TABLE I. Body and Organ Weights of Decorticate Rats Treated with Propylthiouracil.*

Group	No. animals	Initial body wt (g)	Final body wt (g)	Thyroid (mg)	Ovaries (mg)	Adrenals (mg)	Uterus (mg)
I	6	198 ± 4.8†	208 ± 5.2	15.1 ± .6	42.3 ± 5.8	63.2 ± 2.2	271.1 ± 27.8
II	5	207 ± 4.6	198 ± 7	40.5 ± 1.5	48.7 ± 6.2	60.6 ± 2.9	290.7 ± 32.8
III	4	196 ± 3.9	164 ±10	29.2 ± 1.0	35.8 ± 2.7	59.8 ± 3.3	269.3 ± 13.7
IV	4	201 ± 4.1	162 ± 9.4	32.2 ± 1.2	38.3 ± 2.9	58.5 ± 4.1	271.1 ± 18.2
V	3	199 ± 3.2	162 ± 4.0	31.3 ± 2.4	37.2 ± 3.2	53.3 ± 3.0	251.9 ± 15.1

* Group I = Untreated controls; II = propylthiouracil-treated controls; III & IV = decorticate rats allowed one wk for post-operative recovery; V = decorticate rats allowed 5 wk for post-operative recovery. Groups II through V inj. with propylthiouracil suspension for 10 days and killed on 11th day.

† All figures given as mean ± stand. error.

tion, it appears that, whatever extra-hypothalamic nervous pathways may be concerned in modifying hypothalamic activity, none of these are mediated primarily by the neocortex.

Summary. Removal of the neocortex in female rats did not greatly affect the goitrogenic response to propylthiouracil administration nor the weights of the adrenals, ovaries or uteri.

1. Greer, M. A., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 603.

2. ———, *J. Clin. Endocrinol. and Metab.*, 1952, v12, 1259.

3. Bogdanove, E. M., and Halmi, N. S., *Endocrinology*, 1953, v53, 274.

4. Greer, M. A., and Erwin, H., *J. Clin. Invest.*, 1954, v33, 938.

5. Davis, C. D., *Am. J. Physiol.*, 1939, v127, 374.

Received December 3, 1956. P.S.E.B.M., 1957, v94.

In vivo Synthesis of Ascorbic Acid by the Alloxan Diabetic Rat. (23014)

J. V. STRAUMFJORD, JR.* AND E. S. WEST

Department of Biochemistry, University of Oregon Medical School.

The capacity of the albino rat to synthesize ascorbic acid has been known for many years, but only recently has the mechanism of synthesis been partially clarified. King and associates(1) reported that certain compounds accelerate synthesis and excretion of ascorbic acid. One of these compounds, Chloretone, has been used extensively in the study of precursors and mechanism of ascorbic acid synthesis in the rat. Chloretone is capable of increasing urinary excretion from a basal rate of 0.3 mg to 50 mg/24 hours. The evidence that glucose is a precursor for ascorbic acid in the rat is conclusive. Jackel, Mosbach, Burns and King(2) injected uniformly labeled C^{14} glucose into Chloretone treated rats and found 0.3% conversion into ascorbic acid. Degradation studies indicated that ascorbic acid was also labeled uniformly. This work suggested that glucose is the precursor and that the entire molecule is used. Horowitz, Doerschuk and King(3) injected D-glucose-1-C^{14} into Chloretone stimulated rats and found approximately 56% of the total activity in carbon 6 of the excreted ascorbic acid, the position expected according to theory. Horowitz and King(4) similarly showed conversion of glucose-6-C^{14} to ascorbic acid-1-C^{14}. More recently Burns and Mosbach(5) have shown that the normal rat as well as Chloretone-treated animal converts D-glucose-1-C^{14} to ascorbic acid-6-C^{14}.

The purpose of this study was to ascertain whether or not the synthesis of ascorbic acid by Chloretone-stimulated rats is affected by the diabetic state in which the primary meta-

* Present address: Department of Surgery, State University of Iowa, Iowa City.

TABLE I. Urinary Excretion of Ascorbic Acid by Rats before and after Addition of Chloretone to the Diet.*

	Fasting blood sugar, mg %	Urinary ascorbic acid (mg/24 hr)	
		Milk	Milk + Chloretone
Control (11)	81 ± 15	.76 ± .47	22.03 ± 9.89
Alloxan diabetic (12)	284 ± 119.5	3.40 ± 1.21	25.3 ± 5.65
" resistant (6)	111 ± 24	2.03 ± .51	23.5 ± 5.04

Statistical Comparison of Ascorbic Acid Excretion.

	P†	
	Before Chloretone	After Chloretone
Alloxan diabetic *vs* control	<.005	>.10
Alloxan resistant *vs* control	"	"
Alloxan diabetic *vs* alloxan resistant	<.025	

* Values given = mean ± stand. dev. † Level of significance.

bolic deficiency apparently is represented by decrease in the hexokinase reaction and the conversion of glucose to glucose-6-phosphate.

Methods. Twenty-seven male and 4 female rats, weighing approximately 190 g and of the Sprague-Dawley strain, were fasted for 24 hours and then maintained on a diet of evaporated milk until excretion level of ascorbic acid was stabilized and a suitable number of control levels obtained. Animals were kept in metabolic cages and urine collected over 24-hour periods. Three groups of animals were employed: 1. Normal; 2. Alloxan diabetic; 3. Rats given alloxan but which maintained blood sugar levels below 140 mg %. The rats in group 2 were made diabetic by intramuscular injection of 0.1 ml of 10% alloxan solution/100 g body weight. After excretion of ascorbic acid stabilized, 20 mg of Chloretone was added daily to the diet in all groups of animals. Excretion of ascorbic acid in urine was determined on the 1st, 2nd, 4th, and 6th days after addition of Chloretone. There was no appreciable increase in excretion after the 6th day. Ascorbic acid was determined by the Bolin and Book(6) modification of the Roe and Keuther(7) method. Urine was collected as described by Schwartz and Williams(8). Somogyi's (9) method was used for blood sugar determination.

Results. The results for 11 normal control rats are shown in Table I and demonstrate a mean excretion of ascorbic acid of 0.76 ± 0.47 mg/24 hrs. After 6 days of evaporated milk plus Chloretone, they excreted a mean of 22.03 ± 9.89 mg/24 hrs. The 12 alloxan diabetic rats excreted a mean of 3.4 ± 1.2 mg/24 hrs while on evaporated milk and after 6 days of Chloretone, excreted a mean of 25.3 ± 5.65 mg/24 hrs. The third group, consisting of 6 rats, had been injected with alloxan but did not show much elevation in their fasting blood sugar. This group was arbitrarily called alloxan-resistant and was included with the series because they demonstrated an increase in the ascorbic acid excretion while on evaporated milk, giving a mean value of 2.03 ± 0.51 mg/24 hrs. The mean excretion after 6 days of evaporated milk plus Chloretone was 23.5 ± 5.04 mg/24 hrs in this group. Mean fasting blood sugar for the normal control rats was 81 ± 15 mg/100 ml, for the alloxan diabetic animals 284 ± 119.5 mg/100 ml, and for the alloxan-resistant rats 111 ± 24 mg/100 ml.

Statistical comparison of the 3 groups is shown in the table. It is apparent from the level of significance that the alloxan diabetic and normal rats were in statistically different groups relative to excretion of ascorbic acid while on evaporated milk. This was also true of the alloxan-resistant and normal rats. The level of significance is somewhat lower when the alloxan diabetic and alloxan-resistant groups are compared, but the difference is statistically significant. There was no statistical significance between all 3 groups after adding Chloretone to the diet.

Discussion. It is evident that the alloxan

diabetic rat is capable of synthesizing as much ascorbic acid as the normal rat under the stimulus of Chloretone. This suggests that the usual pathways of glucose utilization which are blocked or inhibited in the alloxan diabetic rat are not necessary for the synthesis of ascorbic acid. Thus, the conversion of glucose to glucose-6-phosphate apparently is not an essential reaction for the synthesis of ascorbic acid.

The excretion of ascorbic acid in the 3 groups is much different while on evaporated milk only. The alloxan diabetic rat excreted far greater quantities of ascorbic acid than did the normal rat. Among the possible explanations for this observation are the following: (1) Alloxan has a toxic action on the tubular reabsorption mechanism in the kidney resulting in increased ascorbic acid excretion and stimulus to synthesis. (2) There is competition in the tubular reabsorption mechanism between glucose and ascorbic acid with preference to reabsorption of glucose, thus effecting increased ascorbic acid excretion and, in turn, stimulating increased basal synthesis. Competition between glucose and ascorbic acid reabsorption has been demonstrated in the dog by Selkurt(10). (3) Increased blood sugar by mass action effect stimulates increased synthesis. There is some evidence to show that added glucose feeding does not increase synthesis of ascorbic acid. Perhaps the sustained high level of blood sugar in the diabetic does increase the rate of synthesis. Any one of these explanations would also explain the observation that the alloxan-resistant animal excreted more ascorbic acid than did the normal, but less than the diabetic rat while on evaporated milk only. It seems that the second hypothesis may represent the most likely explanation.

Summary. Synthesis of ascorbic acid is not impaired in the alloxan diabetic rat, indicating that the hexokinase reaction is not necessary for this synthesis. Ascorbic acid excretion by the alloxan diabetic rat and the alloxan-treated but nondiabetic rat is greater than for the normal rat while on evaporated milk only.

1. Longenecker, H. E., Musulin, R. R., Tully, R. H., III, and King, C. G., *J. Biol. Chem.*, 1939, v129, 445. Longenecker, H. E., Fricke, H. H., and King, C. G., *ibid.*, 1940, v135, 497.

2. Jackel, S. S., Mosbach, E. H., Burns, J. J., and King, C. G., *ibid.*, 1950, v186, 569.

3. Horowitz, H. H., Doerschuk, A. P., and King, C. G., *ibid.*, 1952, v199, 193.

4. Horowitz, H. H., and King, C. G., *ibid.*, 1953, v200, 125.

5. Burns, J. J., and Mosbach, E. H., *ibid.*, 1956, v221, 107.

6. Bolin, D. W., and Book, L., *Science*, 1947, v106, 451.

7. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1945, v147, 399.

8. Schwartz, M. A., and Williams, J. M., Jr., *ibid.*, 1952, v197, 481.

9. Somogyi, M., *J. Biol. Chem.*, 1945, v160, 61, 69.

10. Selkurt, E. E., *Am. J. Physiol.*, 1944, v142, 182.

Received December 10, 1956. P.S.E.B.M., 1957, v94.