THE HYPOPHYSIS AND SECRETION OF INSULIN

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The action of the hypophysis on the pancreas has been demonstrated by a number of *direct and indirect* proofs. The latter, first reported from this Institute, are as follows:

(*a*) *Complete hypophysectomy* or anterior (distal) lobe removal produces: (1) Marked sensitivity to the hypoglycemic and toxic action of insulin and other hypoglycemic agents such as phloridzin, fasting, etc. (2) Reduction of intensity of pancreatic and phloridzin diabetes. (3) A rapid fall in blood sugar and tissue glycogen during fasting; of non-pancreatic origin since it occurs after pancreatectomy. The hypoglycemic symptoms can be relieved by prompt administration of glucose, or prevented by a carbohydrate or protein, but not fat diet.

(*b*) *Anterior pituitary extract* produces the following effects in animals with or without pancreas: (1) Increased resistance to the hypoglycemic and toxic action of insulin and other hypoglycemic agents (phloridzin). (2) Aggravation of diabetes in completely pancreatectomized dogs, with intensified ketosis, acidosis, and rapid death. (3) Strongly increased diabetes in animals without pancreas and hypophysis, in Sandmeyer's partial diabetes, and in phloridzin diabetes of hypophysectomized animals. (4) Production of diabetes in normal dogs, and in dogs with partial pancreatectomy; the ease of production being roughly proportional to the amount of pancreas removed.

In acromegaly the hyperfunction of the anterior pituitary might account for the presence of glycosuria in 32 per cent of the published cases (Atkinson, 1936). Some of these acromegalia showed resistance to insulin which was reduced after partial removal of the pituitary adenoma (Davidoff and Cushing, 1927).

The direct action of the hypophysis on the pancreas has been extensively studied after the hypophysectomy and after administration of hypophyseal extracts, with their resultant effects on pancreas weight, cytology of the islands of Langerhans, insulin content, and insulin secretion.

(*a*) *The pancreas after hypophysectomy* showed an increase of islet tissue in *Triturus viridescens* (Adams and Ward, 1936), rats (Krichesky, 1936), and dogs (Bakay, 1940; Porto, 1941). The insulin content was found to be normal in dogs (Chambers, Sweet, and Chandler, 1935) and rats (Haist and Best, 1940) but normal or greater in relation to body weight (Griffiths and Young, 1940). According to Kepinov and Guillaumie (1934, 1935), insulin secretion is increased in the dog, but we have found it to be normal. The hypoglycemic action of systemic blood described by Cowley (1931) was not confirmed in the careful studies of Elena Di Benedetto (1934).

(*b*) *The effects of hypophyseal extracts* on the pancreas are very different and probably depend on the type of extract, the dose, the time of treatment, the species used both for extract and treatment, and finally, on the relative quantity of pancreas present in the animal.

An hypertrophy and neoformation of islet tissue was obtained in 3 days in the rat by Anselmino, Herold, and Hoffmann (1933) and other species (Anselmino, Herold, and Hoffmann, 1935; Anselmino and Hoffmann, 1933, 1936). Other authors have been unable to obtain such rapid effects, but Richardson and Young (1938) found the amount of island tissue was doubled after 2 or 3 weeks of treatment of rats, with an increased insulin content of 130 to 250 per cent (Marks and Young, 1940 *a*, *b*).

A hypoglycemic action of certain hypophyseal extracts failed to appear after pancreatectomy (Hoffmann and Anselmino, 1933; Steppuhn, 1934, 1935; Harrison and Long, 1940). Using pancreatic-jugular anastomoses in the dog, Zunz and La Barre observed increased insulin secretion after pitressin or pitocin (1935 *a*), thyrotropic extracts (1935 *b*), and pancreatotropic extracts (1935 *c*) of the hypophysis. Posterior pituitary extract was without action on insulin secretion after its injection into the pancreatic artery (Foglia, 1931); furthermore, its hyperglycemic activity was the same in normal and in recently pancreatectomized dogs (Houssay and Di Benedetto, 1933).

The diabetogenic action of anterior hypophysis was discovered in this Institute first in the toad without hypophysis and pancreas (Houssay and Biasotti, 1930, 1931) and afterwards in dogs, normal, partially pancreatectomized, or hypophysectomized-pancreatectomized (Houssay, Biasotti, and Rietti, 1932, 1933). The injection of anterior lobe extracts can produce a diabetes which persists after interrupting the injections as was found in dogs with reduced pancreas (Houssay, Biasotti, and Rietti, 1932) or in normal dogs (Young, 1937). After injecting dogs for 3 days with 1.4 gm. per kilo per day of anterior hypophysis extract the pancreas shows a diminished insulin secreting capacity. On grafting it to the neck of a pancreatectomized dog it will not lower the high blood sugar as does a graft of a normal dog pancreas (Houssay and Foglia, 1936).

Richardson and Young (1938) and Richardson (1937, 1940) have found that the first signs of damage in the Langerhans' islets are a partial or total disappearance of the granules of the beta cells; hydropic degeneration of these cells, increased mitosis in some of the islets, and vacuolization of the epithelium of the intralobar ducts. When diabetes does not appear the only changes are the vacuolization of the epithelium of the ducts and a small decrease of the granules of the B cells. When permanent diabetes is produced the islets are transformed into groups of alpha cells with a few altered B cells and, finally, a hyalinization of the islets replaces all the cells or especially the B cells. These damages of the B cells have also been found by Campbell and Best (1938), Campbell, Keenan, and Best (1939), Foglia and De Robertis (1939), Loubatieres (1939), Dohan and Lukens (1939), and Dohan, Fish, and Lukens (1941).

Foglia (1939) with De Robertis found that in 5 dogs injected 2 to 4 days with hypophyseal extract (fasting blood sugar 143 to 191 mg. per cent) the protoplasma of the B cells was vacuolized, A and B cells were atrophic with a dark cytoplasm and pyknotic nuclei.

The diabetogenic extract decreases the insulin content of the pancreas (Campbell

and Best, 1938; Campbell *et al.*, 1939, 1940; Best *et al.*, 1939; Marks and Young, 1940*a*, *b*). The normal insulin content (3 to 4 units per gm.) of the pancreas began to decrease in 24 hours and after 7 days it was as low as 0.14 to 0.47 unit per gm. Eleven days later it was 0.38 in a dog with diabetes and 4.1 units per gm. in another which did not become diabetic. After 7 days of injections the insulin content becomes normal in 4 days (Haist and Best, 1940). In dogs with permanent diabetes the values were of 0.2 unit per gm. or even less and there was no recovery even after 198 days (Best, Campbell, and Haist, 1939).

A simultaneous treatment with protamine-insulin prevents the increase in blood sugar and the glycosuria and also prevents the decrease in the insulin content of the pancreas and the degeneration of the islets (Campbell, Haist, Ham, and Best, 1940). In the partially pancreatectomized cat insulin determines a return to normality of the islets in which hypophyseal extract had produced a hydropic degeneration (Lukens and Dohan, 1940).

The aim of the present work has been to study the insulin secretion of the pancreas of animals either with diabetes produced by hypophyseal extracts or resistant to the extracts. For such purposes those pancreases were grafted by vascular anastomosis to the neck of dogs made diabetic by extirpation of their pancreas. This allowed us to study the capacity of the grafted pancreas to secrete insulin in conditions of overcharge in order to correct a diabetic hyperglycemia.

Materials and Methods

The test for pancreatic secretion in the present studies is the blood sugar lowering action of a duodeno-pancreas preparation from various types of donors, connected by vascular anastomoses with the carotid artery and jugular vein of a pancreatectomized recipient.

Both donor and recipient were anesthetized with 0.8 to 0.10 gm. per kilo of chloralose given intravenously. This anesthetic does not markedly alter blood sugar or insulin secretion. All recipients had been pancreatectomized 20 hours previously. The pancreas and part of the duodenum were removed from the donor, leaving intact the coeliac trunk which was connected with the carotid artery of the recipient by means of a Payr cannula. Blood, passing into the graft (maintained at 38–39°C.) by the pancreato-duodenal arteries, drained by various veins into a section of portal vein which was joined to the jugular vein of the diabetic recipient. The pancreases were maintained over a thermostat at 38–39°C. between pieces of cotton soaked in saline solution and covered with rubber to prevent evaporation. The pancreas and intestine so maintained have shown a normal aspect after 13 hours according to our experience (Houssay, Lewis, and Foglia, 1928). We did not extend our experiments beyond that time.

The diabetic dog (12 to 17 kilos) had been without a pancreas for 20 to 24 hours (exceptionally for 44 to 48 hours). In the latter cases the blood sugar was 350 to 500 mg. per cent instead of 250 to 300 mg. per cent, so that the time necessary to reach normal levels was greater after grafting.

Blood samples for sugar determination were obtained from the carotid artery before and at intervals after grafting. Experiments were discarded if there was evidence of thrombosis, hemorrhage, or poor circulation in the grafted pancreas; also if the recipient died or showed signs of asphyxia before 6 hours of observation.

The pancreas, grafted to the neck by connecting its blood vessels to the carotid artery and jugular vein, continues to live for 10 to 15 hours. It maintains the blood sugar at a normal level, regulates the formation of muscle and liver glycogen, and responds to humoral stimulation by a graduated secretion of insulin. It increases its secretion when blood sugar is high and decreases it when it is low.

When glucose is injected (1 gm. per kilo intravenously) a blood sugar curve similar to that of normal dogs is obtained. If the graft is removed a curve of the diabetic type is obtained. The physiologic insulin secretion maintains the normal sugar concentration in blood and conversely the sugar concentration regulates the insulin secretion (*cf.* Houssay and Deulofeu, 1939). The anesthesia with chloralose does not modify the blood sugar or the insulin secretion.

The donors were of the following types: (1) Hypophysectomized dogs. (2) Normal dogs receiving injections with extract of anterior hypophysis with or without diabetes. (3) Dogs with permanent diabetes produced by injections of anterior hypophysis and persisting after injections were stopped. (4) Dogs maintained in a hyperglycemic state by continuous and prolonged intravenous infusions of glucose.

The anterior hypophyseal extract was prepared weekly from freshly killed beef glands frozen with dry ice (CO_2) for transport to the laboratory. The glands were dissected at low temperature, the anterior lobes separated, and finely ground. Each 800 gm. of gland was added to 2400 ml. of water and 600 ml. of 0.8 per cent NaOH, previously chilled. The container, kept partially submerged in ice water, was repeatedly shaken and then placed in the refrigerator until the following day, when 150 ml. of 0.25 per cent acetic acid was added. After a few minutes 0.8 per cent NaOH was used to bring the solution to a weak alkaline reaction to phenol red. A clear supernatant solution obtained by centrifugation, was divided into 60 ml. portions which were frozen for preservation.

The extract was given intraperitoneally in doses of 7 cc. per kilo per day (1.4 gm. of fresh lobe) in the morning, just after a blood sample for sugar had been obtained from the ear margin. The dogs were kept in metabolism cages to facilitate collection of urine. They were fed 30 to 40 gm. of fresh beef per kilo per day at 2 p.m. Drinking water was not limited.

Chemical analyses were done as follows: blood sugar by Somogyi modification of Hagedorn-Jensen method; urine sugar by Benedict method; and acetone bodies by Van Slyke method.

RESULTS

Normal Pancreatic Graft.—The pancreas of a normal dog, intercalated in the circulation of a dog made diabetic by extirpation of its pancreas 20 to 24 hours previously, corrects the hyperglycemia, bringing back and keeping the blood sugar at normal levels. High blood sugar stimulates insulin secretion, which, however, moderates when normal levels are reached (Houssay and Deulofeu, 1939).

Assuming 120 mg. per cent as the upper limit of the blood sugar in a normal dog, 13 of 15 cases reached this limit in 3 to 5 hours (Table I and Fig. 1). In

TABLE I

Duodeno-Pancreatic Vascular Graft from Normal Dogs

Weight of donor	Blood sugar Hours									Weight of recipient
	Before	½	1	1½	2	3	4	5	6	
kg.	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*kg.*
7	294	268	246	210	184	119	98	95	104	—
8	242	213	210	202	188	121	105	82	77	—
7.2	202	185	165	156	124	119	100	111	101	—
14.0	257	265	257	257	225	162	126	119	100	13.5
12.0	352	296	277	239	226	188	127	105	100	13
11.3	280	221	260	217	—	187	160	138	108	12.5
14.0	239	220	205	198	211	205	185	168	159	15.5
16.5	227	197	179	172	136	—	124	97	97	12
12.0	258	224	222	201	192	142	129	98	89	12
12.5	213	180	176	187	173	149	109	100	87	16
11.5	231	216	199	155	126	132	98	95	91	13
10.0	202	219	195	166	135	109	93	90	82	11
12.3	217	227	239	227	198	125	118	113	119	9
11.0	210	178	167	136	127	108	101	103	104	20.5
9.0	239	227	207	174	147	195	111	101	97	17.5

TABLE II

Duodeno-Pancreatic Vascular Graft from Hypophysectomized Dogs

Hypophysectomized dog			Pancreatectomized recipient									
Days since operation	Weight	Blood sugar	Weight	Blood sugar Hours								
				Before	½	1	1½	2	3	4	5	6
	kg.	*mg. per cent*	*kg.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1571	11.5	91	17	238	229	224	191	158	119	112	112	100
130	7.5	102	16	265	259	238	213	205	158	124	112	103
88	8.0	102	13.5	232	232	218	215	170	105	98	94	84
88	5.5	94	16.5	253	220	215	200	173	124	115	103	81
144	5.5	82	16.5	278	228	236	217	208	189	189	126	104
31	9.0	97	18.5	253	242	230	221	211	193	157	137	107

one animal it took 6 hours and in another a normal level was not reached. This might be due to either a subnormal insulin secretion of this pancreas, or an insulin resistant recipient.

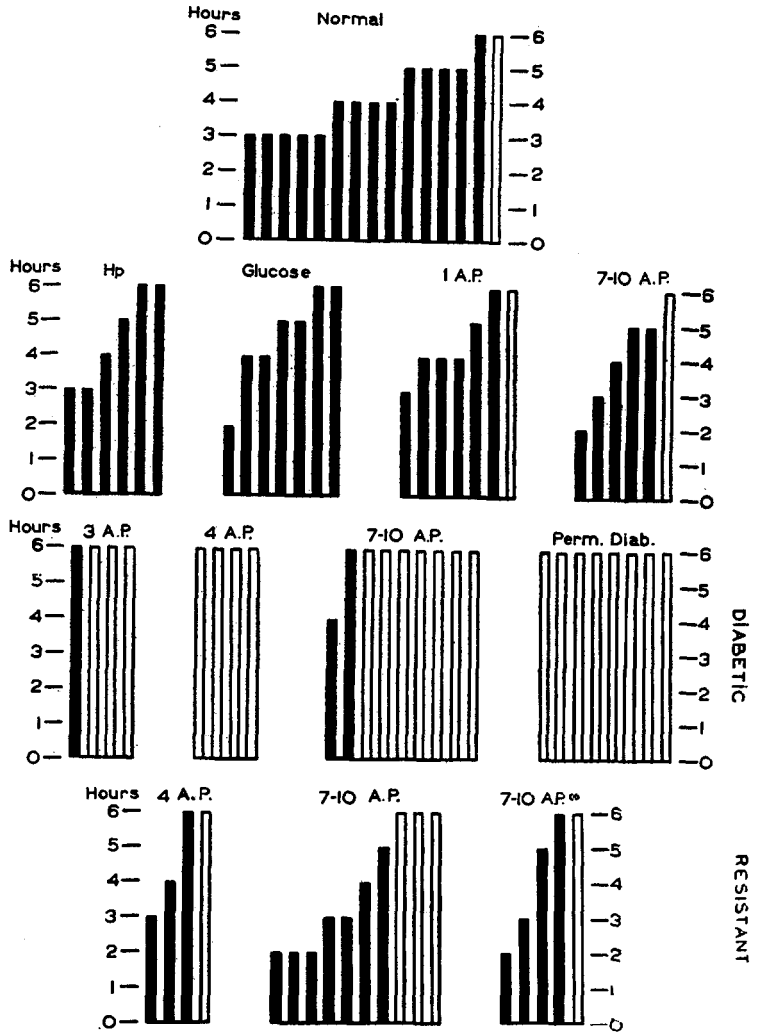

FIG. 1. Dogs pancreatectomized 20 hours before and grafted with a duodeno-pancreas. Each black column shows the time in which the blood sugar of the pancreatectomized dog was lowered to 120 mg. per cent. The white columns indicate that the blood sugar did not decrease to that level within the 6 hours of experiment. The duodeno-pancreases which were grafted belonged to: First row: normal dogs. Second row: Hp = hypophysectomized; glucose = injected continuously during 4 days with glucose (average 1 gm. per kilo per hour); 1 A.P. = normal dogs with one injection of anterior pituitary extract (1.4 gm. per kilo); 7–10 A.P. = injected 7–10 days daily and with diabetes only the last days. Third row: 3 A.P., 4 A.P., and 7–10 A.P. = normal dogs injected during 3, 4, or 7–10 days in the same way and which were diabetic. Perm. diab. = diabetic after suspending treatment. Fourth row: 4 A.P., 7–10 A.P. = injected 4 or 7–10 days without becoming diabetic (resistant); 7–10 A.P.(*) = injected 7–10 days, diabetic between 2nd and 5th days but with normal blood sugar at the end.

Pancreatic grafts from hypophysectomized dogs behaved the same as those from normal dogs, since the hyperglycemia of the recipient was corrected in 3 to 6 hours in all 5 cases (Table II and Fig. 1). The donors had been hypophysectomized for from 31 to 1571 days and were in excellent health. One can conclude that there was no demonstrable diminution or increase in the capacity of the pancreas to secrete insulin in the absence of the hypophysis.

Diabetic action of anterior pituitary extract is at a maximum after repeated daily injections for 3 to 5 days. Blood sugar in fasting animals 24 hours after an injection varies between 120 to 320 mg. per cent (average 176 mg. per cent) for several hundred dogs. Hyperglycemia is accompanied by glycosuria,

TABLE III

Duodeno-Pancreatic Graft from Donor Dogs Receiving a Single Intraperitoneal Injection of Alkaline Extract of Beef Anterior Pituitary (7 Cc. the Equivalent of 1.4 Gm. of Fresh Gland per Kilo per Day)

Donor dog			Pancreatectomized recipient									
Weight	Blood sugar		Weight	Blood sugar Hours								
	Before injection	1 day after		Before	½	1	1½	2	3	4	5	6
kg.	*mg. per cent*	*mg. per cent*	*kg.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
10	—	104	15.5	253	239	205	205	207	185	156	129	109
23	98	129	9.0	261	245	241	213	163	136	110	—	—
16.5	105	133	13.5	267	217	189	180	140	110	102	87	98
16.5	106	140	12.2	273	240	231	208	182	142	112	103	93
12.0	93	149	13.0	390*	346	311	290	249	197	177	163	150
12.8	120	156	14.0	265	236	196	178	143	124	111	118	—
15.0	115	166	15.0	232	230	207	200	180	150	138	115	—

* Pancreatectomized 48 hours before.

increased acetone bodies of blood and urine, and an increased plasma protein. There is also a fall in the alkaline reserve and a slight decrease in plasma chlorides, potassium, and calcium.

If an animal does not eat, the blood sugar fails to rise, or falls if it is high. This indicates that to some extent the hyperglycemia is alimentary. Regardless of the blood sugar level there is an increased resistance to the sugar lowering effect of insulin.

Upon opening the abdomen the omentum appears pink and feels soapy or greasy. The liver appears fatty and has an increased lipid content (Foglia and colleagues, 1937). There is no relation between the fatty appearance of the liver and the blood sugar level.

Pancreatic Grafts from Dogs Receiving Anterior Pituitary Extract.—(a) Dogs

TABLE IV

Duodeno-Pancreatic Vascular Graft from Dogs That Had Received Injections of Alkaline Extract of Beef Anterior Pituitary for 3 or 4 Days (7 Cc. the Equivalent of 1.4 Gm. of Fresh Lobe per Kilo per Day)

Donor dogs

Experiment No.	Weight	No. of injections	Blood sugar Days				
			Before injection	1	2	3	4
	kg.		*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	—	3	90			149	
2	—	3	98			187	
3	—	3	89			251	
4	—	3	94			322	
5	7	4	91	—	150	119	150
6	9.5	4	86	100	137	150	196
7	7	4	88	—	266	100	113
8	9.5	4	110	163	205	223	320
9	10	4	96	131	103	100	108
10	11.5	4	105	111	—	—	121
11	12	4	96	105	99	93	135
12	10	4	109	93	—	—	140

Recipient dogs

Experiment No.	Weight	Blood sugar Hours								
		Before	½	1	1½	2	3	4	5	6
	kg.	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	12	293	278	274	246	262	268	242	246	242
2	10.2	375	325	280	224	190	151	123	122	110
3	10	282	218	230	222	218	246	268	248	—
4	14	278	212	212	182	142	134	184	154	136
5	16	512	474	474	478	466	422	396	300	292
6	15	275	236	226	211	217	232	205	205	221
7	17	258	212	235	214	195	309	288	241	—
8	12.8	380	362	362	350	350	300	328	204	—
9	22	244	216	200	198	—	144	126	122	106
10	—	217	210	206	171	139	131	100	100	110
11	15	265	243	243	257	240	226	191	164	156
12	—	219	187	149	146	130	108	95	73	77

receiving one injection: The pancreas from 6 of 7 animals can be considered normal, since the blood sugar of the recipient was lowered to 120 mg. per cent or less in 3, 4, 4, 4, 5, and 6 hours in dogs with blood sugars of 133, 129, 140, 156, 104, and 166 mg. per cent respectively (Table III). In the remaining

animal, which had been without pancreas for 48 hours, the high value of 390 mg. per cent was reduced to 150 mg. per cent in 6 hours.

TABLE V

Duodeno-Pancreatic Graft from Dogs That Had Received Injections of Alkaline Extract of Beef Anterior Pituitary for 7 to 10 Days (7 Cc. Equivalent of 1.4 Gm. of Fresh Lobe per Kilo per Day). Hyperglycemia of 150 Mg. Per Cent or More Present from 2nd to 4th Day and at Time of Transplanting the Pancreas to the Neck of the Recipient

Donor dogs

Experiment No.	Weight	No. of injections	Blood sugar Days										
			Before	1	2	3	4	5	6	7	8	9	10
	kg.		*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	8	8	78	101	139	—	152	125	100	127	194	—	—
2	8	10	78	78	100	—	182	—	195	242	127	155	162
3	7	8	103	127	122	152	101	—	123	151	151	—	—
4	8	8	88	118	160	121	150	106	110	—	164	—	—
5	6	9	72	86	118	123	162	124	167	126	126	154	—
6	—	9	91	107	155	174	131	106	—	119	139	214	—
7	9.5	7	118	121	—	160	169	202	186	312	—	—	—
8	9.5	8	82	133	—	114	177	151	135	155	137	—	—
9	8	8	102	130	182	—	116	195	202	186	257	—	—

Diabetic recipients

Experiment No.	Weight	Blood sugar Hours								
		Before	½	1	1½	2	3	4	5	6
	kg.	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	17	245	244	209	207	198	158	145	138	124
2	20	255	255	244	202	168	158	160	155	148
3	12.5	385	347	347	310	316	289	223	207	—
4	16.5	283	242	217	178	175	186	180	148	148
5	17.5	429	407	397	304	287	224	224	211	215
6	20.5	320	294	294	246	222	161	116	93	72
7	12	293	289	271	253	255	193	139	134	109
8	13.5	527	489	489	414	338	320	332	332	—
9	22	261	223	221	217	223	184	169	178	200

(*b*) *Dogs receiving 3 injections* and having a marked diabetes (Table IV) yielded pancreatic grafts that had a diminished insulin secretion since the hyperglycemia of the recipient was restored to a normal level in only 1 of 4 cases.

(*c*) *Dogs receiving 4 injections* fell into 2 groups of 4 animals each. In one group the blood sugars rose to 150, 196, 266, and 320 mg. per cent. The pancreas grafts from these dogs failed to secrete enough insulin to restore to normal levels the hyperglycemia of their recipients (Table IV). In the other 4 dogs the blood sugar rose to only 108, 121, 135, and 140 mg. per cent. Pancreas grafts from these animals secreted more insulin than in the previous

TABLE VI

Duodeno-Pancreatic Graft from Dogs That Received Injections of Alkaline Extract of Beef Anterior Pituitary for 7 to 10 Days (7 Cc. the Equivalent of 1.4 Gm. of Fresh Gland per Kilo per Day). These Donors' Hyperglycemia of the 2nd to 5th Day Had Fallen at the Time of Grafting

Donor dogs

Experiment No.	Weight	No. of injections	Blood sugar Days									
			Before	1	2	3	4	5	6	7	8	9
	kg.		*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	6	9	80	115	112	117	188	175	—	95	100	124
2	6	9	83	135	127	118	—	137	152	111	115	120
3	8	9	82	122	126	185	231	275	—	120	130	127
4	7	7	96	98	—	178	147	151	114	134	—	—
5	7	8	78	151	150	151	92	—	119	149	140	—

Diabetic recipients

Experiment No.	Weight	Blood sugar Hours								
		Before	½	1	1½	2	3	4	5	6
	kg.	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	20	282	237	161	121	88	86	89	78	75
2	14.5	265	246	208	175	146	111	88	77	—
3	12	212	199	195	181	178	136	130	120	111
4	16	227	210	210	208	190	183	154	122	104
5	14	328	320	291	261	202	193	191	207	—

hypophyseal diabetic groups, as shown by 3, 4, and 6 hours restoration periods in 3 of them. One failed to reach normal levels in 6 hours (Table III).

(*d*) *Grafts from dogs receiving 7 or more injections* of anterior pituitary extract are divided into 4 groups. There were 9 animals that had a high blood sugar the 2nd to the 5th day up to the moment of the graft (Table V). In these the secretion of insulin was diminished in 7 cases, slightly diminished in 1, and nearly normal in 1.

TABLE VII

Duodeno-Pancreatic Graft from Dogs That Received Injections for 7 to 10 Days of Alkaline Extract of Beef Anterior Pituitary (7 Cc. the Equivalent of 1.4 Gm. of Fresh Gland per Kilo per Day). In These Resistant Donors the Blood Sugar Failed to Rise above 150 Mg. Per Cent at Any Time

Donor dogs

Experiment No.	Weight	No. of injections	Blood sugar Days										
			Before	1	2	3	4	5	6	7	8	9	10
	kg.		*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	7.5	9	98	98	106	95	91	107	—	103	107	112	—
2	10	8	90	—	124	—	—	121	128	116	127	—	—
3	7	7	85	123	—	130	114	106	122	131	—	—	—
4	8	8	96	—	102	—	—	136	135	102	105	—	—
5	11	21	84	114	89	102	—	126	129	122	118	103	115*
6	8	8	110	144	137	—	139	100	118	116	145	—	—
7	7.3	10	102	100	112	138	121	135	124	—	117	140	132
8	7.5	7	80	100	—	91	122	103	103	147	—	—	—
9	8	7	94	121	96	121	119	119	138	127	—	—	—
10	9.5	8	80	—	149	146	—	131	101	99	116	—	—

Diabetic recipients

Experiment No.	Weight	Blood sugar Hours								
		Before	½	1	1½	2	3	4	5	6
	kg.	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	15.5	276	248	187	122	118	96	105	98	82
2	14	229	226	208	143	118	113	88	86	79
3	24	290	250	218	158	110	83	74	84	69
4	14	222	205	205	205	163	120	100	88	—
5	9.5	248	235	229	182	168	117	91	—	—
6	10.5	254	224	194	188	161	143	120	122	105
7	14	229	208	197	191	171	153	148	104	91
8	12	268	288	266	220	231	190	144	136	125
9	12.5	362	303	299	275	273	249	184	170	161
10	14	292	235	237	224	209	179	165	148	207

* Up to the 21st day blood sugar varied between 84 and 117 mg. per cent; values for the last 3 days were 85, 104, and 108 mg. per cent.

In 5 dogs the blood sugar rose to 150 mg. per cent or more between 2 to 5 days, but fell at the time of pancreatic grafting (Table VI). In 2 animals the insulin secretion was normal, in 1 supernormal, in 1 subnormal, and the last definitely inadequate.

Ten dogs injected with anterior pituitary were resistant to its diabetogenic action (Table VII) and in these insulin secretion was found to be supernormal in 3 cases, normal in 4, and subnormal in 3 cases.

In 6 dogs the blood sugar rose only at the end of the period of injections, *i.e.*, at the time of making the graft. In 1 the insulin secretion was supernormal, in 4 normal, and in 1 subnormal (Table VIII).

TABLE VIII

Duodeno-Pancreatic Graft from Dogs That Received Injections for 7 to 10 Days of Alkaline Extract of Beef Anterior Pituitary (7 Cc. the Equivalent of 1.4 Gm. of Fresh Gland per Kilo per Day). The Donors' Blood Sugar Remained Low until the Last Few Days before Grafting

Donor dogs

Experiment No.	Weight	No. of injections	Blood sugar Days									
			Before	1	2	3	4	5	6	7	8	9
	kg.		mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent
1	7.5	9	79	104	134	125	126	144	122	—	154	228
2	8	8	99	109	114	—	152	109	126	104	167	—
3	7	7	94	125	—	109	111	123	172	144	—	—
4	10.5	8	78	85	115	—	108	81	136	143	203	—
5	6	8	98	100	89	—	116	126	112	146	177	—
6	8.5	9	62	70	95	—	89	119	125	133	257	216

Diabetic recipients

Experiment No.	Weight	Blood sugar Hours								
		Before	½	1	1½	2	3	4	5	6
	kg.	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent	mg. per cent
1	12.5	228	211	169	126	101	98	87	62	62
2	16	256	207	201	183	145	108	92	96	89
3	24	239	220	220	163	147	122	94	87	85
4	17	245	194	174	203	180	153	135	106	100
5	20	273	247	219	218	187	142	125	111	117
6	8.5	297	297	287	221	189	157	139	141	128

(*e*) *Duodeno-pancreatic grafts from dogs with permanent diabetes:* In our experience it is exceptional to obtain dogs with permanent diabetes if the pancreas is intact. When anterior pituitary injections raised blood sugar in 2 to 3 days it is easy to maintain an elevation of 200 to 300 mg. per cent but this falls to normal levels a few days after stopping injections.

To overcome this difficulty we used dogs with pancreas reduced to half by

removal of the splenic tail and the free duodenal portion. In these it is easy to obtain and maintain diabetes with lower doses of extracts. We prepared many of these dogs. Several died with intense diabetes, acidosis, and coma; in most of them the blood sugar descended a few days after stopping injections.

Grafts from 7 dogs whose diabetes persisted for 8 to 21 days after cessation of injections showed no ability to correct the hyperglycemia of the recipient during 6 hours of observation (Table IX). This indicates that the insulin secretion is small or completely absent. On the other hand, the pancreas from normal dogs with reduced volume to the half, showed a capacity to reduce hyperglycemia to normal level within 6 hours in 7 of 15 cases.

TABLE IX

Duodeno-Pancreatic Grafts from Donor Dogs Which Had 50 Per Cent Reduced Pancreas and Had Developed Permanent Diabetes after Daily Injection of Alkaline Extract of Fresh, Beef Anterior Hypophysis, the Diabetes Persisting after Cessation of Injections. Hyperglycemia of Diabetic Recipient Was Not Reduced to Normal Even after 6 Hours

Blood sugar of injected dogs	At the time of grafting	Days injected	Days without injection	Weight		Blood sugar of diabetic	
				Donor	Recipient	Recipients at the time of grafting	6 hrs. after grafting
mg. per cent	*mg. per cent*			*kg.*	*kg.*	*mg. per cent*	*mg. per cent*
208-480	303	16	8	7	17	223	167
125-360	165	15	8	7.5	13	577	557
259-313	191	90	8	6.7	14	244	231
308-354	217	131	13	8	17	194	189
292-415	371	11	11	10	15	248	205
237-311	312	20	11	9	12	256	205
207-370	354	17	21	7	21	211	208

The histologic examination of the pancreas of dogs with permanent diabetes showed pronounced lesions of the B cells of the islands with degranulation, vacuolization, or complete disappearance.

RÉSUMÉ AND DISCUSSION

There is no proof of a constant physiologic action of the anterior hypophysis on the pancreas, because (1) the insulin secretion of the hypophysectomized dog is normal; (2) no atrophic lesions have been observed in the islands of Langerhans; on the other hand, they are more developed (Porto, 1941). There is also no proof for the existence of a pancreatotropic anterior pituitary secretion which stimulates or maintains the anatomy or function of these islands.

There is a definite physiologic antagonism well demonstrated between the functions of the anterior pituitary and the pancreas in respect to carbohydrate metabolism. After removal of the hypophysis one may observe (1) a marked sensitivity to insulin; (2) diminution of the intensity of pancreatic or phloridzin diabetes. On the other

hand, an excess of anterior pituitary secretion produces: (1) a marked resistance to the action of insulin, and (2) intensification of pancreatic or phloridzin diabetes and a diabetic state in normal dogs.

In all species of animals lower doses of pituitary extract will produce diabetic symptoms in proportion to the reduction of pancreatic volume. Conversely, insulin injections inhibit or make more difficult the production of diabetic symptoms with anterior pituitary extract and accelerate their disappearance after discontinuing the treatment with the extract.

Anterior pituitary extract produces two effects on the pancreas which are apparently antagonistic; it may stimulate or alter them. In certain species as in the rat, which is resistant to the diabetogenic action, the stimulating action is more apparent with hyperplasia of the islands and the increase of insulin. After extirpation of the pancreas in the rat it is easy to observe the diabetic action. In the toad the protective rôle of the pancreas against pituitary extract is very great, and it is impossible to produce the diabetic action in normal or hypophysectomized animals, but on removal of the pancreas they become extremely sensitive.

The pancreas of the dog is easily altered by pituitary extract which produces diabetes, lesions of the cells of the islands, and diminishes insulin secretion.

The action in the rat and the dog are contradictory; in the rat the extract stimulates the pancreas and in the dog it is inhibited. In reality both actions exist in both species, since one can produce a transitory or permanent diabetes in a rat by partial pancreatectomy, and in the dog in a certain number of cases diabetes fails to appear and on the contrary there is stimulation of insulin secretion as has been described in this paper.

The diabetic action of the anterior pituitary depends on 3 factors: (*a*) the dose and activity of the extracts, (*b*) the extrapancreatic factors, and (*c*) pancreatic factors. The extrapancreatic factors are very important and their existence is shown as follows: (1) anterior pituitary extract produces a marked resistance to the action of insulin (Houssay and Potick, 1929; Di Benedetto, 1932, 1933), injected or from pancreatic graft (Houssay and Foglia, 1936); (2) the diabetic action of the anterior pituitary reaches its maximum intensity in dog or toad without pancreas or hypophysis which is more evident in the absence of the antagonistic action of the pancreas to the pituitary; (3) the hypophysectomized dogs with or without pancreas are extremely sensitive to the hypoglycemic action of insulin. Finally, we may state that anterior pituitary extract produces an increased resistance to insulin but there is no hyperglycemia if the animal does not eat; (4) anterior pituitary extract aggravates the diabetes of a pancreatectomized dog which dies in acidosis and coma; (5) anterior pituitary extract does not produce hyperglycemia in the hepatectomized toad without hypophysis and pancreas (Campos, Curutchet, and Lanari, 1933).

The pancreatic factor in hypophyseal diabetes is manifest in the diminished endocrine function of the islands as follows: (1) The pancreas is able to secrete less insulin than that from a normal animal first observed by Houssay and Foglia (1936) and amply confirmed in this paper. (2) There are cytological lesions of the island cells as shown by Young and Richardson (1937, 1938) and confirmed by Foglia and De Robertis (1939) and Porto (1941). (3) There is a diminished insulin content of the pancreas (Campbell and colleagues, 1938–1940). In our judgment it is more im-

portant to measure the secretion of an organ rather than its contents at death, since the latter does not indicate the secretory intensity, as, for example, in hyperthyroidism. There is an increased secretion of thyroid iodine and yet a diminished iodine content of the thyroid gland.

In the light of all these factors the following interpretation of our experiments is permitted. When anterior pituitary injections produce a diabetes with 3, 4, 7, or more injections, one observes in the pancreas a marked diminution of the capacity to secrete insulin which fails to correct hyperglycemia when grafted into the neck of the pancreatectomized dogs with diabetes. This, we have observed in 14 of the 17 cases studied. In the remaining 3, the diabetes might be due exclusively to extrapancreatic action, whereas in the 14 there was diminution of the insulin secretion. If we include the 6 dogs with permanent diabetes we reach a total of 20 out of 23 cases in which there is an insufficient secretion of insulin to correct the diabetic hyperglycemia.

When animals are hyperglycemic during the first 2 to 5 days with subsequent fall in blood sugar one observes that the pancreatic secretion improves since in 5 cases studied 2 were normal, 1 supernormal, 1 slightly subnormal, and only 1 was definitely inadequate.

In animals resistant to anterior pituitary extract whose blood sugar rises little or not at all, one could observe that in 6 cases insulin secretion was normal, in 3 cases it was definitely supernormal since it corrected the hyperglycemia in only 2 hours, in 1 case there was a slow restoration, and in only 4 cases of these 14 did we observe a diminished secretion. In some of them there were intense lesions of the islands of Langerhans and there must, therefore, have existed extrapancreatic factors which impeded the appearance of diabetes.

In 6 dogs resistant for 6 to 8 days with a terminal rise in blood sugar on the day of grafting, there was normal secretion of insulin in 4 cases, supernormal in 1, and diminished secretion in 1 (Table VIII). The final increase in blood sugar appears, therefore, to be due to extrapancreatic factors.

In dogs receiving only 1 injection of extract there was no alteration of the capacity of the pancreas to correct the hyperglycemia of the recipient; nevertheless, in 3 cases the blood sugar of the donor was 149, 156, 166 mg. per cent (Table III). In the last two groups of dogs mentioned the diabetes lasted only 1 day and did not impair the insulin secreting capacity of the pancreas. The blood sugar rise appears to be due to extrapancreatic factors.

The elevation of blood sugar alone by continuous injection of glucose was not sufficient to alter the pancreas during the short period of our experiments; although the blood sugar had been kept for 4 days at a level similar to that observed in dogs with anterior pituitary diabetes, nevertheless, the pancreas of these dogs demonstrated a normal functional capacity to correct the diabetes of the recipients (Table X). Histologically, there is hyperplasia of the islands and signs of hyperfunction of the B cells as observed by Porto.

Supporting the fact that hyperglycemia is not the only factor to consider, in phloridzin diabetes the pancreas has a diminished insulin secretion (Houssay and Foglia, 1936), and recently Porto has found lesions in the B cells of the islands.

TABLE X

Duodeno-Pancreas of Dogs Which Were Injected Continuously with Glucose (50 Per Cent in Physiological Saline Solution 1 Gm. per Kilo per Hour Intravenously) Grafted to the Neck of Pancreatectomized Dogs

Donor dog

Experiment No.	Weight	Glucose	Glucose total in 4 days	Blood sugar Days				
				Before	1	2	3	4
	kg.	*gm.*	*gm.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	9	1.62	1400	98	124	170	307	271
2	8.5	1.83	1500	96	277	185	139	222
3	10	1.14	1100	94	98	111	163	202
4	9.5	1.00	900	109	158	264	148	196
5	8.5	0.86	700	97	126	125	299	335
6	9.5	0.77	700	94	80	143	212	149
7	9	1.80	1550	95	158	149	188	131

Pancreatectomized recipient

Experiment No.	Weight	Blood sugar Hours								
		Before	½	1	1½	2	3	4	5	6
	kg.	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
1	14	206	202	160	87	86	75	73	66	68
2	14	253	221	202	182	150	141	128	113	113
3	17	232	246	236	219	193	202	154	132	118
4	17.5	333	321	280	231	216	176	158	135	120*
5	15.5	250	231	203	201	163	121	103	89	91
6	17	220	190	174	149	133	129	124	120	110
7	16	260	231	209	176	156	121	114	109	107

* This pancreas showed small hemorrhages during the transfusion.

The damaging action on the islets might be due to: (1) a direct action as is also produced on other tissues, (2) the persistent increase in blood sugar, (3) the increased secretion of insulin which becomes necessary because of the insulin resistance.

Anterior pituitary extract damages the island cells and diminishes insulin secretion. The rise in blood sugar is first due to extrapancreatic activity; this

hyperglycemia aggravates probably the lesions of the pancreatic cells impaired by the damaging action of the extract.

The functional alteration of the islands can persist in animals with intense diabetes even after cessation of pituitary injections. In many cases the

TABLE XI

Summary of Tables I to X

Pancreas from the following types of donor	No. of recipient in which the glycemia fell to 120 mg. per cent or less Hours						Total No. of experiments
	2	3	4	5	6	More than 6	
Normal	—	5	4	4	1	1	15
Hypophysectomized	—	2	1	1	2	—	6
With 1 injection of anterior hypophysis extract	—	1	3	1	1	1	7
With 7 to 10 injections of anterior hypophysis extract. Diabetes final day only	1	1	1	2	—	1	6
With 3 injections of anterior hypophysis extract, diabetic	—	—	—	—	1	3	4
With 4 injections of anterior hypophysis extract, diabetic	—	—	—	—	—	4	4
With 7 to 10 injections anterior hypophysis extract, diabetic from 2nd to 5th day to end	—	—	1	—	1	7	9
With permanent diabetes	—	—	—	—	—	8	8
Total in these four definitely diabetic groups	—	—	1	—	2	22	25
With 4 injections anterior hypophysis extract. No diabetes	—	1	1	—	1	1	4
With 7 to 10 injections anterior hypophysis extract. No diabetes	3	2	1	1	—	3	10
Total in these 2 non-diabetic groups	3	3	2	1	1	4	14
With 7 to 10 injections anterior hypophysis extract, diabetes from 2nd to 5th day but not at end	1	1	—	1	1	1	5
Continuous intravenous injection of glucose, 4 days	1	—	2	2	2	—	7
Total No. of donors 82; equal No. of recipients	—	—	—	—	—	—	84

Dogs were considered diabetic when the blood sugar was higher than 150 mg. per cent.

diabetes gradually disappears with an histological improvement in the islands, but in some a permanent diabetic state may persist.

The histological study of the pancreases of the present animals and others from similar conditions was done by Dr. Porto (1941). He has found a parallelism between insulin secretion, hyperglycemia, and the cytologic conditions of the

cells of the islands. Dogs with anterior pituitary diabetes showed lesions of the islands of Langerhans (in the B cells: degranulation, vacuolization, and afterwards pyknosis; and in the A cells: initial retraction and condensation, and vacuolization afterwards). Besides fatty degeneration was observed in the tubes and acini. The intensity of insular lesions was almost parallel to the level and duration of hyperglycemia. In the dogs injected with anterior pituitary extract, which did not show elevation of their glycemia, signs of hyperfunction of the B cells were observed (the density of the B granules was increased so that the Golgi apparatus could be observed in the unstained preparation). In those dogs which were diabetic but improved in spite of continuing the injection of pituitary extract, a few cellular lesions were observed although most of them appeared normal showing the aspect which Dr. Porto considered that of hyperfunction. In the dogs resistant to the injections and in those whose glycemia rose only the day when the graft was made the same picture of the islands was observed. The dogs which remained permanently diabetic after interruption of the injections of pituitary extract showed destructive lesions of the insular cells: atrophy and disappearance of the islands, or replacement of the same by hyaline tissue formed at the expense of the connective tissue of the island, with very few or no cells.

CONCLUSIONS

The ability of the pancreas, from various types of dogs, to correct diabetic hyperglycemia has been studied (Table XI). The pancreas from one animal was united by a vascular union with the neck blood vessels of another dog which had been pancreatectomized for 20 hours. The time necessary to reduce the blood sugar level to 120 mg. per cent was determined.

1. Pancreas from 6 hypophysectomized dogs produced a normal insulin secretion, showing that an anterior pituitary hormone is not necessary for its production or maintenance.

2. In 14 of 17 normal dogs given anterior pituitary extract for 3 or more consecutive days and presenting diabetes (fasting blood sugar 150 mg. per cent or more) the pancreas showed diminished insulin production.

3. In animals which remained diabetic after discontinuing the injections of hypophyseal extract, the pancreas islands were markedly pathologic and the insulin secretion was practically nil.

4. When hyperglycemia existed on the 2nd to 5th day but fell later, the insulin secretion of 5 dogs was normal in 2, supernormal in 1, and less than normal in 2. Histologic examination showed a restoration of beta cells.

5. In 14 dogs resistant to the diabetogenic action of anterior pituitary extract, as shown by little or no change in blood sugar, the pancreatic secretion of insulin was normal in 6 cases, supernormal in 3, and subnormal in 5 cases. Clear signs of hyperfunction of B cells were observed. In 6 resistant animals a

high blood sugar (150 mg. per cent) appeared shortly before transplanting, but insulin secretion was normal in 4, supernormal in 1, and subnormal in 1 case.

6. With one injection of extract and 1 day of hyperglycemia the capacity of the pancreas to secrete insulin was not altered.

7. A high blood sugar level lasting 4 days does not alter the islets. The hypophyseal extract acts, therefore, by some other mechanism. In normal dogs, the continuous intravenous infusion of glucose for 4 days maintained the blood sugar at levels as high as those after pituitary extract. In these animals the B cells were hyperplastic and insulin secretion normal.

8. Anterior hypophyseal hyperglycemia is due at first to extrapancreatic factors which are the most important, and last only during the injections of extracts. Pancreatic factors appear afterwards and are responsible for permanent diabetes.

Hypophyseal extract produces histological changes in many tissues and damages the Langerhans islands. The coexistent high blood sugar probably exhausts the B cells and exaggerates their injury.

9. In all cases there is a relation between the cytology of the islet B cells and the insulin secreting capacity.

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