glycemic phase is either extrapancreatic in origin as first suggested by Houssay[5] or that insulin is released from the islets by the action of a small quantity of alloxan insufficient to produce detectable histological changes.

After these studies had been completed we learned that Carrasco-Formiguera[6] had conducted experiments in 4 dogs in which alloxan, in dosage of 75 mg/kg, was injected into an intramesenteric vein ". . . while the pancreas was totally excluded from the circulation by clamping all its visible channels of irrigation, previous to, through 6 minutes after the said injection . . .". All 4 dogs developed profound hypoglycemia and in the 2 animals whose tissues were examined histologically the pancreas was said to be normal but the liver showed extremely severe lesions. Carrasco-Formiguera concluded from these experiments that the probable cause of alloxan hypoglycemia was liver damage. However, in our rabbits with occluded pancreatic circulation the alloxan was injected into an ear vein instead of into the portal system and hypoglycemia developed without detectable histological changes in the liver.

Summary. Diabetes was prevented in 9 of 10 rabbits given alloxan by temporary occlusion of the celiac and superior mesenteric arteries. The remaining animal developed moderate, delayed diabetes with mild histological changes in the islets of Langerhans.

Severe hypoglycemia was observed in several of the rabbits which failed to develop diabetes and which failed to show any histological change in the islets of Langerhans or the liver when examined at 2 to 15 days.

These experiments indicate that alloxan diabetes is pancreatic in origin but suggest that the hypoglycemic phase is either extrapancreatic in origin or may be produced by the action of very small quantities of alloxan insufficient to produce detectable changes in the islets of Langerhans.

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5 Houssay, B. A., Orias, O., and Sara, I., *Science*, 1945, **102**, 197.

6 Carrasco-Formiguera, R., *Arch. Biol. Pat. (Univ. de Los Andes)*, 1948, **1**, 111.

17264. Effect of Uric Acid in Glutathione-Deficient Rabbits.*

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The production of diabetes by the administration of alloxan or chemically related compounds, dialuric acid or alloxantin, has provoked considerable speculation whether these may occur in the body as normal or abnormal metabolites in sufficient concentration to damage the islets of Langerhans. Since alloxan can be obtained by the reduction of uric acid *in vitro*, Lazarow[1] suggests that an abnormal purine metabolism may possibly produce alloxan or an alloxan-like compound.

The injection of alloxan produces a profound decrease in blood reduced glutathione in rabbits[2] and rats[3] and the administration of glutathione prior to the injection of alloxan prevents the diabetogenic action of the latter.[1] This suggests that glutathione may represent one of the natural protections of the body against alloxan or alloxan-like substances.

Griffiths[4] artificially lowered the blood glutathione in 4 rabbits from an average of 38 to 18-23 mg/100 cc by feeding a diet de-

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1 Lazarow, A., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 441.

2 Leech, R. S., and Bailey, C. C., *J. Biol. Chem.*, 1945, **157**, 525.

3 Bruckmann, G., and Wertheimer, E., *J. Biol. Chem.*, 1947, **168**, 241.

4 Griffiths, M., *J. Biol. Chem.*, 1948, **172**, 823.

TABLE I.
Effect of a Cystine and Methionine Deficient Diet on Weight, Hemoglobin, and Blood Glutathione in Rabbits.

Rabbit No.	Weeks of diet	Wt, g		Hemoglobin, g/100 cc		Blood reduced glutathione, mg/100 cc	
		Initial	Final	Initial	Final	Initial	Final
D1	8	1759	1050	—	5.0	32	20
D2	8	1710	1410	—	5.0	45	22
(2)*	6	1700	1110	5.4	4.7	25	21
D3	11	1775	1222	—	5.1	46	27
D4	7	1952	1493	—	—	56	27
D5	9	1341	1275	—	6.2	46	28
D6	6	1767	1360	—	6.4	44	21
(2)*	7	2180	1280	9.4	7.1	43	17
D7	5	1720	1555	9.3	4.6	50	15
D8	16	1975	1250	11.3	3.8	49	29
D9	12	2480	1810	10.0	5.0	44	33
D10	4	3120	2640	8.8	7.8	44	19
D11	14	3210	1950	11.1	5.3	48	38
D12	12	2980	3015	10.6	7.9	38	19

* After an interval of 1 to 2 weeks on usual rabbit pellet diet.

ficient in cystine and methionine. Uric acid, in dosage of 1 g/kg, was then injected intraperitoneally and the blood sugar determined. He reports that a moderate hyperglycemia ensued by the 2nd day after the injection and remained for 4-5 days. Thereafter normal blood sugar levels were regained.

This paper describes at attempt to repeat Griffiths' experiments and also to determine if decreasing the blood glutathione by bleeding renders the animal sensitive to the production of hyperglycemia when given uric acid.

Methods and Materials. Male albino rabbits weighing 1.3 to 3.2 kg were used and were fasted for 16 to 18 hours before the injection of uric acid.

Griffiths' modification[4] of a cystine and methionine deficient diet described by Haag and Wright[5] was used and this was fed to each animal for 4 to 16 weeks to lower the blood glutathione. An attempt was made to secure blood glutathione levels under 25 mg/100 cc before the injection of uric acid.

In the hemorrhage experiments varying amounts of blood were removed by repeated cardiac punctures. Approximately 30 cc were removed daily or almost daily and bleeding was continued until a low blood glutathione was obtained in 5 rabbits. Uric acid was then injected.

Control rabbits on the usual laboratory diet of Park and Pollard rabbit pellets were also injected with uric acid.

Uric acid (Eastman Kodak or Coleman and Bell Co.) was administered intraperitoneally in dosage of 1 g/kg in a suspension in 20 cc of water. Blood sugar and blood uric acid concentrations were determined before and at intervals after the uric acid injection.

Blood reduced glutathione was determined by the Potter and Franke[6] modification of the Benedict and Gottschall[7] method adapted to the Leitz photoelectric colorimeter. Blood uric acid was determined by the method of Folin.[8] Blood sugar was determined by the Folin and Malmros[9] micromethod and in some cases, to decrease the reducing effect of the uric acid on the reagents, Nelson's[10] blood sugar method was used.

Results. It was found difficult to lower the blood glutathione in rabbits by feeding the

[5] Haag, J. R., and Wright, L. D., *J. Nutrition*, 1940, **19**, 563.

[6] Potter, V., and Franke, K., *J. Nutrition*, 1935, **9**, 1.

[7] Benedict, S. R., and Gottschall, G., *J. Biol. Chem.*, 1932, **99**, 729.

[8] Folin, O., *J. Biol. Chem.*, 1930, **86**, 179.

[9] Folin, O., and Malmros, H. J., *J. Biol. Chem.*, 1929, **83**, 115.

[10] Nelson, N., *J. Biol. Chem.*, 1944, **153**, 375.

TABLE II.
Effect of Hemorrhage on Blood Reduced Glutathione Concentration in Rabbits.

Rabbit No.	Duration of bleeding period (days)	Amt. of blood withdrawn (cc)	Hemoglobin, g/100 cc		Blood glutathione, mg/100 cc	
			Initial	Final	Initial	Final
H1	6	53	8.4	5.9	35	23
H2	3	78	9.8	5.2	38	19
H3	9	109	10.3	8.1	42	42
H4	11	312	9.8	3.8	33	26
H5	3	85	7.8	3.4	33	17
H6	2	122	9.6	4.1	38	20

sulfhydryl-deficient diet as the animals showed anorexia, loss of weight, marked loss of hair, anemia and generalized weakness. Of 21 rabbits on the diet 9 died before the blood glutathione was lowered to 25 mg/100 cc. The effect of the diet on weight, hemoglobin and blood glutathione in the remaining 12 animals is shown in Table I. Only 5 of these rabbits (D1, 2, 6, 7, 10) showed a lowering of blood glutathione to 22 mg or less in 4 to 8 weeks of diet and 1 (D12) in 12 weeks. The remaining 6 were injected with uric acid although the blood glutathione was still 27 to 38 mg/100 cc after 9 to 16 weeks of the diet. Unless the blood glutathione dropped markedly in the first 4 to 8 weeks it seemed that it was unlikely to fall to 25 mg or less with a prolonged diet.

At the suggestion of Griffiths[11] 18 young rabbits weighing 0.5 to 1.0 kg were put on a modified diet containing one-half the protein of the original diet but none of these survived beyond 4 weeks. Six adult rabbits also failed to survive beyond 4 weeks on this reduced protein diet.

The effect of hemorrhage on the blood glutathione is shown in Table II. In 4 rabbits (H1, 2, 5, 6) there was a reduction to 23 mg/100 cc or less with removal of 53 to 122 cc of blood in 2 to 6 days but 2 others (H3,4) were apparently better able to compensate for the blood loss. This compensation has been observed in patients with anemia.[12] One of our rabbits showed no lowering of blood glutathione after 109 cc of blood were removed in 9 days and in another there was only moderate lowering after removal of 312 cc in 11 days.

Table III shows the effect of intraperitoneal injections of uric acid into control rabbits and into those made sulfhydryl-deficient by diet or bleeding. In each group, including the controls, most of the animals had apparent hyperglycemia for several hours after the uric acid injection but by 24 hours the blood sugar was normal. Such initial hyperglycemia did not occur in those animals which failed to show an elevation of the blood uric acid. In 2 animals (D6, C2) a second injection of uric acid at a later date gave the usual increase in blood uric acid accompanied by apparent hyperglycemia. By the Nelson sugar method which is less affected by a high blood uric acid, the hyperglycemia was less but in several cases quite definite.

After the first 24 hours, when the blood uric acid was always negative, one of the rabbits on the deficient diet (D6) showed a marked transitory hyperglycemia reaching 366 mg/100 cc 2 days after injection of uric acid and one other (D4) a mild hyperglycemia of 200 mg on the 5th day. In the hemorrhage group one rabbit (H4) showed a transient hyperglycemia after uric acid reaching 218 mg on the 3rd day. None of the other animals had blood sugar values significantly higher than the control group injected with uric acid.

Rabbit D6 which developed marked hyperglycemia at 2 days was an animal which had failed to show uric acid in the blood after it had been injected intraperitoneally. When D6 was reinjected 8 weeks later the blood

[11] Griffiths, M., personal communication.

[12] Pickard, R. J., and Marsden, C. S., *J. Lab. and Clin. Med.*, 1933, **19**, 395.

TABLE III.
Effect of Uric Acid Injection on the Blood Sugar in Rabbits.

No.	Group	Blood-glutathione when injected (mg/100 cc)	Highest blood uric acid noted after injection (mg/100 cc)	Blood sugar (mg/100 cc)									
				Time after uric acid									
				Hours				Days					
				0	1	3	5	1	2	3	4	5	6
				Fasting values				Non-fasting values‡					
D1	Diet*	20	28	135	160	120	--	48 dead					
D2	"	22	41	128	205	235	214	102	149	147	165	137	137
	(2)	21	28	124	246	260	218	124	181	118	139	130	139
D3	Diet	27	trace	105	90	80	116	80	114	124	120	—	120
D4	"	27	20	114	160	135	—	105	132	142	160	*200*	160
D5	"	28	15	129	180	165	133	95	133	89	116	—	120
D6	"	21	neg.	107	128	135	139	153	*366*	*352*	*278*	*205*	183
	(2)	15	31	130	211	214	233	114	155	139	114 dead		
D7	Diet	15	trace	135	155	149	139	143	149	135	124	—	125
D8	"	29	10	103	260	354	368	84 dead					
D9	"	33	neg.	124	103	112	67	87	103	95	102	—	95
D10	"	19	7	107	143	147	135	116	145	122	127	127	109
D11	"	38	33	149	185	171	130	178	104	127	27 dead		
D12	"	19	7	105	260	294	240	124	128	130	137	143	129
H1	Hemorrhage	23	16	171	211 died								
H2	"	19	13	141	208	250	239	120	153	130	139	130	
H3	"	42	3	143	155	147	128	122	109	109	112	124	
H4	"	26	7	124		174		124		*218*	194	165	165
H5	"	17	neg.	124	135	128		120	135	120	135		120
H6	"	20	trace	116	128	114	112	133	135	130		130	
C1	Control†	57	11	85	200	165	169 dead						
C2	"	38	neg.	132	124	128	92	118	151	156		137	128
	(2)	38	11	124	188	294	294	114	120	120		124	
C3	"	59	14	92	135	137		101	120	114	120		
C4	"	47	8	95	135	149	143	107	116	109	95		
	(2)	42	14	101	151	153	167	101 reinjected daily blood sugars normal					

* Cystine and methionine deficient diet,
† Park and Pollard rabbit pellet diet.
‡ For convenience in interpretation, all blood sugar values of 160 mg or above at 1 day or later, appear in *italic* type.

uric acid rose to 31 mg/100 cc but no hyperglycemia ensued after 24 hours. Rabbit D4 with a mild hyperglycemia reaching 200 mg did show a blood uric acid elevation to 20 mg/100 cc after injection.

In one rabbit with blood glutathione lowered to 25 mg/100 cc, neutralized sodium urate in 1.2% solution was injected intravenously. Five injections of 0.4 mg/kg each were given in 15 days. The blood sugar remained normal throughout.

Discussion. The cystine and methionine deficient diet theoretically lowers the blood glutathione, a tripeptide containing glycine, glutamic acid and cysteine, by depriving the body of cystine. The rabbits in our experiment did not tolerate this diet well and it was therefore difficult to secure rabbits with lowered blood glutathione for uric acid injection.

No explanation is apparent why one animal showed marked hyperglycemia for 4 to 5 days after uric acid while others which had blood glutathione values as low or lower failed to do so. Only 2 of the 8 rabbits with glutathione of 27 mg or less showed hyperglycemia.

We cannot explain why only a trace or no blood uric acid could be detected after its intraperitoneal injection in 7 instances whereas others attained blood uric acid levels up to 41 mg/100 cc at the same time intervals, nor can we explain the different response in the same animal with a second injection.

A rough correlation existed in most cases between the apparent hyperglycemia observed in the first few hours after injection and the height of the blood uric acid, which may be explained in part by the fact that uric acid reduces the ferricyanide blood sugar reagent. However there were several instances of rising blood sugar with falling uric acid levels and also some high blood sugar values by the Nelson method.

Since all animals became quite anemic on the cystine and methionine deficient diet, and since almost all of the glutathione in normal blood is in the red cell, attempts were made to lower the glutathione by repeated bleedings. In the small group of animals with the blood glutathione lowered in this manner transient hyperglycemia was observed at 3 to 4 days in 1 of 4 rabbits.

Although the results of these experiments were variable and in most cases negative it was thought worthwhile to report them in view of the current interest in glutathione in the prevention of diabetes and the possible role of uric acid or related compounds in the production of diabetes.

In spite of the large proportion of negative results the marked hyperglycemia in one rabbit and the moderate hyperglycemia in 2 others given uric acid with a lowered blood glutathione suggest that further investigation of this subject may be worthwhile. However the impression is gained that if uric acid under these conditions is diabetogenic, its action is very weak.

Summary. Attempts were made to confirm the report of Griffiths that transient diabetes follows the intraperitoneal injection of uric acid into rabbits in which the blood glutathione has been lowered by feeding a diet deficient in cystine and methionine.

In our experiment 21 rabbits were fed the deficient diet. Twelve animals surviving the dietary restriction were injected with uric acid although only 6 had a blood glutathione below 23 mg/100 cc. One of these had a definite transient hyperglycemia for 5 to 6 days after the uric acid injection. No blood sugar changes were seen in the other animals after 24 hours. One rabbit with a blood glutathione of 27 mg/100 cc showed a moderate transient hyperglycemia.

When the blood glutathione was lowered by bleeding instead of diet only one of 4 rabbits injected with uric acid showed a transient hyperglycemia after 24 hours.

It is concluded that if uric acid is diabetogenic under these conditions its action is weak.

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