continued profusely until death. Food was refused after the first day. Hemorrhagic diarrhea appeared within 24 hours and increased in severity progressively. During the course of intoxication animals lost weight (Table III) and developed severe upsets in fluid and electrolyte excretion. Thus, dogs 3 and 4 excreted less than 500 cc/day of urine prior to treatment while during 54 hours of intoxication the former produced 8 l of fluid (urine, vomitus, and loose stool) containing 184 m eq Cl^- and the latter, 5 l containing 247 m eq Cl^-. Intake of water *ad libitum* was increased in proportion. Dogs 1 and 2 succumbed after 40 hours; dog 4, after 54 hours; and dog 3 was sacrificed when moribund at 54 hours.

The remaining dogs (5 to 8, Table III) receiving lower daily doses of diaminopurine evidenced fewer signs of intoxication. Dogs 6, 7, and 8 lost weight. Occasional vomiting and diarrhea without blood occurred only in the last few days prior to their sacrifice (4, 29 and 31 days respectively). Dog 5 recovered after a moderate weight-loss (Table III and Fig. 1).

The course of lethal intoxication in dogs receiving large doses of diaminopurine can be of shorter duration than in animals given supralethal doses of 4-amino-PGA. Protracted emesis is not a feature of intoxication with the latter agent. Moreover, diarrhea does not appear in dogs poisoned with 4-amino-

TABLE III.
Hematological Data from Dogs Injected Intravenously with 2, 6-Diaminopurine.

				Peripheral blood				Sternal bone marrow					
Dog	Day	Daily dosage, mg/kg	Wt, kg	HCRIT, %	RETIC, %	GRCYT, 10-3/mm3	LYCYT, 10-3/mm3	NCC, 10-3/mm3	N-GRCYT, %	EOS-GRCYT, %	MYEL, %	LYCYT, %	ERYTH, %
1	0	50	16.3	43.0	0	17.3	.7	170	32	2	14	8	44
	1		14.6	53.5	0	17.6	0	36	90	1	1	7	1
2	0	50	15.7	54.0	pr	10.6	1.6	245	63	6	18	2	11
	1		14.7	62.5	0	16.0	.2	25	95	1	2	1	1
3	0	40	19.2	41.5	2.2	18.4	1.0		46	4	23	2	25
	2		16.8	59.0	0	5.8	3.7		72	2	3	14	9
4	0	40	19.9	39.0	0	36.0	3.6		49	5	19	4	23
	2		16.2	58.5	.3	38.2	0		97	1	pr	0	2
5	0	20	13.6	44.0	.3	16.7	4.2	45	54	2	12	21	11
	4		13.3	41.5	0	9.8	1.1	10	85	0	5	10	0
6	0	20	10.6	41.0	.6	20.4	1.3	80	67	1	13	8	11
	4		8.2	51.0	0	11.2	.3	14	87	0	8	4	1
7	0	8	18.3	40.0	.7	9.0	1.2	65*	37	3	14	16	30
	18	20	16.7	37.0	0	15.6	.5	80†	70	5	12	8	5
	28		13.8	40.5	0	.1	.1	7	9	3	83	3	2
8	0	8	19.0	48.0	.6	10.8	.8	220*	41	3	8	5	43
	18	20	17.6	45.0	0	7.6	.2	31†	61	7	21	8	3
	28		14.1	50.5	0	5.9	.1	15	80	5	12	2	1

* Value at 6 days.
† Value at 21 days.

HCRIT, hematocrit.
NCC, nucleated cell count.

pr, present, less than 0.5%.
Other abbreviations as in Table II.

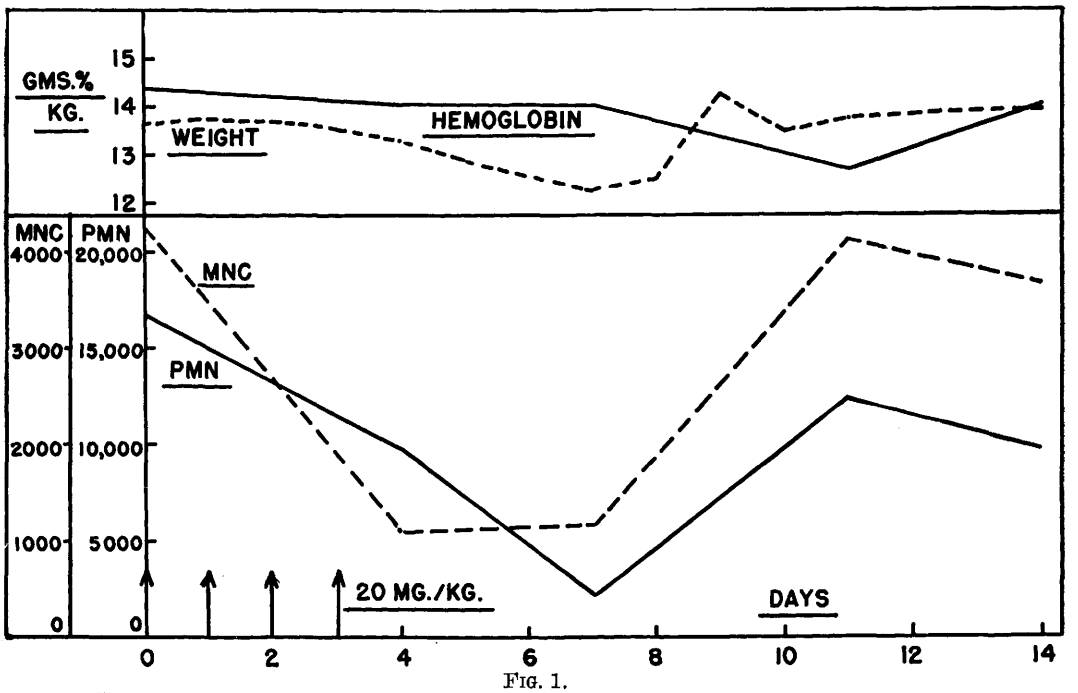

FIG. 1.
Changes in weight and peripheral blood of dog 5, following repeated doses of diaminopurine. MNC, lymphocytes and monocytes/mm³; PMN, granulocytes/mm³.

PGA until after 48 hours and death does not occur before 72 hours.[2]

Lesions in dogs. At autopsy all dogs appeared dehydrated. Bone marrow in sternum, ribs, and vertebrae appeared dry and sparse; femoral marrow, brownish and fatty. The intestinal tract of dogs 1 to 4 was edematous from duodenum to anus. Petechial hemorrhages were visible in ileum and colon and extensive around the anus. The contents of the gut were fluid and blood-stained. Dogs 6, 7 and 8 showed only edema of the intestinal mucosa.

Findings in peripheral blood are noted in Table III. Hemoconcentration developed in dogs 1 to 4 and 6 and might have been masked in dogs 7 and 8 by coexisting anemia. No changes in erythrocyte-volume or hemoglobin-content were noted. Reticulocytes and lymphocytes decreased in numbers in most animals. Granulocytopenia developed in dogs 5 to 8, which had been observed for more than 48 hours. Details of changes in peripheral blood of dog 5 are noted in Fig. 1.

Bone marrow was studied with aspirations from sternum and both iliac crests as well as with tissue-blocks taken at autopsy from ribs and femur. Only values from sternal marrow are reported in Table III since they corresponded closely to samples from other sites. A reduction of total nucleated cells was noted in all animals when measured. This observation was confirmed by study of smears and sections which contained only scattered islands of hematopoietic tissues. The outstanding feature of the depletion was the marked reduction of erythropoietic tissues. Surviving cells of the erythroid series included the naturally occurring erythrocytes with nuclear remnants, normoblasts, and erythroblasts. Although myeloid cells shared in the general depletion, all dogs with one exception evidenced increased percentages of polymorphonuclear granulocytes with relative decreases of immature myeloid forms. The marrow of the exceptional dog, 7, contained at 28 days, a high percentage of myelocytes in association with an intercurrent infection. Details of marrow findings in dog 5 are shown in Fig. 2.

Histological study of the gastrointestinal

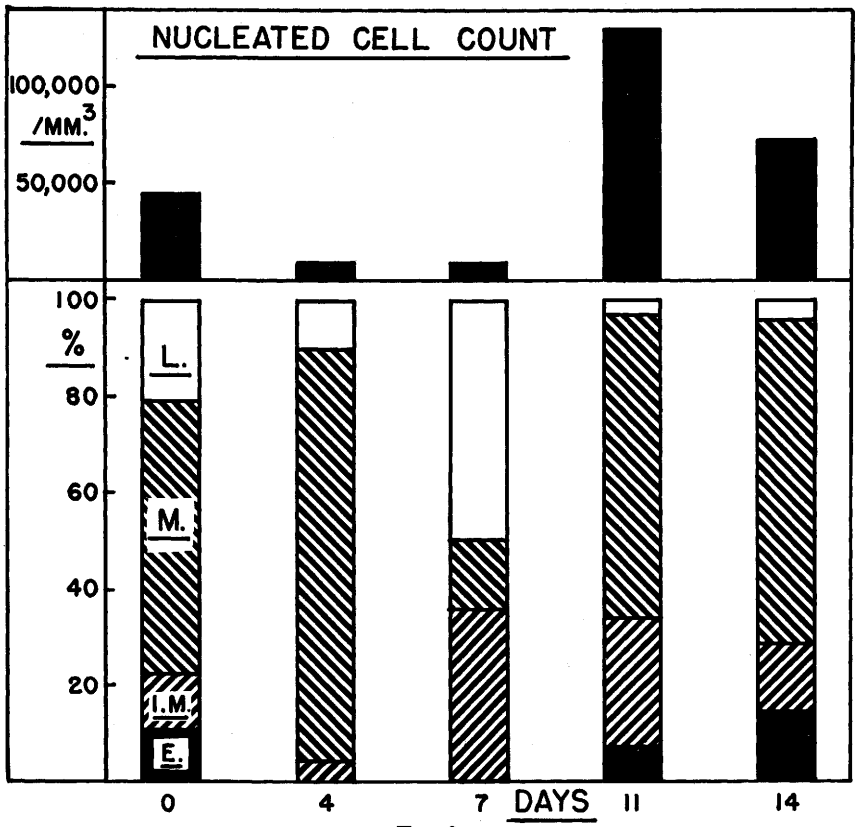

FIG. 2.

Changes in cell-count and differential count of nucleated cells from sternum of dog 5, following repeated doses of diaminopurine. L., lymphocytes; M., polymorphonuclears; IM., myeloblasts, myelocytes and metamyelocytes; E., red cells.

tract of dogs 1 to 4 revealed changes similar to those described above in rats receiving 100 mg/kg/day of diaminopurine. In addition, superficial ulcers of mucosa and extensive matting of villi occurred in ileum and colon. The remaining animals showed only edema and hyperemia of the intestinal mucosa. Changes in other tissues resembled those seen in rats.

Discussion. Since other purines such as caffeine[10] or adenine[11] are known to cause a variety of acute pharmacological actions, it was not unexpected that large doses of 2,6-diaminopurine should result in death within a few hours after administration. Indeed, the gross behavior of rats and mice is similar following equivalent doses of either 2,6-diaminopurine or adenine (6-aminopurine) greater than 500 mg/kg.[12] On the other hand 4-amino-PGA is inert from the standpoint of acute pharmacological effects.[1,2] At present it does not appear pertinent to compare diaminopurine and 4-amino-PGA on the basis of such actions which bear no known relationship to metabolite-antagonism. More appropriate is a comparison of lesions in intestines and bone marrow caused by both agents. Their effects on mucosa of ileum and colon of rats and dogs cannot be differentiated by histological studies. The depletion of hematopoietic tissues following effective doses of either agent can be of similar severity. However, certain features of the disturbance in hematopoiesis caused by 4-amino-PGA in dogs are unique

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[11] Drury, A. N., *Physiol. Rev.*, 1936, **16**, 292.

[12] Philips, F. S., Thiersch, J. B., and Bendich, A., unpublished observations.

such as the appearance of abnormal mitosis and "nuclear explosions" in normoblasts, alteration of the nuclear pattern of mature and primitive erythroid cells and formation of cells resembling megaloblasts, and production of bizarre, giant metamyelocytes.[2] Similar pathological cells have not been observed in dogs receiving diaminopurine. Therefore, it cannot be inferred that the agent acts as an antagonist of PGA in the metabolism of hematopoietic tissues.

Summary. The administration of large doses of 2,6-diaminopurine in mice and rats can result in death within a few hours. In dogs, large doses caused protracted vomiting, hemorrhagic diarrhea, dehydration and death within 48 hours. Doses permitting survival of rats and dogs for 24 hours or longer produced depletion of bone marrow and damage to epithelium of colon and ileum. The course of intoxication and changes in bone marrow permit a differentiation between the actions of 2,6-diaminopurine and a folic acid-antagonist like 4-aminopteroylglutamic acid.

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Effect of Mercury on Renal Tubular Transfer of p-Aminohippurate and Glucose in Man. (17449)

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The depressant effect of intravenously administered salyrgan (mersalyl) on the maximum renal tubular secretion of p-aminohippurate (Tm_{PAH}) in man has been demonstrated.[1,2] The inability of the mercurials, salyrgan and meralluride (mercuhydrin), to depress the Tm_{PAH} in the dog has also been shown.[2,3] That the maximum tubular reabsorption of glucose (Tm_G) in the dog is unaffected by mercury chloride or meralluride in dosages of 4 mg of bound mercury per kilogram of body weight has been established recently.[3] With regard to the Tm_G in man, Weston *et al.*[4] have reported that the intravenous administration of 2 cc of mercuzanthin or thiomerin, "immediately after several ten minute control periods generally resulted in a 40-80% decrease."

Thus the current concept is that intravenously administered mercurials depress the renal tubular transfer systems for PAH and glucose in man but leave unaffected these systems in the dog. It is the purpose of this study to re-examine the effect of a mercurial, mercuzanthin, on the Tm_{PAH} and Tm_G in man.

Method. Ten fasting male subjects were used in the Tm_G observations. Five of the 10 subjects used for the Tm_G observations plus an additional subject were used in the Tm_{PAH} determinations. All of the subjects were free of demonstrable renal dysfunction. The study pattern was the same for all of the subjects. Using the saturation methods outlined by Goldring and Chaisis[5] 3 control urine collection periods, each of 12 minutes duration, were obtained. Two cc of mercuzanthin* were injected intravenously at the end of the third control period. Immediately following the third period, 5 consecutive 20

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* One cc of mercuzanthin represents 135 mg of the sodium salt of b-methoxy-hydroxy-mercuri-propylamide of cyclopentane dicarboxylic acid equivalent to 39 mg of mercury and 35 mg of anhydrous theophylline.