COMPARATIVE DIABETOGENIC ACTION OF THE HYPOPHYSIS FROM VARIOUS ANIMALS

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The rôle of the anterior portion of the hypophysis in the production or intensification of diabetes was discovered in this Institute in 1929 and 1930 and since then has been studied continuously. An abstract of this work up to 1935 was published by Houssay in 1936. Some of the extracts of anterior hypophysis in animals without hypophysis and pancreas and with mild diabetes, intensified the hyperglycemia and glycosuria to a degree exceeding the intensity of the diabetes associated with pancreatectomy alone. This was observed in the toad (Houssay and Biasotti, 1930), and in the dog (Houssay and Biasotti, 1932; Houssay, Biasotti, and Rietti, 1932; Houssay, Biasotti, Di Benedetti, and Rietti, 1932). These latter observations suggested a possible method of biological assay of the diabetogenic factor. The present experiments were undertaken to demonstrate the relative potency of the diabetogenic principle in extracts of the anterior hypophysis from various species.

Test Animals

(*a*) *Dogs.*—The hypophysectomized-pancreatectomized dog is difficult to maintain in good condition because the injection of anterior hypophyseal extract profoundly aggravates its condition. On the other hand insulin cannot be used since they are so sensitive to this agent they develop fatal hypoglycemia from doses as low as 1 to 3 units per day. Normal dogs, while not showing this marked insulin sensitivity, require such large amounts of anterior hypophyseal extract (between 1 and 2 gm. per kilo per day for several days) as to prohibit its extensive use for titrating the diabetogenic principle. Mention should be made that this diabetes in normal dogs, characterized by hyperglycemia, ketonemia, hyperlipemia, acidosis, etc., (Houssay, 1936) is produced specifically by anterior hypophyseal extracts, extracts of other organs being inert. The hyperglycemia which is preceded and accompanied by increased insulin resistance, appears between the 2nd and 5th days after starting with the injections. Upon discontinuing the injections, blood sugar returns to normal in 1 to 3 days. If the animal fasts, the injections produce insulin resistance without hyperglycemia.

Dogs with subtotal pancreatic resection demonstrate Sandmeyer's diabetes if less than 3 gm. of pancreas are left. This attenuated diabetes is not permanent since it

may either improve or advance progressively to a fatal termination. In many cases this diabetes disappears with hypophysectomy if the hyperglycemia and glycosuria are not intense and if the animal is not in a cachectic condition. The injection of anterior lobe extract in a dog having Sandmeyer's diabetes rapidly aggravates the diabetic symptoms and results in death (Houssay, Biasotti, and Rietti, 1932).

If 3 to 6 gm. of pancreas are left in the dogs, they remain normoglycemic and in good condition, but none the less very sensitive to the diabetogenic action of the anterior hypophyseal lobe extract (Houssay, Biasotti, and Rietti, 1932). We have demonstrated that as the pancreatic tissue is reduced surgically, smaller doses of anterior hypophyseal lobe become necessary. The difficulty with the very large pancreatic resections is that the dog may remain permanently diabetic if large or repeated doses of anterior pituitary extract are given. In these partially pancreatectomized dogs (retaining 3 to 6 gm. of pancreas) with normal glycemia, a permanent diabetes was first obtained with anterior lobe extract. This diabetic state persisted in spite of discontinuance of injections of anterior hypophyseal extract and ultimately resulted in cachexia and death (Houssay, Biasotti, and Rietti, 1932). This phenomenon has since been confirmed in the rat by Long (1937, 1939), and in the cat by Dohan and Lukens (1939), and Lukens and Dohan (1940). Young (1937) later made the important observation that when the normal dog was injected with progressively increased doses of anterior hypophyseal extract, diabetes was produced and persisted even though the hypophyseal extract injections were discontinued.

The dogs with subtotal pancreatic resection but with normal glycemia are very sensitive to anterior hypophyseal extracts but they present two difficulties: (1) permanent hypophyseal diabetes may be obtained in some cases (4 cases of the first 12 dogs studied); (2) very exceptionally hyperglycemia and glycosuria may be obtained from large doses of extracts of tissues other than hypophysis, once in the case of muscle and of thyroid gland in our large report of experiments. These two difficulties may be met by: (1) leaving at least 4 gm. of pancreas; (2) injecting the animals no longer than 4 days; and (3) allowing 7 to 10 days to elapse before beginning a new series of injections.

Dogs with extensive pancreatic resection when hypophysectomized, maintain a great sensitivity to the anterior hypophysis extract without showing non-specific diabetogenic reactions. When the anterior hypophysis treatment is suspended, the glycemia rapidly returns to the normal level and with few exceptions the dogs recover from diabetes, providing the rules mentioned in the preceding paragraph are followed. The animals do not require special care and maintain excellent general health and have been successfully used by Houssay and Biasotti (1938) in their investigations and in the present study.

In the normal dog the anterior hypophyseal lobe of the ox has a diabetogenic action. From 1932 to the present we have injected this substance into more than 500 animals. The bovine anterior lobe showed diabetogenic action in normal animals such as dogs, cats, pigeons, and guinea pigs (listed in order of decreasing action). The action is still less on rats, rabbits, and white mice. It has no action on either toads or serpents (Houssay, Biasotti, and Rietti, 1933). According to Young (1938) this action is constant in dogs but inconstant in cats and rabbits. Rats, mice, and guinea pigs fail to show a diabetogenic response.

(*b*) *Toads.*—In terms of response to the diabetogenic action of hypophyseal extract, the toad deprived of hypophysis and pancreas is the most sensitive animal yet observed. However, the animal lives but a short time after removal of the pituitary and pancreas, and there are marked individual and daily variations in response to the injections of anterior hypophyseal extract. Comparative experiments must be done in many animals on the same day and under identical conditions with adequate controls. Groups of 15 to 20 toads were used in each series.

The diabetogenic action of the hypophysis of different species of animals has been studied in the toad and in other batrachians deprived of the hypophysis and pancreas. In the toad, *Bufo arenarum* Hensel, the hypophysis of the same toad is diabetogenic (Houssay and Biasotti, 1930) as well as that of fish, chicken, ox, dog, and man (Houssay and Biasotti, 1931) and also that of *Xenopus laevis*, *Leptodactyllus ocellatus*, and *Bufo paracnemis*. Conversely in *Bufo paracnemis* there was a response from *Bufo arenarum* Hensel. In the frog, *Leptodactyllus ocellatus* (L.) Gir., *Ceratophrys ornata*, and *Bufo d'Orbigny* without hypophysis and pancreas, the hypophysis of the same animal has diabetogenic action. In the toad, extracts of other organs of toads and of other species (amphibian, bovine, or human) are not diabetogenic.

The neurointermediate lobe of the hypophysis of the toad (equivalent histologically and physiologically to mammalian posterior lobe) has a diabetogenic action, although it is less than that of the pars distalis (equivalent histologically and physiologically to mammalian anterior lobe). Likewise the bovine posterior lobe of the hypophysis has some action but it is less active and more toxic than that of the anterior lobe of the same species (Houssay and Biasotti, 1930).

EXPERIMENTAL DATA

The diabetogenic action of the hypophysis from various animals (fish, batrachian, birds, mammals) was studied in normal dogs, in hypophysectomized dogs with 4 gm. of pancreas and in toads deprived of their hypophysis and pancreas. The diabetogenic action of the serpent hypophysis was not studied in the dog because it had given poor results in the toad, and because it was not possible to obtain sufficient amounts for use in mammals.

Experiments on Dogs

Methods.—In a few instances normal dogs were injected with human hypophysis in order to verify its diabetogenic action in the animal not deprived of any organ. In the remainder of the experiments 9 hypophysectomized male dogs, with pancreas reduced to about 4 gm., were used to test the diabetogenic principle.

Under ether anesthesia with midline incision partial pancreatectomy leaving the adherent portion to the duodenum with the main excretory duct was first performed. Several days later, when the dog had recovered from the operation, total hypophysectomy was done under ether anesthesia using the temporal route. The animals were not injected with hypophyseal substance until they had completely recovered from the surgical procedures, and only when they were in excellent general health, usually several months after hypophysectomy. Two dogs were used after almost 4 years (Table I).

The dogs were mostly males with an average weight of 10 kilos (from 7.5 to 11.8 kilos) and were kept in metabolism cages and fed raw beef (35 to 40 gm. per kilo per day). Fasting blood specimens were taken in the morning preceding the day on which the course of injections was started and on the 4 subsequent days before the injections were given, *i.e.*, 18 hours after meals and 16 hours after the preceding injection. A small cut on the margin of the ear gave the necessary amount (0.2 cc.)

TABLE I

Date of Operations and Sensitivity of Dogs Used

Dog No.		Terminal state	Weight	Amount of bovine anterior hypophysis exerting diabetogenic action per day	
				Fresh	Dry
			kg.	*mg. per kg.*	*mg. per kg.*
5-77	Pancreatectomized 8-4-1937 Hypophysectomized 9-25-1937	Died of diabetes 9-6-1940	10.5	40	9.4
5-78	Pancreatectomized 8-18-1937 Hypophysectomized 9-25-1937	Died of distemper 6-4-1938	9	20	4.7
5-88	Pancreatectomized 11-20-1937 Hypophysectomized 12-15-1937	Died of diabetes 4-2-1938	9.5	40	9.4
5-93	Pancreatectomized 12-31-1937 Hypophysectomized 3-12-1938	Died of distemper 12-4-1939	10	20	4.7
6-03	Pancreatectomized 10-20-1938 Hypophysectomized 11-15-1938	Died of diabetes 12-29-1939	11.8	40	9.4
6-07	Pancreatectomized 10-14-1938 Hypophysectomized 11-21-1938	Alive 6-15-1941	10.1	40*	9.4
6-10	Pancreatectomized 12-16-1938 Hypophysectomized 3-3-1939	Alive 6-15-1941	8.6	20	4.7
6-26	Pancreatectomized 9-27-1939 Hypophysectomized 10-21-1939	Sacrificed 10-16-1940	9.1	40‡	9.4
6-27	Pancreatectomized 9-27-1939 Hypophysectomized 10-21-1939	Sacrificed 10-21-1940	7.5	20‡	4.7

* Later there was not diabetes with 500 mg. per kilo per day.

‡ Later there was not diabetes with 150 mg. per kilo per day.

for the Hagedorn-Jensen procedure with deproteinization by the Somogyi technique, for determination of the blood sugar.

Hypophyses of various species of animals were used (human, bovine, sheep, dog, rat, guinea pig, chicken, serpent, toad, and fish). The human hypophysis was obtained from the Pathology Department.[1] The other hypophyses were taken from

[1] The authors gratefully acknowledge the courtesy of Professor P. I. Elizalde and Professor D. Brachetto Brian, and of Dr. O. del Piano and Dr. J. A. Pique in making the human hypophyseal material available.

adult normal animals of both sexes. The glands were removed immediately after the death of the animals, except for those of human and fish origin, which were removed a longer period after death, and those from chickens which were removed a few hours postmortem. Hypophyses of the rats and guinea pigs were utilized fresh. Some were dried on a watch crystal (toad), and others, such as those of human beings and dogs were placed in acetone, pulverized after 2 days and then kept in sulfuric acid vacuum. The hypophyses of hens and fish were used as whole glands, but those from the other species of animals were used as separate lobes as is noted in each particular case. The glands were triturated in a mortar, suspended in physiological saline solution, and finally stored in the refrigerator until used.

TABLE II

Species	Number of glands used	Anterior lobe			Posterior lobe	
		Fresh	Dried	Acetone powdered	Fresh	Dried
		mg.	*mg.*	*mg.*	*mg.*	*mg.*
Human beings	10	346	83	66	71	15.3
Dogs	40	43	10	7.09	12.8	3
Bovine	1836	2000	470	435	400	86
Guinea pigs	3	13.33	2.83	2.60	4.33	0.97
Rats	30	4.57	1.097	0.83	1.43	0.345
Toads	100	3.3	0.55	—	2.1	0.37
		Whole hypophysis				
"Merluza"	6	8.3	1.3			
"Corvina"	6	5.9	0.9			
Chickens	7	6.3	1.3	1.04		

Toad (*Bufo arenarum* Hensel).

The fish "merluza" (*Merluccius hubbsi*, Marini, 1939).

The fish "corvina" (*Micropogon opercularis*, Quoy and Gaimard, 1824).

The weight of the whole gland or the weight of its lobes, was determined for the various types used in fresh or dried state and placed in sulfuric acid vacuum for preservation (Table II).

The bovine anterior hypophysis extract was prepared once a week. The gland was removed immediately after the animal was slaughtered and was placed at once in carbon dioxide snow. In the laboratory they were kept at a low temperature and the anterior lobe was separated and reduced to pulp with a meat grinder. Water (2,400 cc.) and 0.8 per cent sodium hydroxide (600 cc., previously cooled) were added to 800 gm. of prepared gland. The flask was shaken continuously while submerged in ice water and then placed in the refrigerator until the next day. The mixture was then acidified with 150 cc. of acetic acid (2.5 per cent), then alkalinized with NaOH (0.8 per cent) until a light alkaline reaction to phenol red indicator was observed. After centrifuging the mixture a clear supernatant portion was drawn off and stored in the frozen state in 60 cc. containers.

All injections were given by the peritoneal route, twice a day for 4 consecutive days, using physiological saline solutions (0.8 per cent) as a vehicle for the hypophyseal substance. The volume used varied in relation to the dose of hypophysis admin-

istered but always was less than 5 cc. All possible precautions for asepsis were taken and we have not observed any kind of infection although the sterilization of the extract could not be perfect because of the nature of the material injected.

On each test dog the diabetogenic action of the bovine anterior hypophyseal lobe extract was determined by injecting the same dose daily for 4 consecutive days. If the fasting blood sugar determination reached 150 mg. per cent or more it was considered as a positive result. When the test was completed the animal was kept at rest (in normoglycemia) from 7 to 10 days before being used for further determinations. If the initial dose was inactive, larger doses were given 4 to 7 days later until a positive result was obtained.

After a rest interval the diabetogenic action of the hypophysis of another animal species was determined in the same manner.

Results.—Bovine Hypophysis: The dog's sensitivity to bovine anterior hypophysis was fairly constant (see Table III). A positive result was obtained with 20 mg. per kilo per day in 3 dogs and with 40 mg. per kilo per day in the other 6 animals. From time to time this potency was verified by test. It did not change in 3, it increased in 3, the dogs remaining permanently diabetic and in the other 3 a definite decrease of such sensitiveness was observed. Not all the anterior hypophyseal extracts have the same activity. Nevertheless, it is possible to notice that the dog's sensitivity itself changed little during the long experimental periods (Table III).

The bovine anterior hypophysis produced a diabetogenic action on the dog with its pancreas intact. A positive result was observed with 500 mg. per kilo per day for 4 consecutive days in 14 per cent of the animals; with 1,000 mg. per kilo per day in 53 per cent; and with 1,500 mg. per kilo per day in 70 per cent. On the hypophysectomized and partially pancreatectomized dogs (with 4 gm. of pancreatic tissue) doses of 20 mg. of extract per kilo per day in 3 dogs and 40 mg. per kilo per day in 6 dogs were sufficient to obtain diabetogenic action. In other words, they were 25 to 40 times more sensitive than normal dogs.

Human Hypophysis: The diabetogenic action of human hypophysis was verified in normal as well as in hypophysectomized and pancreatectomized dogs (with 4 gm. of pancreatic tissue left). From autopsy material 170 human hypophyses were assembled, the lobes separated, stored in acetone, and dried. An alkaline extract was prepared from this material in powder form by the described technique and placed in the refrigerator.

Three normal dogs were injected intraperitoneally. In the first, the extract from 59 anterior hypophyseal lobes was given in 4 days; the remaining 2 received the extract from 55 lobes in 2 days. The diabetogenic action was definite in the second dog and incomplete in the first and third dogs (Table IV).

In the dogs deprived of their hypophyses and of a part of their pancreas the diabetogenic action was obtained with 13 mg. (or a little over that amount) per kilo per day of fresh anterior lobe extract (Table IV). This finding demon-

strates that human hypophysis has a marked diabetogenic action notwithstanding the fact that two unfavorable factors diminished the activity: (1) the

TABLE III

Dogs Deprived of Hypophyses and with Only 4 Gm. of Pancreatic Tissue, Injected Peritoneally Twice a Day for 4 Days with Alkaline Extract of Fresh Anterior Lobe of Bovine Hypophysis (33 Per Cent in Physiological Solution). Blood Sugar Determined from Capillary Blood from Ear Margin, Animals Being without Food for Previous 12 Hours

Date	Dog No.	Weight	Injected hypophysis per kg. per day Weight		Blood sugar Days				
			Fresh	Dry	0	1	2	3	4
		kg.	*mg.*	*mg.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
11-23-37	5-77	10.2	20	4.70	26	—	88	127	106
12-26-39	5-77	10.5	20	4.70	67	108	84	62	74
6-27-40	5-77	10.5	20	4.70	93	139	133	196	107
3-10-38	5-77	10	40	9.41	92	—	204	—	255
12-11-39	5-77	10.5	40	9.41	97	147	201	300	—
7- 1-40	5-77	10.5	40	9.41	78	116	177	206	121
11-15-37	5-77	10.3	100	23.52	102	131	—	290	—
10-19-37	5-77	10.6	400	94.08	98	276	—	—	—
	5-78	9	20	4.70	96	—	—	155	—
	5-78		40	9.41	94	—	210	—	225
	5-78		100	23.52	98	106	—	255	—
	5-78		400	94.08	91	—	—	—	280
3-10-39	5-88	9.5	40	9.41	98	—	236	—	272
3-21-38	5-93	10	20	4.70	119	—	—	—	177
3-10-38	5-93	10	100	23.52	102	131	—	290	—
2- 3-39	5-93	10	100	23.52	104	105	115	195	280
12-11-39	6-03	11.8	40	9.41	147	207	259	335	—
9-18-39	6-03	11.8	200	47.04	90	—	88	99	—
12-26-39	6-07	10.1	20	4.70	103	101	95	80	119
12-11-39	6-07	10.1	40	9.41	111	138	169	240	—
12-26-39	6-10	8.6	20	4.70	76	172	259	211	247
12-11-39	6-10	8.6	40	9.41	104	238	221	236	—
6-27-40	6-10	8.2	20	4.7	84	122	253	265	—
12-26-39	6-26	9.1	20	4.7	69	106	—	77	92
12-11-39	6-26	9.1	40	9.41	100	93	195	209	—
7-22-40	6-26	11.0	80	18.80	86	91	94	99	89
8- 2-40	6-26	11.0	150	35.25	81	102	104	108	129
12-26-39	6-27	7.5	20	4.7	92	106	—	209	191
12-11-39	6-27	7.5	40	9.41	113	129	138	161	—
7-22-40	6-27	8	40	9.40	79	94	90	89	101
7-29-40	6-27	8.5	80	18.80	88	95	96	104	107
8- 5-40	6-27	8.5	150	35.2	93	103	108	113	138

glands were obtained postmortem from individuals who had been ill, and (2) 6 to 24 hours had elapsed before the gland was available for extract.

Rat Hypophysis: The anterior hypophysis of the white rat produced diabetogenic action in doses of 100 glands in 4 days, *i.e.*, 25 glands a day (11.43 mg. of fresh glands per kilo per day) on a dog previously responding to 20 mg. bovine gland per kilo per day (Table V). There was no action with 40 nor with 20

glands of anterior hypophysis of the rat injected in 4 days. The posterior lobe had no action in doses of 40 lobes in 4 days.

Dog Hypophysis: The hypophysis of the dog showed little diabetogenic activity in our few experiments. Diabetogenic action was obtained with 100 glands, but 50 glands injected in 4 days failed to produce any action in 1 dog which had responded to 80 mg. per kilo per day of bovine anterior hypophyseal lobe extract (Table V). Because of the few animals used, and also because of

TABLE IV

Normal and Test Dogs Injected with Human Hypophysis

Date	Dog No.	Weight	Injected hypophysis per kg. per day					12 hr. fasting blood sugar				
			No. of lobes injected		Weight			Days				
			Anterior lobe	Posterior lobe	Fresh	Dry	Acetone dried and powdered	0	1	2	3	4
1940		*kg.*			*mg.*	*mg.*	*mg.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
Normal dogs												
6-29	—	6.0	2.36	—	850	200	160	100	98	119	121	110
11-25	—	7.5	3.66	—	1260	300	230	90	112	173	—	—
11-28	—	6.7	4.10	—	1410	340	260	80	79	110	—	—
Dogs with reduced pancreas (4 gm.) and without hypophysis												
5-17	6-27	7.5	0.025		8.70	2.09	1.66	84	89	114	87	82
4-15	6-07	10.1	0.026		9.17	2.20	1.75	71	73	102	98	93
5-6	6-07	10.1	0.031		10.48	2.52	2.00	92	97	82	102	81
4-1	5-77	10.5	0.037		13.04	3.13	2.38	80	112	167	235	212
5-6	5-77	10.5	0.037		13.04	3.13	2.38	97	111	177	200	139
4-15	6-10	8.6	0.044		15.25	3.66	2.91	89	170	286	280	239
4-15	6-27	7.5	0.046		15.72	3.77	3.00	74	91	94	100	128
3-11	6-07	10.1	0.075		26.20	6.29	5.00	80	83	147	318	298
3-17	6-26	9.1		0.044	3.15	0.68		76	89	76	69	69
4- 1	6-26	9.1		0.18	12.71	2.74		71	80	83	74	82
3-11	6-10	8.6		5	7.74	1.66		81	95	104	213	265
5-13	6-10	8.6		8	13.50	2.91		92	81	89	98	88

the increasing resistance of the test animal, a final conclusion regarding the minimal active dose cannot be reached. On the other hand, in toads without hypophysis and pancreas, the diabetogenic action of the dog hypophysis is extremely intense. There was no action with 93 posterior hypophyseal lobes of the dog injected in 4 days.

Sheep Hypophysis: We have verified the action of the sheep anterior hypophyseal lobe in normal dogs (1,500 mg. per kilo per day), but we have not titred it in our partially pancreatectomized animals.

Guinea Pig Hypophysis: Fresh anterior lobes of 36 guinea pigs (13.3 mg. per kilo per day) had no diabetogenic action nor did posterior hypophyseal lobes of 36 guinea pigs (6.0 mg. per kilo per day) in dogs sensitive to 150 mg. per

kilo per day of bovine hypophysis extract. Larger doses might have been active but were not used.

Chicken Hypophysis: Diabetogenic action was obtained with 237 whole hypophyses of chickens (35 mg. per kilo per day) injected in 4 days. There was a rise of blood sugar to 144 mg. per cent with 180 hypophyses of chickens

TABLE V

Dogs without Hypophysis and with About 4 Gm. of Pancreas, Injected Intraperitoneally Twice Daily for 4 Days with Hypophysis of Various Animals

Date	Dog No.	Weight	Hypophysis infected per kg. per day					Blood sugar				
			No. of lobes		Weight			Days				
			Anterior lobe	Posterior lobe	Fresh	Dry	Acetone and powdered	0	1	2	3	4
		kg.			*mg.*	*mg.*	*mg.*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
Toad *Bufo arenarum* Hensel (dried pars distalis)												
10- 9-39	6-10	8.6	1.45	—	4.8	0.8		71	102	106	105	115
9- 5-39	6-07	10.1	2.50	—	8.2	1.4		96	107	96	129	131
9-27-39	6-10	8.6	2.90	—	9.6	1.6		112	153	122	146	283
9-22-39	6-10	8.6	5.81	—	19.2	3.2		70	103	182	328	297
Chicken (fresh whole hypophysis)												
2-27-40	5-77	10.5	0.48	—	3.00	0.62	0.495	83	94	96	95	85
3-11-40	5-77	10.5	2.38	—	15.00	3.09	2.47	82	79	92	108	144
4-15-40	5-77	10.5	5.64	—	35.55	7.33	5.87	91	86	133	142	150
Rat (fresh anterior lobe)												
10- 5-38	5-93	10.0	0.50	—	2.28	0.55	—	98	—	102	95	—
12-11-40	6-26	9.1	1.11	—	5.08	1.22	—	86	79	95	82	101
9- 5-38	5-93	10.0	2.50	—	11.43	2.74	—	98	—	—	208	243
3-11-40	6-27	7.5	—	1.33	1.91	0.46	—	76	78	81	74	90
Dog (acetone dried and powdered)												
4-1-40	6-07	10.1	0.40	—	17.02	3.96	4.32	87	80	101	113	82
5-13-40	6-27	7.5	0.90	—	38.70	9.00	8.00	81	92	109	98	93
7-16-40	6-10	8.0	1.59	—	67.0	15.15	10.9	88	97	95	95	106
9-30-40	6-07	9.0	2.77	—	119.0	27.7	15.6	65	82	112	243	286
4-1-40	6-10	8.6	—	0.43	5.58	1.31	1.16	91	89	85	88	81
5-13-40	6-26	9.1	—	0.72	9.24	2.17	2.77	78	83	91	96	81
10-14-40	6-07	9.0	—	2.50	33.0	7.7	5.22	73	84	97	95	97

(15 mg. per kilo per day) injected in 4 days in a dog sensitive to 40 mg. of bovine anterior hypophysis (Table V). The equivalent diabetogenic potency of chicken hypophysis compared to 20 mg. bovine anterior hypophysis was between 7.5 and 15 mg.

Toad Hypophysis (Bufo arenarum Hensel): The diabetogenic action of the pars distalis of the toad hypophysis (equivalent to the mammalian anterior lobe) has been studied by Foglia (1940). An intense diabetogenic action was obtained with 200 lobes in 1 animal and with 100 lobes in another injected in 4 days. In 1 dog sensitive to 20 mg. per kilo per day of bovine anterior hypoph-

ysis the same sensitivity was obtained by the use of 9.6 mg. per kilo per day of toad pars distalis (Table V). Taking into consideration the difference in weight of the fresh organ, the diabetogenic activity of toad hypophysis as compared with that of the ox is 3 to 5 times greater with the same weights of dried gland.

Fish Hypophysis: Hypophyses were obtained from two species of fish in the Buenos Aires market, caught on the preceding day in Mar del Plata. The glands cannot be considered fresh although the fish were shipped on ice. The "merluza" (*Merlucius hubbsi*, Marini, 1933), and the "corvina" (*Micropogon operculatis*, Quoy and Gaimard, 1824) were used. We have injected 20 and 115 "merluza" hypophyses during 4 days in dogs (dogs 6-07 and 6-27) without modifying their blood sugar. Hypophyses of 40 "corvinas" injected in 4 days were not effective (dog 6-10). 815 hypophyses (479 of "merluza" and 336 of "corvina") were then injected into dog 6-27 in 4 days with negative results.

Experiments on Toads

In 1930 and 1931, Houssay and Biasotti demonstrated for the first time the diabetogenic action of the hypophysis of fishes, batrachians, birds, oxen, dog, and man, on the toad without hypophysis and pancreas. The pars distalis of the toad is definitely more active than the neurointermediate lobe (pars neuralis plus pars intermedia). Furthermore, the anterior lobe of oxen, dogs, and rats is definitely more active than the posterior lobe.

Methods.—Bufo arenarum Hensel toads in groups of 15 to 20 toads of the same size, age, and sex, were anesthetized with ether under a bell jar. Pancreatectomy was done by abdominal incision and the pars distalis of the hypophysis removed by the oral route, the sphenoid and parasphenoid bone being cut but not removed. This left both skeletal and mucous membrane protection of the operative sites. Control animals had similar operative procedures except for the organ removal. The test animals were injected with a saline suspension of finely triturated hypophyses. The blood sugar was determined 24 hours after injection by the Hagedorn-Jensen method on blood taken from the heart and deproteinized by the Somogyi method.

Results.—The various mammalian anterior hypophyses and the whole hypophysis of the chicken were diabetogenically active in the toad (Table VI). The hypophyses of the human being, dog, toad, rat, guinea pig, chicken (entire hypophysis), and ox can be listed in a decreasing order of activity.

The whole hypophyses of fish and serpents were but slightly active. To groups of hypophysectomized and pancreatectomized toads 3 glands were injected in each toad on the same day; the hypophysectomized and pancreatectomized control group had an average blood sugar of 54 mg. per cent; those injected with "corvina" hypophyses *Micropogon operculatis* (Quoy and Gaimard, 1824) had 79 mg. per cent; those injected with "merluza" *Merlucius*

hubbsi (Marini, 1933) had 72 mg. per cent; and those injected with serpent hypophyses, *Constrictor constrictor* (L) had 97 mg. per cent.

Subsequently, we studied on one dog the action of the hypophyses of 475 "corvina" *Micropogon opercularis* about 8 hours after being caught in the ocean at Mar del Plata. These fish were kindly supplied by Messrs. Biedma, Aguilar, and Motti from the aquarium of that city. It will be noted that these hypophyses proved less active than those of the other animals studied (see Table VI).

TABLE VI

Diabetogenic Action of the Anterior Lobe of Hypophysis of Different Species of Animals on the Toad Bufo arenarum Hensel, without Hypophysis and Pancreas (Each Number Represents the Average of a Group of 15 to 20 Toads)

Species Acetone-dried anterior lobe	Average blood sugar after 20 hrs.				
	Experiments with 3 mg. per toad		Experiments with 1 mg. per toad		
	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
Controls	88	60	69	53	106
Man	205	—	183	—	—
Dog	169	—	176	—	—
Toad	163	183	160	107	205
Rat	170	—	137	—	—
Guinea pig	—	134	—	107	—
Chicken*	161	—	—	97	—
Ox	—	141	—	89	—
"Corvina"‡	—	—	—	—	115§

* Entire hypophysis.

‡ "Corvina" is the fish *Micropogon opercularis* (Quoy and Gaimard, 1824).

§ The same day with 5 mg., 151 mg. per cent.

DISCUSSION

Dogs from which the hypophysis and all but 4 gm. of pancreas had been removed were used as test animals for the diabetogenic action of the hypophysis. Fixed daily doses of the extract of the anterior lobe of the hypophysis were injected intraperitoneally for 4 consecutive days. Blood sugar determinations were made before feeding and before the daily injections of extract of the hypophysis. Levels of 150 mg. per cent or more were considered positive evidence of diabetogenic action. When there was hyperglycemia there was likewise glycosuria and increased urinary excretion of ketone bodies.

A diabetogenic action was noticed with the anterior hypophysis of the human, toad, rat, and chicken (whole hypophysis) in doses of between 9 and 13 mg. of fresh lobe per kilo per day in these dogs. These glands were equivalent in action to about 20 mg. of fresh bovine hypophysis.

The anterior hypophysis of the dog was less active in our experiments since we obtained a positive result with 119 mg. per kilo per day (a total of 50 glands).

However, we hesitate to make this conclusion final since (*a*) the number of experiments was small; (*b*) the test animals showed a rapid loss of sensitivity at the time of the test; and (*c*) the anterior hypophysis of the dog has been found to be very active diabetogenically when tested on the toad.

We did not obtain a positive result with either anterior hypophysis of guinea pig or with the whole hypophysis of fish, probably because we did not use sufficient doses. The anterior hypophysis of the guinea pig is definitely active on toads and the hypophysis of the fish has a definite action on the same animal although it is inferior compared with that of the other animals studied.

The anterior hypophysis of man has been shown to be the most active in partially pancreatectomized dogs as well as in hypophysectomized and pancreatectomized toads. But we obtained in addition a positive diabetogenic action in normal dogs with pancreas intact, with a daily dose of 1260 mg. per kilo per day of fresh human anterior hypophysis lobe extract, in 2 days time.

The diabetogenic power of the anterior hypophyses which were studied can be placed in the following order of decreasing activity, tested on toads deprived of their hypophysis and pancreas: human, dog, toad (*Bufo arenarum* Hensel), white rat, guinea pig, chicken (whole hypophysis), ox, snakes, "corvina" (*Micropogon opercularis*). We found in the dog a similar order (human, toad, rat, chicken, and ox). The activity of the dog hypophysis on the dog was not established with precision and insufficient amounts of the hypophysis of the guinea pig and "corvina" were injected to demonstrate their action.

The diabetogenic action of the posterior lobe was also tested but smaller doses than that of the anterior lobe were injected. Nevertheless, it was verified by Houssay and Biasotti (1930 and 1931) on the toad and by Houssay, Biasotti, and Rietti (1932, 1933) on mammals that with equal weights its action is much less intense than that of the anterior lobe. Thus 12.7 and 13.5 mg. per kilo per day of human posterior hypophysis and 33 mg. per kilo per day of posterior hypophyseal lobe of the dog did not produce any diabetogenic action. In one instance, the diabetogenic action was obtained with 7.7 mg. per kilo per day of human hypophysis in dog No. 6-10, but this result was due probably to an abnormal sensitivity of the dog on that date because it was not observed 2 months later in the same dog when 13.5 mg. per kilo per day were given.

The diabetogenic action of the anterior hypophysis must be considered as specific and peculiar to this organ, as has been demonstrated by Houssay, Biasotti, and Rietti (1932, 1933) because the extracts of other organs did not have such action in 4 days on the same dogs nor 660 mg. per kilo per day of the liver or kidney of the toad (including the adrenals), nor 400 to 1000 mg. per kilo per day of kidney, liver, spleen, testicle, muscle, and thyroid of dogs or oxen.

Neither 30, 40, or 60 mg. of corticosterone nor 80 to 200 mg. of desoxycorti-

costerone, injected in 2 days produced the diabetogenic effect. Houssay and Biasotti (1938) did not obtain any action with prolactin (100 mg. in 4 days) or with adrenotropic (adreno-cortico-tropic) extract sent by Collip (250 units), follicle stimulating hormone (F.S.H.) (150 mg.), and luteinizing hormone (L.H.) (100 mg., *i.e.*, 100 units).

CONCLUSIONS

Of all the anterior hypophyses tested, those of the human produced the most marked diabetogenic action in the dog with its pancreatic tissue reduced to 4 gm., and in the hypophysectomized and pancreatectomized toad. The human hypophysis also produced diabetogenic action in the normal dog on daily doses of 1.26 mg. per kilo per day for 2 days.

The hypophysectomized dog with its pancreas reduced to 4 gm. is very sensitive to the anterior hypophyseal diabetogenic action and is the best test animal for demonstrating such action in mammals.

The anterior hypophysis of man, toad, rat, and chicken produces in such animals a diabetogenic action with doses of from 10 to 15 mg. per kilo per day. The bovine anterior hypophysis has identical action in 20 mg. doses. That of canine origin was much less active in a few though inconclusive experiments.

It was impossible to demonstrate a diabetogenic action with either guinea pig hypophysis or with that of fish probably because insufficient doses were injected.

The diabetogenic action was not obtained by the injection of other organ extracts of toads, dogs and oxen, of corticosterone (30, 40, and 60 mg. in 4 days) or of desoxycorticosterone (80 mg. and 200 mg. in 4 days).

The toad (*Bufo arenarum* Hensel), deprived of its hypophysis and pancreas is the most sensitive biological reactor for testing the diabetogenic action. In this animal the diabetogenic action of anterior hypophyses from varied sources decreased in the following order: man, dog, toad (*Bufo arenarum* Hensel), white rat, guinea pig, chicken (whole hypophysis), ox, serpent (*Constrictor constrictor* (L.)), the fish "corvina" *Micropogon opercularis* (Quoy and Gaimard, 1824), and "merluza" *Merlucius hubbsi* (Marini, 1933).

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