the pressures tend to fluctuate in spontaneously hypertensive rabbits. This is also true of normal rabbits, whereas rabbits with experimentally induced hypertension have more stable pressures. Lastly, the mean systolic pressure for our colony becomes significantly higher at 6 or 8 months compared to the mean value at 4 months. Normal rabbits also show a tendency to have higher pressures as they get older. The ordinary New Zealand White laboratory rabbit is considered adult at 4 months at which time it can be reproductive. It continues to grow and gain weight for the first 6 months to a year of its life. However, it can be stated that some animals in our colony which did not continue to gain weight as rapidly as others, tending instead to level off at 4 or 6 months, nevertheless had increasing blood pressure with age.

Summary. We have bred and raised a colony of spontaneously hypertensive rabbits.

TABLE II. Typical Protocol of Spontaneously Hypertensive Rabbit.

Pd-3 (male) Born: 7/11/53

Date	Wt (lb)	1st reading	2nd reading (5 min. later)
10/15/53	5½	150/105	150/100
11/ 7	6¼	170/130	165/120
11/30	6⅛	170/135	160/125
1/13/54	6½	135/ 90	140/100
1/28		Cuff pressure:	160/110
		Femoral puncture:	150/115

TABLE III. Mean Systolic Pressures of Female and Male Rabbits in the Spontaneously Hypertensive Colony.

Age (mo)	3-4		5-6		7-8	
	♀	♂	♀	♂	♀	♂
No. of animals	43	53	29	33	23	29
Mean systolic pressure, mm Hg	145	150	155	160	165	160

It is concluded that there is a hereditary factor in hypertension and that the characteristic changes of blood pressure in spontaneously hypertensive animals are similar to the normal rabbit population. These findings would indicate that spontaneously hypertensive rabbits have characteristics in common with man in his development of essential hypertension.

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Effect of Dietary Aminopterin and Sulfasuxidine on Biosynthesis of Ascorbic Acid in the Rat.* (21256)

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Williams(1) reported that dietary aminopterin markedly reduced liver ascorbic acid concentrations in the rat. Subsequently, Schwartz and Williams(2) observed that the depression in liver ascorbic acid was not the result of an increased rate of urinary excretion of this vitamin. It has been further reported (3) that succinylsulfathiazole (sulfasuxidine) when fed at a 2% level in a purified diet also has a depressant action on rat liver ascorbic acid.

In the present work we have investigated various possible methods by which amino-

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TABLE I. Ascorbic Acid Catabolism in Liver Homogenates* from Normal and Aminopterin-Fed Rats. (2-hr incubation at 37°C.)

Ration of animals	No. of exps.	Ascorbic acid in μg/flask (a) Avg initial conc.	Avg final conc.	(b) Avg destruction of ascorbic acid	% destruction of ascorbic acid (b/a × 100)
Basal†	7	1033	757	276	26.7 (14.2-36.5)‡
Basal + aminopterin	6	1004	807	197	19.4 (11.3-32.0)

* Detailed flask contents are given in text.

† Avg destruction of ascorbic acid in Krebs-Ringer phosphate buffer alone was 3.6% with a range of 0-9.1%.

‡ Range of values.

pterin and sulfasuxidine may depress liver ascorbic acid. Four major possibilities exist: 1) Aminopterin and sulfasuxidine may inhibit the biosynthesis of ascorbic acid in the rat; 2) they may deplete the liver of ascorbic acid by inducing an abnormally high excretion of the vitamin; 3) they may increase the rate of ascorbic acid catabolism in the liver; or 4) they may cause a translocation of ascorbic acid so that, while the liver is depleted of the vitamin, other organs are enriched with it. The direct verification of the first hypothesis is difficult because as yet no adequate *in vitro* system for demonstrating ascorbic acid synthesis in animal tissues has been reported. Previous work(2) has shown that the second hypothesis is not valid since there is no abnormally high urinary excretion of ascorbic acid in aminopterin-fed rats. If the 2 remaining hypotheses could be ruled out, the effect of aminopterin and sulfasuxidine on rat liver ascorbic acid could probably be explained by the first hypothesis, *i.e.*, that these substances exert their effects on the biosynthesis of ascorbic acid. In the present paper, results obtained in studying these possible mechanisms by which aminopterin and sulfasuxidine decrease liver ascorbic acid in the rat are reported.

Experimental and results. Effect of dietary aminopterin on ascorbic acid catabolism in rat liver homogenates. Adult male rats of the Sprague-Dawley strain were used as experimental animals. One group of rats was fed a basal ration consisting of 18% casein, 4% salts IV(4), 5% corn oil, 2% vitamin mixture (5) which was supplemented with one μg of vit. B_{12} per g of mix, and 71% sucrose. Another group was fed the basal ration to which aminopterin was added at a level of 4 mg/kg of ration. After about 2 weeks the group receiving aminopterin exhibited the typical aminopterin toxicity syndrome: porphyrin secretion around the eyes and nose, diarrhea, and general inactivity leading to death. When these symptoms appeared, the rats were sacrificed and 1:4 liver homogenates in Krebs-Ringer phosphate buffer, pH 7.4, were prepared. Twenty-five ml Erlenmeyer flasks containing one ml of liver homogenate from control or aminopterin-fed rats, one ml of Krebs-Ringer phosphate buffer, 0.5 ml of freshly neutralized ascorbic acid solution (2 mg/ml), and 0.5 ml of distilled water were incubated at 37°C. One ml aliquots of the reaction mixture were taken at the beginning of the incubation and again after 2 hours. After deproteinizing with 5% metaphosphoric acid in 10% acetic acid, the aliquots were analyzed for ascorbic acid by the method of Roe and Keuther(6) as modified by Bolin and Book(7). A further modification by us included the substitution of a mixture of 3 parts concentrated HCl and 2 parts of phosphoric acid (85% by volume) for the 85% sulfuric acid of the original method. The results of this experiment are presented in Table I. It should be noted that the osazone method of ascorbic acid analysis, which was used here, detects ascorbic acid, dehydroascorbic acid, and diketogulonic acid. This means that the disappearance of ascorbic acid registered by this method indicates the amount of ascorbic acid which has been degraded beyond diketogulonic acid, presumably to compounds arising from a rupture of the carbon chain. Since the

TABLE II. Effect of Dietary Aminopterin on Liver, Adrenal, Spleen, and Kidney Ascorbic Acid.

Organ	Basal group		Aminopterin-fed group	
	Asc. acid conc. in μg/g organ	Total organ asc. acid in μg	Asc. acid conc. in μg/g organ	Total organ asc. acid in μg
Liver	359* (318–426)†	Not d‡	173 (132–252)	Not d‡
Adrenal	4431 (3647–5979)	67 (40–83)	3241 (1298–4411)	68 (40–110)
Spleen	579 (421–657)	446 (349–582)	601 (420–772)	290 (176–333)
Kidney	183 (164–218)	192 (161–234)	120 (90–160)	109 (76–168)

* Each figure represents avg value obtained from analysis of 5-7 organs.
† Range of values.
‡ Not determined. Other studies showed that livers of aminopterin-fed rats are somewhat smaller than those of rats fed a basal ration. Therefore, the size of the livers could not compensate for the decreased ascorbic acid concentrations.

data of Table I indicate that ascorbic acid in Krebs-Ringer phosphate buffer is stable at 37°C for 2 hours, the destruction observed upon incubation in the presence of liver homogenates must be effected by the homogenates. The table indicates that there is slightly less destruction induced by liver homogenates from aminopterin-fed rats then that induced by control rat liver homogenates. Therefore, it is clear from the data that dietary aminopterin does not cause an abnormally high metabolic breakdown of ascorbic acid.

Effect of aminopterin on the translocation of ascorbic acid within the rat. The same rats used in the work described above were also utilized for this study. The ascorbic acid content of the liver, spleen, kidney, and adrenal gland of both groups of rats was determined. The spleen, kidney, and adrenal weights were recorded and these organs were homogenized in 5% metaphosphoric acid-10% acetic acid mixture. The deproteinized extracts were analyzed for ascorbic acid by the modified method of Roe and Keuther noted above.

In Table II are presented both ascorbic acid concentrations and values for the total organ ascorbic acid content. Previous results indicating decreased liver ascorbic acid in aminopterin-fed rats(1,2) have been confirmed by these data. From the table it can also be seen that aminopterin depresses both adrenal and kidney ascorbic acid concentrations but has no significant effect on spleen ascorbic acid concentrations. However, the total spleen ascorbic acid content of the aminopterin-fed group was considerably lower than that of the control group. This was due to the large difference in the weight of the spleens of the 2 groups of rats. The difference in average adrenal weights also explains why the total adrenal ascorbic acid in both groups is approximately equal. Since there is no significant increase in spleen, kidney, or adrenal ascorbic acid, it can be concluded that the decrease in liver ascorbic acid is not associated with a translocation of this vitamin from the liver to other organs in the rat. Since the possibility of increased urinary excretion of ascorbic acid in the aminopterin-fed rat has previously been ruled out(2), and the present experiments invalidate the increased catabolism and translocation possibilities, the first hypothesis, namely, that aminopterin inhibits the biosynthesis of ascorbic acid, is considerably strengthened.

Guinea pig experiments. If aminopterin and sulfasuxidine inhibit the biosynthesis of ascorbic acid, then upon feeding these compounds to guinea pigs which receive an adequate dietary supply of ascorbic acid, no depression in the liver levels of ascorbic acid should occur. Therefore, the effect of these 2 substances on liver ascorbic acid levels in young, male guinea pigs was studied. In the first experiment, one group of guinea pigs was fed the purified ration of Roine, Booth, Elvehjem, and Hart(8), while a second group received the same ration plus an oral supple-

TABLE III. Influence of Dietary Aminopterin and Sulfasuxidine on Liver Ascorbic Acid Levels in Guinea Pigs Receiving Adequate Dietary Ascorbic Acid.

Diet fed	Exp.	Feeding period	No. of animals	Liver ascorbic acid, μg/g
Basal	I	10–12 days	6	51 (27– 79)*
" + aminopterin		10–12 "	5	78 (51– 98)
Basal	II	10 "	7	39 (21– 51)
" + aminopterin		10 "	5	37 (15– 92)
Basal	III	6 wk	7	73 (44–104)
" + sulfasuxidine		6 "	8	77 (42–108)

* Range of values.

ment (by pipette) of 3 mg of aminopterin per guinea pig per day(9). To insure an adequate dietary intake of ascorbic acid, this vitamin was administered to both groups at a level of 10 mg of ascorbic acid/guinea pig/day. After about 2 weeks the aminopterin-fed guinea pigs exhibited a marked decline in weight, which within a few days terminated in death. This weight loss was also associated with leukopenia as demonstrated by leukocyte counts performed on blood removed from the ears of the animals. During this period of rapid weight loss, the guinea pigs were killed and the livers removed for ascorbic acid analysis.

The results of the first experiment, Exp. I, are presented in Table III. It can be observed that there was no depression of liver ascorbic acid induced by orally administered aminopterin. A repetition of this experiment (Exp. II) gave essentially similar results although the absolute values obtained in this second experiment were lower than those previously obtained.

In the third experiment, the guinea pigs were fed sulfasuxidine at a level of 2% in the purified ration of Roine *et al.* From Table III it can be seen that sulfasuxidine, after a 6-week feeding period, also had no effect on liver ascorbic acid in the guinea pig.

The above findings which show that there is no depresesion in guinea pig liver ascorbic acid induced by dietary aminopterin or sulfasuxidine is considered as added evidence that the decrease in liver ascorbic acid observed in rats fed either of these 2 compounds is due to an inhibition of the biosynthesis of ascorbic acid in the rat.

Discussion. Recently, Sauberlich(19) has confirmed our earlier findings with respect to the aminopterin-induced depression in liver ascorbic acid in the rat. Sauberlich has further reported that the *Leuconostoc citrovorum* factor overcomes this depressant effect of aminopterin on liver ascorbic acid. Therefore, aminopterin, in lowering liver ascorbic acid, apparently acts in its well-known role as a folic acid antagonist. The action of sulfasuxidine is more obscure. We have found that sulfasuxidine exerts its depressant effect even in the presence of folic acid(3).

Our results indicate that both sulfasuxidine and aminopterin inhibit the biosynthesis of ascorbic acid, although the major portion of the present studies were concerned with aminopterin. The effect of sulfasuxidine has been dealt with extensively in a previous communication(3). If sulfasuxidine acted to depress liver ascorbic acid in the rat in any manner other than inhibition of biosynthesis, a depression in guinea pig liver ascorbic acid would be expected. As reported above, this depression was not found.

Sulfasuxidine is very poorly absorbed through the intestinal wall(10). This means that the inhibition of ascorbic acid biosynthesis in the rat by sulfasuxidine, and perhaps by aminopterin also, probably occurs in the intestinal tract. Since aminopterin and sulfasuxidine are bacterial inhibitors(11-15), they may exert their depressant action on liver ascorbic acid by inhibiting the growth of certain intestinal micro-organisms which produce some necessary component of the ascorbic acid synthesis system. With respect to this possibility, it is interesting to note that Reyniers(16) has reported that germ free suckling rats require a dietary source of ascorbic acid.

Aminopterin and sulfasuxidine have also been reported to cause morphological changes in the intestinal wall(17,18); and they may act, then, by interfering with some reaction taking place there, a reaction which is vital in the chain of reactions ultimately producing ascorbic acid.

Summary. Possible mechanisms by which dietary aminopterin and sulfasuxidine decrease levels of liver ascorbic acid in the rat have been investigated. In previous studies an increased rate of ascorbic acid excretion after feeding aminopterin had been found not to occur. In the present studies, dietary aminopterin has been found not to induce an increased destruction of ascorbic acid by liver homogenate or a translocation of liver ascorbic acid to other organs. Also, aminopterin and sulfasuxidine do not depress liver ascorbic acid in guinea pigs receiving an adequate daily supply of ascorbic acid. Therefore, all of these findings are considered as evidence that aminopterin and sulfasuxidine decrease liver ascorbic acid of the rat by inhibiting the biosynthesis of the vitamin. Possible mechanisms of this inhibition are discussed.

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Influence of Photodynamic Effects on Diffusion in Rabbit Dermis.* (21257)

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Castellani(1) stressed the importance of an unknown photodynamic effect on mucopolysaccharides of connective tissues, namely hyaluronic acid. *In vitro* he observed a steady reduction of viscosity of hyaluronic acid mixed with hematoporphyrin and exposed to visible light, as compared with hyaluronic acid mixed with hematoporphyrin and not exposed to light. According to the author, such reduction of viscosity is due to a depolymerization of this mucopolysaccharide.

In view of the importance which this phenomenon might have for interpretation of the pathogenesis of cutaneous diseases caused by

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