critical. At concentrations below the critical level the dyes had little or no effect upon the coagulation system studied. At concentrations above the critical level all the dyes produced a progressively increased incoagulability of the system. This anticoagulant effect of the dyes is related to concentration and the action is on some substance other than heparin.

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Toxicity of Some Synthetic Pteridines in Rats.* (17593)

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This is an extension, into the field of vitamin analogs, of the research started in this laboratory(1) on the effect of amino acid analogs on tumor-bearing rats and mice. While there is considerable literature on the anti-folic acid activity and tumor-inhibiting effects of such folic acid analogs as 4-amino-pteroylglutamic acid and 4-amino-N-10-methyl pteroylglutamic acid, less work has been done with the substituted pteridines themselves. The importance of the 4-amino substitution for folic-acid antagonism has been demonstrated in much of this work.(2) In this connection, the anti-folic acid activity and tumor-inhibiting effect of 2,6-diamino-purine is of interest.(3) This compound is identical with 2, 4-diamino-pteridine except for the extra carbon atom in the pteridine ring. In view of these facts, several 4-amino substituted pteridines were prepared in this laboratory.(4) A study of the toxicity of these compounds in normal rats was undertaken before investigating their effects on tumor-bearing animals.

Procedure. For this study, 56 female rats of the Long-Evans strain weighing about 150 g were used. They were divided into 7 groups of 8 animals each. Two groups served as controls, while each other group received one of the 5 different pteridines. They were kept on the regular stock diet during the experimental period. The compounds were dissolved in 0.1 M phosphate buffer, pH 7.4, and the concentrations used, except for 2,4-diamino-pteridine, approached the maximum solubility.

Of the eight animals used for each compound studied, 4 received 3 ml intraperitoneally daily (Low Dose), and 4 received 3 ml twice daily (High Dose). One control group was not injected; the other was given the same volume of phosphate buffer as the experimental animals. The injection period was for 10 days. Half of each group was killed on the eleventh day (Group A), and the rest were killed 6 days later (Group B). At the time of sacrifice, blood was taken for hemoglobin, white blood count and blood smear. The kidneys and liver were weighed, and pieces of kidney, liver and sternal marrow were fixed in formalin. A bone marrow smear was made by

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TABLE I.
Total Doses of Pteridines Injected.

Compound	Concentration (mg/100 ml)	Total dose in 10 days	
		Low (mg)	High (mg)
2-thiol-4-amino-pteridine	4	1.2	2.3
8-thiol-4-amino-6,7-dimethyl-pteridine	5	1.5	2.9
2-thiol-4-amino-6,7-dihydroxy-pteridine	100	30	57
2,4-diamino-pteridine	100	30	57
2,4-diamino-6,7-dihydroxy-pteridine	4	1.2	2.3

TABLE II.
Effects of Injection of Pteridines on Animal Weight.

Compound injected	Group and No. of animals, per group	Initial wt, g*	Gain during 10-day inj., g*	Gain in following 6 days, g*
Controls, no inj.	A + B (8)	154 ± 2.6	21.3 ± 3.6	
	B (4)	154 ± 5.4	26.0 ± 3.6	7.0 ± 2.0
Controls, buffer	A + B (8)	144 ± 2.4	22.8 ± 2.8	
	B (4)	142 ± 4.2	24.5 ± 3.5	7.0 ± 3.0
2-thiol-4-amino-pteridine	A + B (8)	156 ± 1.6	18.9 ± 2.2	
	B (4)	158 ± 2.7	22.8 ± 2.6	3.8 ± 2.7
2-thiol-4-amino 6,7-dimethyl-pteridine	A + B (8)	146 ± 2.3	20.9 ± 4.5	
	B (4)	145 ± 2.1	20.3 ± 4.6	9.0 ± 2.0
2-thiol-4-amino-6,7-dihydroxy-pteridine	A + B (8)	158 ± 4.7	19.6 ± 2.3	
	B (4)	160 ± 4.4	20.5 ± 4.8	7.8 ± 2.2
2,4-diamino-pteridine	A + B (8)	152 ± 2.3	7.9 ± 1.8	
	B (4)	150 ± 3.0	11.0 ± 2.7	8.0 ± 1.7
2,4-diamino-6,7 dihydroxy-pteridine	A + B (8)	143 ± 2.7	21.3 ± 2.4	
	B (4)	138 ± 3.5	21.8 ± 4.3	—11.5 ± 1.8

* Values represent arithmetic mean, ± the standard error of the mean.

forcing a drop of plasma through a segment of rib. In some animals the kidney tissue was also fixed in Carnoy's solution (chloroform, alcohol, and glacial acetic acid).

Results. Effects on animal body weight. The animals were weighed every other day. Table II shows the average initial weight, the average gain during the injection period, and the average change in weight during the following 6 days.

The significant findings were, 1) the 2,4-diamino-pteridine group gained less weight during the injection period but continued to gain at the normal rate during the following 6 days, and 2) the 2,4-diamino-6, 7-dihydroxy-pteridine group lost weight during the 6 days following the injections.

Effects on kidney and liver weight. No compound produced a consistent increase or decrease in the weight of either organ. However, because of the number of animals used, such effects would not be detected unless they were striking.

Hematological effects. Hemoglobin was determined by the acid hematin method, using a Klett photoelectric colorimeter. No tendency toward anemia was observed in any group. For the differential count 200 cells were examined. No significant effects were seen in the white blood counts nor the differentials. However, two of the 2,4-diamino-pteridine animals showed a depressed total white blood count and a relative granulocytopenia. Because of this, another group of 6 similar animals, with 6 controls, was given the high dose of 2,4-diamino-pteridine under the same

experimental conditions, except that blood counts were done on every third day during the injection period. No effects on the white counts nor the differentials were observed in this group.

Histological effects. No pathological changes were found in any of the liver sections nor in the bone marrow smears and sections. The important changes were confined to the kidneys of those animals receiving 2,4-diamino-pteridine. All showed deposits of yellow, burr-like crystals within the renal tubules. The deposits were detectable grossly, on the cut surface of the kidneys containing the greatest amounts of crystals, as a yellow discoloration most marked in the outer zone of the medullary pyramid or at the cortico-medullary junction. This area fluoresced strongly under ultra-violet light. Microscopically, none of the spherical, radially striated crystals were found in the glomeruli. They appeared in increasing numbers in the lower parts of the nephron, and were most numerous in the collecting tubules. Yellow intracellular deposits, not definitely crystalline, were found in an occasional tubule cell. In some tubules the lumen was completely blocked by an accumulation of the crystals, and in such areas the epithelial cells were flattened, the nuclei pyknotic, and inflammatory cells had infiltrated the region. No evidence of increased mitosis was found.

The amount of crystalline deposits and the extent of the renal lesions were greater in those animals receiving the higher dose and in those killed immediately after the injection period. The deposits were better preserved with Carnoy's fixation than with formalin since the latter dissolved away some of the crystals.

Discussion. The weight loss in the animals receiving 2,4-diamino-pteridine was correlated with the degree of crystalline deposits in the kidneys. This was observed even more clearly in a previous experiment using a wider range of dosage. It appears probable that the observed effect of this compound on body weight can be ascribed either entirely or principally to the renal damage. Crystal deposits within the renal tubules have been reported following administration of xanthopterin (2-amino-4,6-dihydroxy-pteridine)(5) and high doses of folic acid itself.(6)

Since this was a study of general toxicity rather than an investigation of antifolic acid action of the compounds, no attempt was made to lower the level of folic acid in the diet nor to reduce the intestinal bacterial flora. Swenseid, *et al.*(7) reported leukopenia but no anemia in succinyl-sulfathiazole-treated weanling rats receiving 2,4-diamino-6,7-diphenyl-pteridine at a level of 50 mg per 100 g in the diet. However, the dimethyl-substituted compound, 2,4-diamino-6, 7-dimethyl-pteridine, was much less toxic. It is not unreasonable to expect that 2,4-diamino-pteridine itself would have even less effect on the hematopoietic system. Daniel, *et al.*(8) found no anemia nor growth impairment in chicks fed 2,4-diamino-pteridine at a level of 200 mg per 100 g in the diet, along with approximately 15 γ of folic acid per 100 g of diet.

Summary. Five 4-amino-pteridine compounds were studied for toxicity in rats. The 2-thiol-substituted pteridines were not found to be toxic in the doses used. The 2,4-diamino-6,7-dihydroxy-pteridine and 2,4-diamino-pteridine interfered with the growth rate. The latter compound caused crystalline deposits in the renal tubules, and the extent of the deposits correlated well with the body weight effect. No histological effects were noted in the liver and bone marrow,‡ and none of the compounds altered the blood picture.

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