TABLE I.
Toxicity of Various Sodium Chloride Solutions Administered Intravenously at Different Rates.

	Rate of administration cc/minute													
	4.0-3.5		2.9-2.5		2.4-2.0		1.9-1.5		1.4-1.0		0.9-0.5		0.5-0.2	
% NaCl	cc/20 g	D*	cc/20 g	D*	cc/20 g	D*	cc/20 g	D*	cc/20 g	D*	cc/20 g	D*	cc/20 g	D*
0	4.7†	4/4	4.6†	3/3	5.1	1/1	4.5†	1/1	4.2†	1/1	3.9†	1/1	4.6†	2/2
0.425	5	3/10	5	0/9	5	0/6	5	0/3	5	0/2	5	0/1		
									"Safe" range					
0.85	5	4/6	5	2/8	5	0/3	5	0/4	6.4‡	0/3	5.0	0/6	5.0	0/1
1.70	3.9†	1/1	4.0†	2/2					4.2†	1/1	5.0	1/3	5.0	1/3
2.55	2.2†	1/1					1.8†	1/1	2.6†	1/1	2.9†	3/3	5.1	2/2

* D = No. dead / No. dosed
† Death before 5 cc injected.
‡ Two of these animals received 7 cc per 20 g.

16676

Failure of Xanthopterin to Influence Hematopoiesis and Growth in Rats.*

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Tschesche and Wolf[1] reported that rats fed goats' milk develop an anemia which responds to xanthopterin. Simmons and Norris[2] and Simmons[3] observed that anemia in Chinook salmon responds to injections of xanthopterin. Totter and Day[4] reported that in rats with the deficiency induced by feeding a purified diet containing succinylsulfathiazole, administration of 20 μg of xanthopterin per day caused a leukocytosis and a marked weight gain, but in subsequent experiments they failed to confirm this observation. Wright and Welch,[5] Daft and Sebrell,[6] and Axelrod, *et al.*,[7] also failed to note any effect when xanthopterin was fed to rats deficient in pteroylglutamic acid (PGA). Totter and co-workers[8,9] reported that favorable hematopoietic responses occur when xanthopterin is given to vitamin M-deficient monkeys. In this laboratory, 2 macrocytically anemic swine have shown a submaximal hematopoietic response to large parenteral doses of xanthopterin (10 mg daily),[10] a phenomenon which is being investigated further.

* This investigation was supported, in part, by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

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1 Tschesche, R., and Wolf, H. G., *Ztsch. f. physiol. Chem.*, 1937, **248**, 34.

2 Simmons, R. W., and Norris, E. R., *J. Biol. Chem.*, 1941, **140**, 679.

3 Norris, E. R., and Simmons, R. W., *J. Biol. Chem.*, 1945, **158**, 449.

4 Totter, J. R., and Day, P. L., *J. Biol. Chem.*, 1943, **147**, 257.

5 Wright, L. D., and Welch, A. D., *Science*, 1943, **98**, 179.

6 Daft, F. S., and Sebrell, W. H., *Public Health Rep.*, 1943, **58**, 1542.

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9 Totter, J. R., Shukers, C. F., Kolson, J., Mims, V., and Day, P. L., *J. Biol. Chem.*, 1944, **152**, 147.

Cerecedo and his associates[11,12] found that xanthopterin, like PGA, when added to highly purified diets fed to mice or rats, causes a significant improvement in lactation performance. Fraenkel and Blewett[13] have reported that the growth of the larvae of the insect, *Ephestia kuehniella,* on an artificial diet which contains all the known factors of the vitamin B-complex in pure form, is markedly accelerated by the addition of PGA, and that xanthopterin, in concentrations about 1000-times greater, has a comparable effect.

Recently Norris and Majnarich[14,15] have studied the effects of xanthopterin in experiments conducted both *in vitro* and *in vivo*. Studies *in vitro* involved the use of cultures of bone marrow from rats and other animals. Xanthopterin in low concentrations was reported to cause an increase in the rate of red and white cell proliferation. In rats fed a purified diet containing sulfathiazole (1%), it was claimed that xanthopterin is hematopoietically active. They reported that xanthopterin *in vitro* and *in vivo,* is more effective than PGA, since it appeared to act in smaller doses and more rapidly. They concluded that bone marrow cannot use PGA directly in hematopoiesis while xanthopterin can be so utilized.

Findings with xanthopterin have been so variable, and the claims made by Norris and Majnarich are so remarkable, that in this study a further attempt has been made to determine whether xanthopterin can play a role in hematopoiesis and growth in rats. The data obtained do not support the conclusions drawn by Norris and Majnarich.

Methods. Weanling female albino rats‡ were kept singly in cages with wire mesh floors. The basal diet had the following composition (in g per 100 g): purified casein, 20; glucose ("Cerelose"), 48.7; hydrogenated vegetable oil ("Primex"), 18; corn oil, containing vitamins A and D,§ 2; salt mixture (U.S.P.X. No. 2), 2.8; accessory salts‖ 0.2; cellulose ("Cellu-flour"), 5; choline chloride, 0.2; inositol, 0.1; thiamine hydrochloride and pyridoxine hydrochloride, each, 0.0005; riboflavin, 0.001; nicotinamide, 0.005; calcium pantothenate, 0.0055; ascorbic acid, 0.01; 2-methyl-1,4-naphthoquinone, 0.001; biotin, 0.00001.

Group I. Sixteen of the weanling rats were placed on the above basal diet with 1% *sulfathiazole* incorporated. At the end of 28 days, 3 rats were dead and 3 rats showed anemia without leukopenia. During the following 21 days, 5 more rats became anemic. In those rats that developed anemia during the latter period, leukopenia also was present. During this 21-day period, 3 rats died before developing an anemia of a level suitable for experiment; at the end of this period an erythrocyte level exceeding 5,000,000 per cm was found in 2 rats. Each of 3 rats (No. 78, No. 80 and No. 82) was given 100 μg of xanthopterin parenterally per day for 6 days; also one (No. 81) was given 200 μg per day, and another (No. 76) was given 400 μg per day, for 6 daily injections. Each of 5 rats (No. 69, No. 73, No. 80, No. 82, and No. 84) was given 50 μg of PGA daily for 6 injections; 2 of these rats (No. 80 and No. 82) previously had been treated with xanthopterin. The variable effects of xanthopterin and PGA are considered in the section entitled *Results.*

Groups II and III. Forty rats were placed on the basal diet in which 2% *succinylsulfathiazole* was incorporated. At the end of 28 days, when the rats showed marked im-

10 Pritchard, J. A., Welch, A. D., and Heinle, R. W., unpublished data.

11 Mirone, L., and Cerecedo, L. R., *Arch. Biochem.,* 1948, **15**, 324.

12 Sica, A. J., Allgeier, A. M., and Cerecedo, L. R., *Arch. Biochem.,* 1948, **18**, 119.

13 Fraenkel, G., and Blewett, M., *Biochem. J.,* 1947, **41**, xviii.

14 Norris, E. A., and Majnarich, J. J., *Am. J. Physiol.,* 1948, **152**, 175.

15 Norris, E. A., and Majnarich, J. J., *Am. J. Physiol.,* 1948, **152**, 178.

‡ Obtained from Blaine Biological Gardens, Media, Pa.

§ Two grams of the corn oil preparation contained vitamin A, about 4500 units and vitamin D, about 560 units.

‖ Accessory salts contained in 0.2 g (expressed in mg): KCl, 100; NaCl, 87.2; $FeSO_4$, 10; $MnSO_4$, 1; $ZnSO_4 \cdot H_2O$, 1; NaI, 0.3; NaF, 0.1; $CoCl_2 \cdot 6H_2O$, 0.01.

pairment of growth, leukocyte and hemoglobin levels were determined. Twelve severely leukopenic rats were assigned to group II. In these rats the effects of xanthopterin and of PGA on leukopoiesis and growth were studied. Each of 6 rats was injected with 50 μg of PGA daily for 5 days. Into the remaining 6 rats, xanthopterin was injected daily for 5 days; the daily dosage was 100 μg in one rat, 200 μg in 4 rats, and 400 μg in the sixth rat. Leukocyte counts and weighings were made at 3-day intervals. The positive effects of PGA and the lack of effect of xanthopterin are considered under *Results*.

The remaining 21 rats, of the group given the purified diet plus 2% succinylsulfathiazole, composed group III. In this group a study was made of the effects of xanthopterin and of PGA on hemoglobin synthesis, as well as on growth and leukopoiesis. Under these conditions, severe anemia failed to develop during a period of 28 days. However, an anemia was induced by means of hemorrhage, using a technic similar to that described by Kornberg *et al.*[16] Blood, in an amount equivalent to approximately 2% of the weight of the rat, was removed at each bleeding. Sufficiently low hemoglobin levels were produced by 2 to 4 bleedings of each rat, carried out at intervals of 2 days. Two rats died during the course of the bleeding, and 2 rats died 2 days following the last bleeding. Seventeen rats were available for experiment, of which 4 served as untreated controls. Each of 4 rats was given parenterally 50 μg of PGA daily for 5 days. Nine rats were given xanthopterin parenterally; 5 of the 9 were given 100 μg each, daily for 5 days, while 4 were given twice this dose. Weighings, leukocyte counts and hemoglobin measurements were done at 3-day intervals. Hemoglobin was determined as oxyhemoglobin using a Klett-Sumerson photoelectric colorimeter. The findings are presented in Figure 1 and are discussed under *Results*.

Results. Group I (1% sulfathiazole). In rats given a purified diet containing succinyl sulfathiazole, anemia is rarely seen. However, the use of sulfathiazole in a purified diet induced, during a 7-week period, an anemia in 8 of 10 surviving rats.

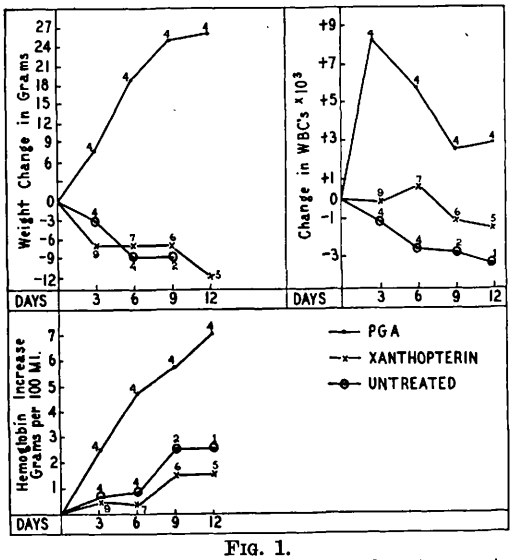

FIG. 1.
Changes in hemoglobin levels, leukocyte counts, and weight in rats fed a purified diet containing 2% succinylsulfathiazole and bled 2 to 4 times. One group received pteroylglutamic acid, one group received xanthopterin, and a third group served as the untreated control. The numbers at each point in the figure represent the number of surviving rats represented in the average.

The data presented here indicate clearly that a markedly variable *hemolytic anemia* has occurred in rats fed sulfathiazole in a purified diet. Reticulocytoses of 10 to 25% were noted in many of the anemic rats before therapy. Those rats in which anemia developed earlier did not show leukopenia. Therefore, leukopoiesis and erythropoiesis, as judged by reticulocytosis, certainly was occurring. It is evident that the anemia was due to factors other than one related directly to the lack of an essential hematopoietic factor or factors. The feces of many of the rats were observed to contain great amounts of bile pigments. One rat (No. 69), while receiving a total dose of 300 μg of PGA parenterally, showed a decrease in the number of circulating erythrocytes from 4.0 million to 1.7 million per cmm in 6 days, yet during this same period, the leukocyte count increased from 3,700 to 68,500 per cmm; on the sixth day, a recticulocytosis in excess of 80% was present. The marked anemia, with

[16] Kornberg, A., Tabor, H., and Sebrell, W. H., *Am. J. Physiol.*, 1944, **142**, 604.

a marked leukocytosis and reticulocytosis, was maintained. The rat died on the twelfth day after the start of therapy. A similar fall in erythrocyte count was noted in a rat (No. 76) which received a total dose of 2400 μg of xanthopterin. The red blood cell level fell from 4.0 million to 2.9 million per cmm; a reticulocytosis of 20% was present on the sixth day. During this 6-day period, the leukocyte count increased from 4,900 to only 6,700 per cmm. The rat died 15 days after treatment with xanthopterin was started.

Four rats (No. 78, 80, 81 and 82) showed a slight increase in the number of circulating erythrocytes after treatment with xanthopterin. During the 9 days following the start of xanthopterin therapy, an average increase in erythrocytes of 1.4 million per cmm occurred; the largest increase was 1.8 million (No. 80). The leukocyte counts fell in all 4 rats. No rat receiving xanthopterin showed a positive growth response.

In the 5 rats given PGA, variable effects on growth and circulating erythrocyte levels were obtained. A marked leukocytosis occurred in all PGA-treated rats. The largest increase in erythrocyte count was 3.3 million per cmm (No. 82). The small increase in erythrocyte counts that occurred in rats given xanthopterin, did not occur earlier than in those given PGA.

Group II (2% succinylsulfathiazole). The effect of xanthopterin and of PGA on growth and leukopoiesis were compared in leukopenic rats with arrested growth. Six rats given a daily dose of 50 μg of PGA parenterally for 5 days showed favorable leukocyte and growth responses during the 12 days of study. Six days after the start of therapy an average increase in white blood cells of 12,900 per cmm was noted. Six rats receiving 2 to 8 times as much xanthopterin parenterally showed an average increase of 1,300 per cmm. At the end of 12 days those rats given PGA all were alive and an average gain in weight of 21 g was observed. The xanthopterin-treated rats showed an average loss in weight of 11 g during this same period. One rat given xanthopterin died on the sixth day. No differences were observed in leukocyte and growth responses to different dose levels of xanthopterin. "Spectacled" eyes and ruffled fur developed in rats during the period of administration of xanthopterin. The lack of effect of xanthopterin on growth and leukopoiesis is similar to that previously observed.[5-7]

Group III (2% succinylsulfathiazole plus hemorrhage). It was felt that the study of the comparative effects of xanthopterin and PGA on erythropoiesis could be conducted more satisfactorily in deficient rats made anemic by hemorrhage than in rats with an anemia due to an active hemolytic process.

Definite hematopoietic and growth responses followed therapy with PGA. Little difference between the responses occurring in the xanthopterin-treated anemic group and in the untreated anemic control group was noted.

In the 4 rats given PGA, the average hemoglobin concentration at the start of therapy was 6.6 g per 100 ml of blood. During the 12 days after the start of therapy, an increase of 7.1 g to an average concentration of 13.7 g per 100 ml occurred. This was accompanied by an average growth response of 26 g. An average increase in leukocytes of 8,300 per cmm was noted on the third day. All the PGA-treated rats were alive at the end of the experiment.

Nine anemic rats were given xanthopterin in doses 2 to 4 times larger than the dose of PGA used. The average hemoglobin concentration at the start of therapy was 6.3 g per 100 ml of blood. The hemoglobin concentration at the end of 12 days in the 5 surviving rats was 8.1 g. No favorable leukopoietic response was noted. Loss of weight continued in spite of xanthopterin therapy. The 5 surviving rats lost an average of 12 g during the 12 days following the start of therapy. "Spectacled" eyes and ruffled fur developed in spite of the administration of the pterin. Three of the 5 surviving rats died during the 3 days following the last blood determinations. No significant difference was noted between those rats given 200 μg and those rats given 100 μg of xanthopterin per day.

Four anemic rats were not treated. Their average hemoglobin concentration was 8.2 g

per 100 ml at the start of the observation period. The average hemoglobin level closely paralleled that of the xanthopterin-treated anemic rats during the 12-day observation period. Only 1 rat was alive at the end of 12 days. Weight and leukocyte changes also were similar to those noted in the xanthopterin-treated rats.

Discussion. Norris and Majnarich[15] have not indicated the mechanism of production of the anemia they observed in rats fed a purified diet containing sulfathiazole. Sulfathiazole has been reported by Richardson[17] to cause hemolytic anemia and the hemolytic anemia-producing properties of related sulfonamides have been studied by Latven and Welch.[18] That such a hemolytic process operated in the rats of Norris and Majnarich[15] is indicated by the production of an anemia within 22 days after the diet was started, and by the rapid fall in erythrocyte counts noted after the brief rise following therapy. Against a hemolytic process are the apparently very low reticulocyte levels which were reported by them; it would seem certain, however, that a measurable erythrocytic response following therapy in any type of severe anemia would produce greater reticulocyte responses than the 0.8 and 1% maximum values that they observed. It is suggested that the reticulocyte counts reported by Norris and Majnarich[15] are in error.

The effects shown here of PGA and xanthopterin in rats made anemic by feeding a purified diet containing sulfathiazole do not confirm the observations of Norris and Majnarich.[15] No positive leukocytic responses were noted after therapy with xanthopterin, although marked responses occurred after the administration of PGA. Doses of xanthopterin larger than the doses they used failed to produce the distinct increases in circulating erythrocytes that they recorded. Highly variable results also were obtained when PGA was given to those rats fed a purified diet containing sulfathiazole; however, those PGA-deficient rats made anemic by hemorrhage, and then given PGA, all showed nearly equal, marked erythropoietic responses.

Summary. 1. A purified diet containing sulfathiazole produced a hemolytic anemia of variable intensity which could not be completely reversed either by pteroylglutamic acid or by xanthopterin in any of the doses used.

2. Rats fed a purified diet containing succinylsulfathiazole developed leukopenia and an inhibition of growth which responded to PGA, but not to xanthopterin.

3. In similar rats fed a purified diet containing succinylsulfathiazole, but made anemic by repeated hemorrhage, PGA induced definite erythropoietic, leukopoietic and growth responses, while xanthopterin was inactive.

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18 Latven, A. R., and Welch, A. D., *J. Pharmacol. and Exp. Therap.*, 1944, **81**, 301.

16677

Stripping Film Technics for Histological Autoradiographs.

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There are now three general technics of autoradiography. The first method developed was that of placing the specimen in contact with a photographic plate, removing the specimen, and developing the plate. London[1] studied anatomical specimens in 1904 and Lacassagne and Lattes,[2] histological specimens

1 London, E. S., *Arc D'elec. Med.*, 1904, **12**, 363.

2 Lacassagne, M. A., and Lattes, J. S., *Compt. rend. Acad. Sci.*, 1924, **178**, 488.