4. Treatment of experimental *B. pyocyaneus* infections of the cornea: Ulcers of uniform severity were produced by inoculation of the cornea with a 24-hour broth culture of a virulent strain of *B. pyocyaneus*. Treatment consisted of 3 applications (drops) at 2-hour intervals of streptomycin solution containing 10,000 μg per ml. This form of treatment afforded complete protection when commenced within 6 hours following inoculation. The infected control eyes uniformly progressed to complete destruction of the cornea.

Summary. 1. The penetrability of streptomycin through the cornea may be increased by abrasion, inflammation, ion-transfer and wetting agents. 2. No local toxic effects were noted when saline solutions of streptomycin containing up to 10,000 μg per ml were used. With concentrations of 50,000 μg or as a dry powder, delayed healing occurred. 3. Intraocular injection, in amounts up to 1,000 μg of streptomycin in 0.1 ml saline were well tolerated. Smaller amounts (25 to 300 μg) were therapeutically effective up to 6 to 8 hours against a virulent strain of *Str. pyogenes,* though transient or negligible vitreous opacities occurred with these concentrations. 4. Experimental corneal ulcers produced by injection of *B. pyocyaneus* were prevented when treatment was started within 6 hours after inoculation by 3 applications at 2-hour intervals of a saline solution of streptomycin containing 10,000 μg per ml.

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Further Studies on the Mechanism of Alloxan Diabetes, Pancreatectomy and Alloxan.*

MARTIN G. GOLDNER AND GEORGE GOMORI.

From the Department of Medicine, The University of Chicago.

The experiments to be presented here were performed in an attempt to investigate further the significance of the initial blood sugar fluctuations after alloxan and the character of the ensuing diabetes. To be more specific, they concern the problems of the pancreatic origin of the alloxan hypoglycemia and the interrelationship of external and internal pancreatic secretion in the development of alloxan diabetes.

The typical triphasic fluctuation of the blood sugar after a diabetogenic dose of alloxan is well known. An initial hyperglycemia is followed within a few hours by a secondary hypoglycemic phase which may last from 6 to 10 hours and after which the final persistent hyperglycemia and glycosuria develops. It is important to emphasize that the 2 initial phases, especially the secondary hypoglycemia, vary in severity with different species. In the dog for instance these fluctuations are rather mild and asymptomatic, while the rabbit is thrown into severe hypoglycemic shock and convulsions which it survives only if large amounts of glucose are given repeatedly over several hours.

The hyperglycemic phase is absent in adrenalectomized rabbits[1] and depends to a certain degree on the nutritional state of the animals and the glycogen stores of the liver. It is absent, too, in hepatectomized animals.[2] The explanation that it is extrapancreatic in origin[1] has found general confirmation and acceptance.

The secondary hypoglycemia starts at about the same time when histological examination of the pancreas begins to discover degenerative changes in the beta cells as

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1 Goldner, M. G., and Gomori, G., *Endocrinology,* 1944, **35**, 241.

2 Houssay, B. A., Orias, O., and Sara, T., *Science,* 1945, **102**, 197.

TABLE I.
Blood Sugar Fluctuation Following the I.V. Injection of a Diabetogenic Dose of Alloxan into Dogs Which Were Pancreatectomized Several Days or Weeks Prior to the Experiment.

Dog N R	Days after pancreatectomy	Insulin treatment	Fasting blood sugar	Dose of alloxan, mg/kg	Blood sugar level (Hr after injection of alloxan) 1	2	3	4	5	6	8	24	48
122	11	+	156	50	151	155	157	175	—	165	—	161	136
214	12	+	168	50	190	194	214	—	218	—	212	198	151
142	7	0	297	50	—	315	—	295	—	254	—	312	278
528	6	0	332	50	329	249	334	324	317	338	—	340	—
186	30	0	288	50	288	321	324	311	—	—	—	304	314
132	6	0	266	60	—	290	—	286	—	266	—	268	306
221	21	0	258	75	269	238	—	208	—	211	—	283	264
168	40	+	219	75	227	238	—	203	—	220	—	247	186
123	6	0	273	100	322	337	—	307	302	—	290	303	244

pyknosis, cloudy swelling and degranulation. On the basis of this observation and others, as for instance that the fall of the blood sugar fails to occur only then when the dose of alloxan is insufficient to induce necrosis of the islet cells, it was suggested that the secondary hypoglycemia is pancreatic in origin and due to stored and preformed insulin leaking out of the degenerating beta cells.[1] This explanation has found confirmation by many workers, but of late has been challenged by others. Particularly Houssay and his co-workers[2,3] have reported that they have observed hypoglycemic reactions in dogs that were pancreatectomized shortly prior to the injection of alloxan. They therefore ascribed the secondary hypoglycemia to an extrapancreatic mechanism and suggested that it may be due to a primary effect of alloxan upon the liver. Since they do not deny the direct effect of alloxan upon the islet cells nor the pancreatic origin of the ensuing diabetes, one would have to conceive that alloxan produces hypoglycemia simultaneously by 2 independent actions upon 2 different target organs, *i.e.* in the pancreas by release of insulin, in the liver by prevention of glycogenolysis. Unlikely as this coincidence may appear theoretically, we felt necessary to review and to extend our own experiments on the effect of alloxan upon depancreatized dogs. We have failed to find any alloxan hypoglycemia in such animals. Our observations were made on 4 different groups of experiments. Table I shows the fluctuation of the blood sugar when a diabetogenic dose of alloxan is given to dogs which had been depancreatized several days or weeks prior to the acute experiment. The first group of dogs whose diabetes was treated adequately with insulin and the second group of dogs with uncontrolled diabetes showed the same negative reaction of their blood sugar, the initial blood sugar level being the only difference. No doubt, the glycogen stores of the livers differ greatly in these 2 groups, yet hypoglycemia did not occur in either one. Similar results were obtained in 2 series of acute experiments (Table II). In the first one, the animals were depancreatized 30 minutes prior to the injection of alloxan. This is the procedure with which the South American workers obtained their positive results. Here too, no hypoglycemia occurred in our animals. The last group are animals in which a functional pancreatectomy was performed. The pancreas was freed and its blood supply interrupted by clamping of all vessels for 5 minutes during and after the injection of alloxan. It has been shown in earlier experiments that alloxan is rendered innocuous to the islet cells within 5 minutes after injection.[4] Thus any fluctuation of the blood sugar after alloxan, observed in these animals, must be extrapancreatic in origin—if the clamping of the vessels is complete. All 4 animals showed a moderate initial hyperglycemia and no hy-

[3] Houssay, B. A., Orias, O., and Sara, T., *Rev. de la Soc. Arg. de Biologia*, 1945, **21**, 30.

[4] Gomori, G., and Goldner, M. G., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 232.

TABLE II.

Fluctuation of Blood Sugar Following I.V. Injection of Diabetogenic Dose of Alloxan in Dogs, the Pancreas of Which Had Been Either Extirpated 30 Days Prior to the Experiment or Clamped for 5 Days After the Injection of Alloxan.

Dog N R	Blood sugar fasting	Type of operation	Blood sugar after operation	Dose of alloxan, mg/kg	Blood sugar level (Hr after injection of alloxan) 1	2	3	4	5	6	8	10	24	48
409	108	Total pancre-	176	60	192	196	272	276	216	—	252	—	332	356
94	106	atectomy	148	60	202	228	280	322	328	—	312	240	284	296
188	72		104	60	98	107	110	124	121	—	152	—	186	318
478	94		89	60	116	140	154	194	166	—	160	—	276	—
165	78	Pancreas clamped	—	60	100	74	72	75	80	73	—	—	84	112
503	96	for 5 min.	—	60	186	116	102	70	61	60	68	—	70	188*
88	90	after inj. of	—	60	144	125	113	89	115	99	101	—	116	104
141	94	alloxan	—	75	122	134	120	94	90	110	112	100	96	96

* This dog developed diabetes. Histological examination revealed that the clamping had been incomplete.

TABLE III.

Blood Sugar Fluctuation Following the I.V. Injection of a Diabetogenic Dose of Alloxan in Rabbits After Partial Pancreatectomy.

Rabbit N R	Time after partial pancreatectomy	Dose of alloxan, mg/kg	Blood sugar level (Before and hours after injection of alloxan) 0	1	2	4	6	8	10	24	48
	Days										
29	12	200	110	232	280	262	120	70	—	180	304
43	10	200	75	184	299	236	82	66	72	195	258
44	21	200	90	210	326	278	56	58	80	212	336
56	21	200	106	170	257	302	124	60	—	198	282
	Min.										
62	30	200	94	144	196	165	102	80	—	182	254
63	30	200	103	175	244	254	148	63	—	206	290
75	30	200	89	160	220	208	94	70	—	240	312
22	Intact control	—	113	232	304	82	47	31*	—	—	—

* Died in hypoglycemic convulsions.

poglycemia occurred in 3 of these animals—none of which developed diabetes. Only one dog, NR. 503, showed a secondary hypoglycemia, but also developed diabetes. Biopsies from the pancreas of this dog showed typical islet cell degeneration and it must be concluded that here the interruption of the blood supply was incomplete. If the hypoglycemia was due to hepatic dysfunction it should not have been followed by diabetes and it should have occurred in the 3 others which did not develop diabetes.

It has been mentioned above that the secondary hypoglycemia is much more marked in rabbits than in dogs. It seemed, therefore, desirable to repeat these experiments in this species. Unfortunately, total pancreatectomy in rabbits is an almost impossible procedure, in a one-stage operation. A 2-stage operation, on the other hand, precludes acute experiments. We therefore have confined ourselves to observations on partially depancreatized rabbits. The operation was performed under sodium pentothal and the splenic part of the pancreas was removed as far as possible. The part close to the portal vein and the duodenum remained *in situ*. Controls with anesthetized rabbits which were not operated upon showed that anesthesia did not modify significantly the blood sugar fluctuation. Table III represents the values of 3 acute and 4 chronic experiments as compared with the reaction of the intact control animal and demonstrates that partially depancreatized rabbits develop after alloxan a less severe hypoglycemia than intact animals.

TABLE IV.
Ligation of Part of the Pancreatic Duct Does Not Protect the Islets in the Ligated Part of the Pancreas Against the Degenerative Effect of Alloxan.

Dog N R	Days after partial ligation of pancreatic duct	Dose of alloxan, mg/kg	Blood sugar level (Hr after injection of alloxan)											Degenerative changes in islets	
			Fasting	1	2	3	4	6	8	10	24	48	72	Ligated part	Nonligated part
519	16	60	72	108	74	92	120	44	91	—	152	197	215	+	+
419	22	200	100	84	98	107	113	128	116	116	61	456	912	+	+
524	20	75	94	110	106	132	110	90	64	78	102	240	276	+	+

None of these animals showed symptoms of hypoglycemic shock, none required glucose infusions and all survived and became diabetic. Similar experiments have been reported by Banerjee.[5] These observations seem to warrant the conclusion that the secondary alloxan hypoglycemia depends on the amount of islet cell tissue present or of insulin available. It seems to us that supportive evidence for this conclusion can be drawn also from the fact that the rabbit with its severe alloxan hypoglycemia has a relative high pancreatic insulin content (5-9 units per g) whereas in the dog the pancreas contains relatively less insulin (2-4 units per g) and the alloxan hypoglycemia is mild. We are, however, aware of the fact that this relationship between alloxan hypoglycemia and pancreatic insulin content does not hold true for every species and that the absolute weight of the pancreas and the rate of production of insulin may play a significant role.

Table IV shows results of experiments in which alloxan was given to dogs with partial ligation of the pancreatic duct. Walpole and Innes[6] in England have reported that duct ligation protects the islet cells against alloxan degeneration and have discussed the possibilities that either a normal acinar tissue may be necessary for the action of alloxan, or that the fibrosis prevents alloxan from reaching the islets—like clamping of the blood supply does. Our experiments rule out this latter mechanical possibility. We ligated only a part of the pancreatic duct, then permitted the ligated part to undergo fibrosis and after a period of 15-21 days gave a diabetogenic dose of alloxan. All 3 animals developed diabetes. Biopsies were taken from both parts of the pancreas and showed that the islet cell necrosis was equally severe in the fibrotic and in the normal part of the gland.

We have not performed total duct ligations. Pancreatic fibrosis, however, is not uncommon among dogs. Thus by coincidence we found a fibrotic pancreas in a dog which had received alloxan and 2 weeks later was pancreatectomized. This dog had developed alloxan diabetes as any other dog and histological examination made it very likely that the fibrosis had existed long before alloxan had been given.

Summary. (1) Further evidence has been presented to support the hypothesis that the secondary alloxan hypoglycemia is pancreatic in origin. (2) Fibrosis of part of the pancreas does not protect the islets in this part against the degenerative action of alloxan.

[5] Banerjee,, S., *J. Biol. Chem.*, 1945, **158**, 547.

[6] Walpole, A. L., and Innes, J. R. M., *Brit. J. Pharm. and Chemotherapy*, 1946, **1**, 174.