disease with giant bilateral apical cavities. On February 4, 1948, an anterior stage thoracoplasty was done on the left side. On May 28, 1948, intracavitary drainage was established in the left apical cavity. The poor general condition of the patient prevented further collapse therapy. On September 22, 1948, the left apical cavity was entered into through the drainage tract. Cavernostomy was done, and the depths of the cavity were lined with a split-thickness skin graft. This was repeated on October 20, 1948, November 17, 1948 and on February 9, 1949; on the last occasion pinch grafts were used. In all instances mentioned, at least a 75% "take" of the graft was obtained. The cavity on the left side diminished and gradually was covered with skin; the bronchial openings, which at first were large, gradually closed. At autopsy, May 8, 1949, there was no evidence of an open bronchus leading to the skin surface. The surface skin on the chest wall showed a depression about 1½″ in diameter which entered into the lung. The cavity seemed to be completely covered by skin and was entirely exteriorized. The cavity on the right side remained unchanged. Two other cases have been treated. In both skin grafting has been successful and the cavities seem to be healing.

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17263. Effect of Alloxan in Rabbits with Temporary Occlusion of the Arteries to the Pancreas.*

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Numerous investigations have shown that the injection of alloxan into various animals causes destruction of the pancreatic islets of Langerhans with the production of diabetes. After the injection of a diabetogenic dose of alloxan intravenously into a rabbit hyperglycemia appears which reaches its peak within 2 hours. This in turn is followed in 2 to 9 hours by profound hypoglycemia frequently with convulsions. Finally within 24 to 48 hours diabetes develops and although most agree that the diabetes results from the destruction of the islets of Langerhans, the initial hyperglycemic phase and especially the hypoglycemic phase have caused much speculation and disagreement.

Although diabetes usually is considered to result from an action of the drug on the pancreatic islets, Jimenez-Diaz[1] reported that clamping the renal blood vessels immediately before the injection of alloxan prevents the development of diabetes. Others[2] have not been able to confirm this.

In the present investigation alloxan was injected into rabbits with the blood supply to the pancreas occluded temporarily in order (1) to confirm that alloxan diabetes is pancreatic in origin, and (2) to determine if the hypoglycemia is dependent upon alloxan producing histological changes in the islets of Langerhans.

Materials and Methods. Male albino rabbits weighing 2.0 to 2.7 kg were used and each was fasted for 16 to 18 hours before an experiment. Anesthesia was obtained by 18 to 20 mg/kg of 5% sodium pentobarbital intravenously supplemented by ether given by open mask as required. A transverse incision was made in the left upper quadrant of the abdomen extending quite far laterally. The celiac axis and the superior mesenteric artery were exposed and clamped close to the abdominal

* This work was aided by grants from the American Cyanamid Co., and the Diabetic Fund.

1 Jimenez-Diaz, G., Grande-Covian, F., and DeOya, J. C., *Nature*, 1946, **158**, 589.

2 Martinez, C., Gitter, S., and Covian, M. R., *Rev. Soc. argent de biol.*, 1947, **223**, 81.

aorta with rubber-tipped bulldog clamps. Each animal was then given 150 mg/kg of 5% alloxan (Eastman Kodak) into an ear vein during a 2 minute period and the clamps were left in place for a further period of 8 minutes. The clamps were then removed and the incision closed. Blood samples were obtained immediately before the alloxan injection and at frequent intervals thereafter, and the blood sugar was determined by the Folin and Malmros micromethod.[3]

The animals were sacrificed by an intravenous injection of sodium pentobarbital. A part of the pancreas was removed immediately and fixed in Bouin's fluid. The remainder of the pancreas and portions of other organs were fixed in Helly's fluid. Gomori's chrome alum hematoxylin stain[4] was used to differentiate beta and alpha cells in the islets of Langerhans, and hematoxylin and eosin was used on the other tissues.

Preliminary Experiments. In some preliminary experiments the celiac artery alone was clamped. Of 8 such animals given alloxan 2 developed severe diabetes at 24 hours with typical destruction of the pancreatic islets. Two others developed mild, transient, delayed hyperglycemia after 6 days but when studied histologically at 19 and 21 days no abnormalities were detected in the pancreas. The remaining 4 failed to show hyperglycemia in 19 to 37 days of study and on histological examination the pancreas appeared normal. Of the 6 animals which failed to develop typical diabetes 5 had shown definite early hypoglycemia with convulsions in 3 cases.

When India ink (20 cc of a 1 to 5 dilution) was injected into an ear vein with only the celiac artery clamped small quantities of the ink were detected in the pancreas indicating that the blood supply was not completely occluded. However, when both the celiac and mesenteric arteries were clamped and India ink injected no ink could be detected with certainty in the pancreas of 2 animals while in a 3rd only very small quantities were present. Control sections of the liver and kidney

TABLE I.
Effect of Alloxan on Blood Sugar of Rabbits with Celiac and Superior Mesenteric Arteries Occluded.

Rabbit No.	Blood sugar in mg/100 cc																		Notes
	Control† 0	Min. 20	Min. 40	Hr 1	Hr 2	Hr 3	Hr 4	Hr 5	Hr 6	Hr 7	Hr 8	Hr 9	Days 1	Days 2	Days 3	Days 4	Days 5	Days 6	Days 7	Days 12-15	
1	127	177	152	142	133	87	124	106	97				70	137		93	104		99	105	Sacrificed 15 days
2	114	142	122	122	118	92	94	84	92				124	116		135	121		127	118	" 15 "
3	103	87	87	103	84	66	47*	65	65				96	108	114						" 3 "
4	114	127	137	153	148	139	87	70	57		43*		124	116	122						" 3 "
5	84	92	89	148	171	131	81	41*	50				159	—	139	171	258	230			" 6 "
6	94	93	84	87	75	45	34*						59	118							Died 2 "
7	101	109	114	109	150	101	88	73	37*				79	129	85				116	109	Sacrificed 12 "
8	99	120	128	131	114	112	93	82	69				96	105	92				108	116	" 12 "
9	89	81	85	90	120	127	105	106		62	52	46	106	116	112		108				" 5 "
10	103	81	101	112	135	118	97	68		59	60		175	156	153		123				" 5 "

* Hypoglycemic reaction.
† After induction of anesthesia, immediately before alloxan injection.

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

[4] Gomori, G., *Am. J. Path.*, **1941, 17, 395.**

showed large quantities of the ink.

In control animals with both the celiac and superior mesenteric arteries occluded but no alloxan or ink injected the blood sugar remained within a normal range.

Results. Table I shows the blood sugar of 10 rabbits given alloxan just after clamping the celiac and superior mesenteric arteries.

An initial hyperglycemic phase usually seen after the injection of alloxan in normal animals was present but not striking in most of these experiments.

Six of the 10 rabbits developed severe hypoglycemia within 3 to 9 hours after alloxan with blood sugar values of 34 to 47 mg/100 cc and 2 others had blood sugar values of 59 and 69 respectively. The remaining 2 failed to develop hypoglycemia by 6 hours when they were fed and the possibility exists that hypoglycemia might have occurred had they been fasted longer.

Nine of the 10 animals failed to develop diabetes. Eight were sacrificed in 3 to 15 days and one rabbit, which had unusually prolonged hypoglycemia, died of an undetermined cause 2 days after the injection with a normal blood sugar. In the remaining rabbit (No. 5) the blood sugar was normal for 3 days, reached 171 mg on the 4th day and 258 and 230 respectively, on the 5th and 6th day at which time the animal was sacrificed. Such a delayed onset of hyperglycemia occurs occasionally in unoperated rabbits given alloxan but usually definite hyperglycemia develops within 24 to 48 hours.

For histological examination 2 animals were sacrificed at 3 days, 3 at 5 days, 2 at 12 days and 2 at 15 days. No significant findings were noted in the liver, spleen, adrenals or kidneys in any of the animals. Seven rabbits (Nos. 2, 3, 6, 7, 8, 9, 10) showed no definite histological changes in the pancreas. The tissues of one animal (No. 1) were inadvertently destroyed. In the remaining 2 (Nos. 4 and 5), mild alterations in the pancreas were found.

In rabbit No. 5 which developed delayed hypoglycemia the islets showed (1) an apparent reduction in the total number of beta cells, some islets consisting solely of alpha cells; (2) a definite variation in size of the nuclei of beta cells, with some large hyperchromatic forms; (3) a total of 4 mitotic figures in beta cells found in examination of 2 different sections; and (4) a pallor of the cytoplasm of the beta cells, suggesting degranulation but without definite vacuoles. Rabbit 4, which had a normal blood sugar and appeared well when sacrificed 3 days after alloxan, showed the same type of histological changes but to a considerably less degree.

It should be pointed out that the changes described above are in marked contrast to the massive necrosis of beta cells which usually follows a diabetogenic dose of alloxan. The findings do suggest a mild injury of the beta cells with evidence of regeneration.

Discussion. These experiments strongly suggest that alloxan diabetes is pancreatic in origin for we have not observed definite diabetes when the pancreatic blood supply was interrupted sufficiently to prevent detectable changes in the islets of Langerhans.

Although very mild, transitory, delayed hyperglycemia was observed in 2 rabbits with only the celiac artery occluded and no islet changes were seen, which appears contradictory, it must be emphasized that these animals were sacrificed at 19 and 21 days after the administration of alloxan and it is well known that slight pancreatic islet damage observed in the first 2 or 3 days after alloxan injection may not be visible several days later. Since very small amounts of India ink were present in the pancreas of 1 of 3 animals with both celiac and superior mesenteric arteries occluded, and since 1 rabbit developed delayed hyperglycemia accompanied by slight but definite pancreatic changes, it is not certain that clamping these 2 vessels always eliminates completely the blood supply to the pancreas.

From these experiments it is impossible to say that no alloxan reached the pancreas and that the hypoglycemic phase was extrapancreatic in origin. However, hypoglycemia was observed to follow the injection of alloxan without the subsequent development of diabetes and without detectable changes in the islets of Langerhans even in 2 animals whose pancreas was examined at 2 and 3 days when alloxan damage to the islets should be severe.

These experiments suggest that the hypo-

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-

* This work was aided by grants from the American Cyanamid Co., and the Diabetic Fund.

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.