

The results of the present investigation supplement, and in a way, confirm, the earlier findings in our laboratory concerning the lowered resistance against diphtheria toxin that characterizes a low intake of vitamin C. Tooth structure²⁰ and glucose tolerance¹² have been considered in the earlier papers.

Conclusions. Accompanying the anemia of scurvy in guinea pigs there was a progressive decrease in both hemoglobin and erythrocytes and a slight increase in leucocytes.

Guinea pigs receiving 3.0 mg of vitamin C per day and given subcutaneous injections of diphtheria toxin showed less disturbance in their blood cell count than similarly injected animals receiving 0.5 mg or less of the vitamin per day. The changes in the low-vitamin, toxin-injected groups were characterized by chromatophilia, anisocytosis and poikilocytosis.

10835

Antihormone Effects in Pancreatic Diabetes.

F. C. DOHAN AND F. D. W. LUKENS.

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

Collip and Anderson¹ observed that during the prolonged injection of anterior pituitary extract (thyrotropic) its effect upon the metabolic rate was not sustained. They also noted^{1, 2} that the serum of refractory animals inhibited the response of normal animals to thyrotropic hormone. Similar inhibitory effects have been observed in the case of the gonadotropic hormone. The relation of such antagonistic principles to species has been further studied.^{3, 4, 5}

Relatively few reports are available on the development of principles which antagonize the diabetogenic action of the anterior pituitary, although refractoriness has long been recognized. Collip⁶ re-

²⁰ Swanson, W. F., Sigal, A., and King, C. G., *J. Am. Dental Assn.*, 1936, **23**, 2089.

¹ Collip, J. B., and Anderson, E. M., *Lancet*, 1934, **1**, 76.

² Anderson, E. M., and Collip, J. B., *Lancet*, 1934, **1**, 734.

³ Thompson, K. W., and Cushing, H., *Proc. Roy. Soc. (London)*, series B, 1937, **121**, 501.

⁴ Thompson, K. W., *Proc. Soc. Exp. Biol. and Med.*, 1937, **35**, 637.

⁵ Anderson, E., and Evans, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 797.

ported one dog which was injected with pituitary extract and depancreatized; its diabetes resembled that of the Houssay animal. Similar experiments have been performed by Campbell, Keenan and Best.⁷

We have used depancreatized dogs to study the effect on the diabetes (a) of prolonged treatment with pituitary extract and (b) of the injection of the serum from a normal dog treated with anterior pituitary extract. The influence of these procedures on the excretion of glucose, nitrogen and acetone bodies of the depancreatized animals during fasting and the omission of insulin has been recorded. When these criteria manifest the full diabetic response it is regarded (since Houssay) as the result of pituitary activity, and we have consequently assumed that the amelioration of diabetes indicates an "inhibition of the diabetogenic principle(s)."

Methods and Results. A. Intraperitoneal injections of a crude saline anterior pituitary extract (beef) were administered daily, with few exceptions to a female mongrel dog. During the tenth and eleventh month of injections blood serum was obtained and injected intraperitoneally into 2 completely depancreatized dogs. The serum was administered to the diabetic dogs the day before and the first 2 days of fasting. The results are shown in Table I. The injection of the serum from the donor dog was accompanied by a marked decrease in glycosuria and a slight decrease in urine nitrogen from the control levels. The blood sugars during the fasting periods

TABLE I.
Fasting Metabolism of Various Depancreatized Dogs—3-day Periods.

Dog	Date*	Wt, kg*	Total serum injected, cc	Glucose, avg g/day	Urine Nitrogen, avg g/day	Ketones avg mg/day
Control Periods.						
A-2	10/6/38	4.50	0	12.8	3.8	28
A-5	11/24/38	10.25	0	18.9†	4.2†	0†
A-6	12/24/38	6.05	0	14.8	5.0	593
A-8	4/14/39	7.15	0	22.9	7.8	1301
During Injection of Serum from Normal Dog.						
A-6	1/4/39	5.45	84	25.7	5.6	136
A-7	3/3/39	7.20	87	17.1	7.6	90
During Injection of Serum from Dog Treated 10 Months with Ant. Pituitary Extract.						
A-2	11/4/38	4.35	70	2.1	2.6	17
A-5	12/6/38	9.35	84	3.7	3.2	0

* 1st day of period.

† Loss of some urine during this period.

Insulin omitted during all periods.

⁶ Collip, J. B., *Glandular Physiology and Therapy*, Chicago, 1935, p. 105.

⁷ Campbell, J., Keenan, H. C., and Best, C. H., *Am. J. Physiol.*, 1939, **126**, 455.

were within the range 132-372 mg per 100 cc, which may be found in the hypophysectomized-depancreatized dog. Injections of serum from normal dogs were not associated with a decrease in the glycosuria or urine nitrogen excretion.

It may be noted that Dog A-2 died from insulin shock 9 days after receiving the serum from the donor dog. At this time it was receiving a daily dosage of 18 units of insulin and eating 100 g of pancreas, 400 g of beef and 60 g of sugar a day. Dog A-5, after the last injection of serum from the donor dog, showed a marked reduction in insulin requirement. Seventeen days after this last injection it excreted only 0.5 g of sugar per day during a 2-day fast without insulin. The dog's appetite then failed, and it was sacrificed. The donor dog died from acute distemper several weeks after completion of this portion of the experiment.

The autopsies on the diabetic dogs as well as their fasting control periods indicated that the pancreatectomies were complete.

Autopsies. Dog A-2, November 13, 1938; wt. 3.55 kg. No pancreatic tissue was found. There was very little body fat. The liver was not grossly fatty. Microscopic sections of the pituitary, thyroid, ovary, adrenal and liver failed to reveal anything unusual.

Dog A-5, December 30, 1938; wt. 7.5 kg. No pancreatic tissue was found. There was ample abdominal fat. Liver glycogen 1.87%. Microscopic sections, as in Dog A-2, failed to reveal any definite abnormality.

B. It was the purpose of these experiments to determine the effect of prolonged intraperitoneal anterior pituitary extract injections in 2 completely depancreatized dogs. With rare exceptions they were maintained on a constant diet and insulin was given. The effect of cessation of anterior pituitary extract injections and insulin upon the fasting metabolism of these dogs is shown in Table II. There was a marked reduction in the fasting glycosuria and a slight decrease in nitrogen excretion when compared to the control periods. The blood sugars of dog A-8 during the 3-day fasting period starting 6/23/39 ranged from 158 to 168, and those of dog A-6 during the period starting 3/23/39 from 228 to 99 mg per 100 cc.

During the course of the daily injection of 2 cc of anterior pituitary extract into Dog A-8 there was first a marked diabetogenic effect so that the insulin was raised from 30 to 70 units. However, by the 15th day the insulin requirement was slightly less than before starting the extract. Dog A-6 also showed an increased and then a decreased diabetic response to anterior pituitary during the injections as measured by the insulin requirement.

TABLE II.
Effect of Prolonged Anterior Pituitary Extract Injection on the 3-day Fasting Metabolism of Depancreatized Dogs.

Date*	wt, kg	Period of injection ant. pituitary, days	Time† from last ant. pit. injection, days	Glucose, avg g/day	Urine nitrogen, avg g/day	Ketones, avg mg/day
No. A-6; Female; Pancreatectomy December 19, 1938.						
12/24/38	6.05	control	—	14.8	5.0	593
3/5/39	5.32	30	1	7.0	7.1	34
3/23/39	5.25	48	2	1.7	3.8	0
No. A-8; Female; Pancreatectomy April 10, 1939.						
4/14/39	7.15	control	—	22.9	7.8	1301
4/27/39	6.70	control	—	27.3	8.0	3267
6/23/39	6.35	38	4	0	3.7	36

* 1st day of period.

† To 1st day of period.

Insulin omitted during all periods.

After the final fasting periods, both dogs were fed but insulin was withheld. One showed a moderate glycosuria when fed in spite of the low sugar excretion during fasting. This implies that the fasting glycosuria was due to an inhibition of the production of glucose and not to its normal utilization. The other had only a slight glycosuria on feeding. Both dogs resembled the Houssay animal.

The autopsies as well as the control periods indicated that the pancreatectomies were complete.

Autopsies. Dog A-6, April 5, 1939; wt. 4.40 kg. Died in insulin shock. No pancreatic tissue found. Three small cysts were noted in the intermediary lobe of the pituitary; ample anterior lobe tissue of normal appearance was present. The thyroid gland was composed of thick epithelial cells and there was less colloid than usual. The appearance suggested thyroid stimulation of moderate degree. Adrenals, ovaries and liver were not remarkable; liver fatty acids were 1.83%.

Dog A-8, June 28, 1939; wt. 5.70 kg. No pancreatic tissue found. Small fat depots. The liver appeared grossly normal and microscopic examination showed some vacuolation; liver fatty acids were 4.94%; glycogen 0.37%. The thyroid showed slight evidence of stimulation; adrenals and ovaries not remarkable although ovarian follicles were large.

Sera from both dogs were kindly tested by D. J. Ingle for anti-diabetogenic effect in fed, partially depancreatized rats. Due to changes in food intake the results were inconclusive, but certainly there was no marked effect such as that obtained by Long, Thompson and Fry⁸ with their antiserum from a normal dog.

In addition, 5 cats were treated with pituitary extract for 2 months and were depancreatized a week after the last injection. For the first 3 days after operation the average glucose excretion was 1.75 g per kg per day, in contrast to 3.2 g per kg per day excreted by 16 untreated diabetic cats. The urine of all the injected cats was negative for acetone.

Discussion. Two depancreatized dogs given serum from a donor treated with pituitary extract had a diminished excretion of glucose and nitrogen. This did not occur when the serum of a normal dog was used. It appears that the passive transfer of a principle antagonistic to the diabetogenic hormone(s) of the anterior pituitary is demonstrated. This is comparable to the results obtained by others in the case of the thyrotropic and gonadotropic hormones. Since the action of the serum occurred during the absence of the islands functional changes in insulin secretion are eliminated and the "antifactor" thus appears to act on the pituitary or on the tissues.

A similar response occurred when the antifactor was actively induced by the administration of the extract to two depancreatized dogs. Here, too, any adaptation of the pancreatic islands such as might occur in an intact animal had been eliminated.

The cysts in the pituitary gland of Dog A-8 were probably not significant, since there was abundant anterior pituitary tissue present, and since there were no cysts in the pituitaries of the other dogs.

Conclusions. 1. Two completely depancreatized dogs given intraperitoneal injections of serum from a dog treated over 9 months with anterior pituitary extract showed a marked decrease in the degree of the glycosuria. (2) Two completely depancreatized dogs injected with anterior pituitary extract for 30 or more days showed, after cessation of extract injection, a much less severe diabetes during fasting than before the injections. (3) The metabolic behavior of these 4 dogs was much like that of the depancreatized-hypophysectomized animal.

⁸ Long, C. N. H., Thompson, K. W., and Fry, E. G., personal communication.