Effects of ACTH, Cortisone and Adrenal Cortical Extract on Carbohydrate Metabolism of Hypophysectomized Dogs.*† (19618)

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The Addisonian patient and the adrenalectomized animal exhibit severe disturbances in carbohydrate metabolism(1,2), which can be rectified to a great extent by administration of cortisone(1,3). Massive doses of either ACTH or cortisone in the normal animal can produce a diabetes-like condition(4). The hypophysectomized animal exhibiting severe adrenal cortical atrophy also shows evidence of derangements in its carbohydrate metabolism; this animal manifests hypersensitivity to the hypoglycemic action of insulin(5-7), a secondary hypoglycemia in the glucose tolerance test(7), and a diminished response to the hyperglycemic action of adrenaline(8,9). This paper is concerned with the effects of 1) adrenal cortical extract (ACE), 2) cortisone, and 3) ACTH upon these defects of carbohydrate metabolism in the hypophysectomized dog. In this way the importance of the adrenal cortical atrophy in the disturbed carbohydrate metabolism of the hypophysectomized dog may be further clarified.

Methods. The investigations were performed on 11 trained, unanesthetized male and female hypophysectomized dogs. The technic of hypophysectomy(8), the after-care and the feeding(7) of these animals have been described elsewhere.§ As a prerequisite for inclusion in this study, the dogs had to exhibit a high degree of insulin hypersensitivity and marked resistance to the hyperglycemic action of adrenaline. All experiments were done in the postabsorptive state. Insulin sensitivity was determined by the hypoglycemic response to 0.025 unit/kg insulin (Lilly),§ given intravenously (designated hereafter as the test dose); blood sugar changes were followed for 4 hours. Glucose tolerance was determined by the blood sugar response to a 10-minute intravenous infusion of glucose, 0.075 g/kg/min.; blood sugar changes were again followed for 4 hours. The response to the hyperglycemic action of adrenaline was tested by a 5-minute intravenous infusion of adrenaline hydrochloride, 0.0035 mg/kg/min. The ACE used was kindly supplied by Dr. E. C. Kendall; 1 cc of this extract was prepared from 150 g adrenal gland. The usual dose of ACE was 25 cc; the route and time of administration were varied, but the optimal effects with this dose were achieved when given as follows: 5 cc intramuscularly 17 to 18 hours before the experiment, 10 cc intramuscularly one hour prior to the injection of insulin, and again 10 cc intravenously immediately before insulin. Cortisone acetate (Cortone acetate, Merck) was given intramuscularly twice daily in doses ranging from 20 to 50 mg/day for periods of 65 to 86 days. Most of the ACTH preparations used were prepared and kindly supplied by Dr. R. W. Bates of E. R. Squibb and Sons. The following Squibb preparations were used: Lot No. P232Ac (20 IU/mg), C15Pr (3 IU/mg), P273Ac (10 IU/mg), and D13Pr (4 IU/mg). The daily dosage of these preparations ranged from 3 to 10 IU/kg and was administered intramuscularly in 4 divided doses (9 A.M., 1 P.M., 5 P.M., and 9 P.M.) for periods of 23 to 125 days. In addition, a few dogs received Armour ACTH§ preparations in daily dosages of 1 to 3.5 mg/kg for periods of 49 to 75 days prior to the adminis-

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TABLE I. Effect of Adrenal Cortical Extract (ACE) on the Insulin Response of Hypophysectomized Dog H-5.

	Time, min	Control Blood sugar in mg %	ACE-Rx* Blood sugar in mg %
(A) First 4-hr test	0	71	78
	Insulin (.025 μ/kg) intrav. at 0 time		
	10	58	62
	20	49	45
	30	46	42
	40	39	42
	50	38	41
	60	38	47
	75	35	51
	90	36	56
	120	39	67
	150	43	72
	180	43	77
	210	48	78
	240	43	79
(B) Second 4-hr test	0 (240)	43	79
	Insulin (.025 μ/kg) intrav. at 0 (240) time		
	10 (250)	46	73
	20 (260)	42	61
	30 (270)	38	57
	40 (280)	36	54
	50 (290)	34	51
	60 (300)	33	53
	75 (315)	31	59
	90 (330)	30	61
	120 (360)	33	71
	150 (390)	37	75
	180 (420)	39	78
	210 (450)	45	80
	240 (480)	47	82

* Dose of ACE (Kendall): 25 cc i.m. (see text for schedule).

tration of any Squibb ACTH preparation. The Armour ACTH preparations used were lot No. 58-9-50 and 212-103 (both having potencies of 1 mg = 2 La-I-A Armour Standard).

Results. I. *Experiments with ACE.* Sixteen insulin sensitivity tests and 12 adrenaline experiments were performed on hypophysectomized dogs 233 to 456 days post-hypophysectomy. ACE, in the above dosage, exerted a marked anti-insulin effect in all the experiments, *viz.*, it prevented the fall of the blood sugar to the low level seen in the untreated hypophysectomized state and accelerated the recovery of the blood sugar to the pre-insulin injection levels. This is illustrated in Part A of Table I which records the insulin response of hypophysectomized dog H-5. Since the maximal effect of the 2 injections of ACE (given within the 1-hour period prior to insulin) seemed to occur 4 to 6 hours later, an 8-hour insulin sensitivity test was devised in which a second injection of insulin was given at the conclusion of the first 4-hour period and blood sugar changes again followed for 4 hours. Thus, 2 successive 4-hour insulin sensitivity tests were conducted after the ACE injections. Table I records such a typical 8-hour test in which Part A represents the first 4-hour test period and Part B, the second 4-hour test period. As can be seen, the maximal effect occurred in the second 4-hour test period (Part B, Table I). In no case did ACE, in the dosages given, completely abolish the insulin hypersensitivity of the hypophysectomized dog. At the same time, such signs as ataxia, inability to stand and photophobia, present in the untreated hypophysectomized animal after insulin, did not appear after ACE administration. In the above experiment, the effect of ACE on the post-absorptive blood sugar was slight, but in other experiments, ACE was seen to elevate the postabsorptive blood sugar to a greater degree. The glycemic response to adrenaline in these animals showed only a small increase after ACE administration, and no animal had a response comparable to that of a normal dog.

II. *Experiments with cortisone.* Twenty experiments, testing the insulin sensitivity, glucose tolerance and adrenaline resistance,

TABLE II. Insulin Sensitivity Tests in Hypophysectomized Dog H-3 before and during Cortisone Administration.

Time, min	Days of cortisone-Rx: 0 Blood sugar in mg %	15* Blood sugar in mg %	40† Blood sugar in mg %
0	64	70	72
Insulin (.025 μ/kg) intrav. at 0 time			
10	55	70	71
20	51	64	67
30	43	61	63
40	39	60	70
50	37	61	72
60	38	63	74
75	40	69	75
90	44	71	78
120	45	73	80
150	56		
180	56		
210	58		
240	58		

* Dose of cortisone: 20 mg/day i.m.
† " " " increased to 50 mg/day 11 days prior to exp.

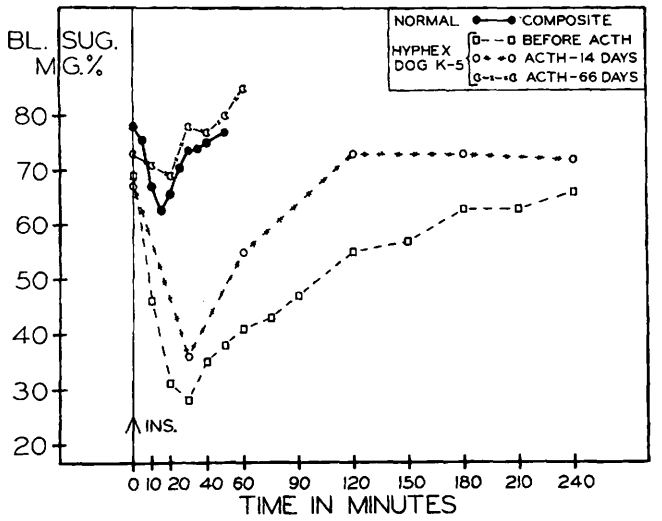

FIG. 1. Blood sugar curve produced by insulin (.025 μ/kg i.v.) in normal dogs compared with those of hypophysectomized (hyphex) Dog K-5 before and during ACTH regimen. Armour ACTH #212-103 was given for 49 days and followed by Squibb ACTH #C15Pr for 17 days.

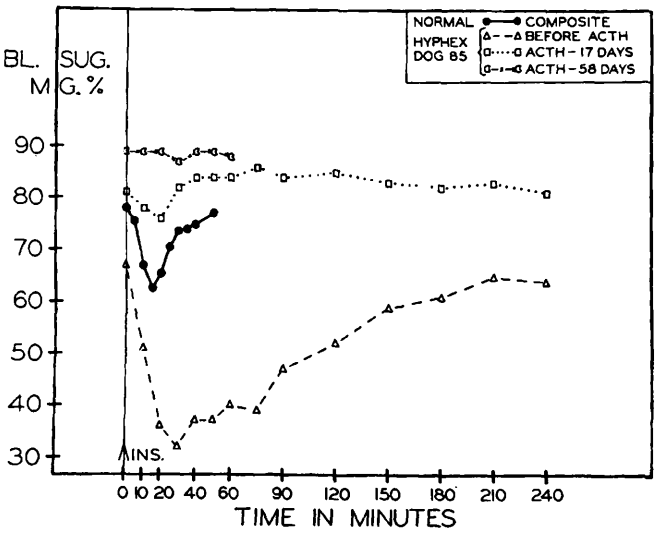

FIG. 2. Blood sugar curve produced by insulin (.025 μ/kg i.v.) in normal dogs compared with those of hypophysectomized (hyphex) Dog 85 before and during ACTH regimen. Only Squibb ACTH #C15Pr given for 58 days.

were done on a series of hypophysectomized animals 71 to 237 days post-hypophysectomy. The animals first were given 20 mg of cortisone per day, and their dosage was raised gradually to 50 mg/day. The changes in insulin sensitivity produced by cortisone in a representative hypophysectomized dog H-3 are recorded in Table II. As can be seen, 15 days of cortisone administration to dog H-3 (20 mg/day): 1) produced a rise in the post-absorptive blood sugar to normal, 2) diminished considerably the previously marked blood sugar fall in response to insulin, and 3) returned the blood sugar to the pre-injection levels in a short period of time. Thus, cortisone exerted a marked anti-insulin effect at this time. The dose of cortisone was increased and an experiment done on the 40th

day revealed a further change in the blood sugar curve so that there was complete abolition of the insulin hypersensitivity of dog H-3. This response to the test dose was similar to that seen in normal animals. The other dogs used in this phase of the investigation exhibited similar results in that cortisone administration produced a marked anti-insulin effect; the degree of response varied directly with the amount and duration of the dosage. Concomitant with the abolition of the insulin hypersensitivity in these hypophysectomized dogs, cortisone changed the glucose tolerance so that the secondary hypoglycemia characteristic of the hypophysectomized dog was either significantly reduced or abolished. In addition, the glycemic response to adrenaline during cortisone administration approached or exceeded the normal range.

III. *Experiments with ACTH.* A total of 17 insulin sensitivity tests, 10 glucose tolerance tests, and 9 adrenaline tests were performed on a series of hypophysectomized dogs in order to assess the effect of ACTH on their carbohydrate metabolism. The ACTH regimens were begun 85 to 570 days post-hypophysectomy. In all the animals, continued administration of ACTH first diminished the exaggerated response to insulin, then elevated the post-absorptive blood sugar and abolished the insulin hypersensitivity thereby producing a normal response to insulin (Fig. 1). Prolonged administration of ACTH produced a resistance to the test dose of insulin inasmuch as no fall at all in the blood sugar was evident after the injection of insulin and usually elevated the postabsorptive blood sugar to above normal (Fig. 2). It is of interest to note that a potent Squibb preparation of ACTH was able to produce complete abolition of the insulin hypersensitivity in some animals as early as 8 days after the onset of the regimen. In other dogs this effect was seen only after longer periods of administration of the same preparation. Likewise, resistance to the test dose of insulin appeared at varying intervals after the ACTH regimen was begun; this was true when the same preparation was tested in different animals.

Concomitant with these effects, ACTH changed the glucose tolerance of these dogs

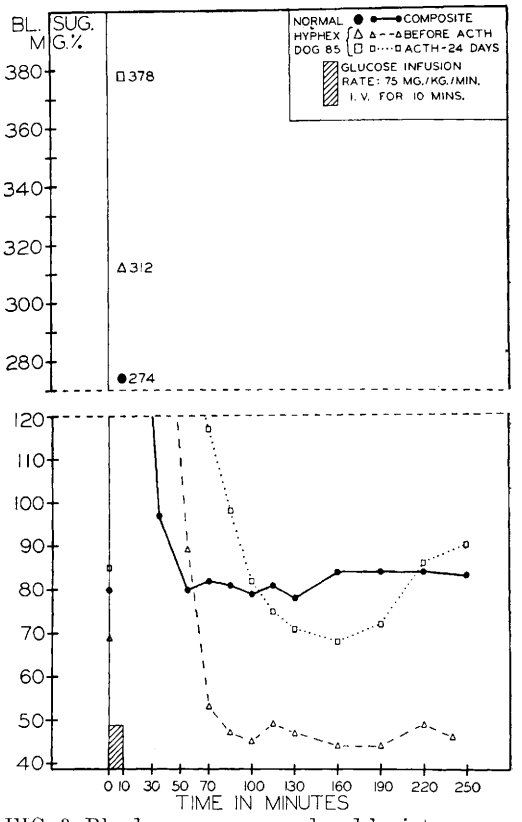

FIG. 3. Blood sugar curve produced by intravenous glucose (.75 g/kg) in normal dogs compared with those of hypophysectomized Dog 85 before and during ACTH regimen. Only Squibb ACTH #C15Pr given for 24 days.

in that there was always evident, at the end of the intravenous glucose infusion, an elevation in the peak of the blood sugar curve which was above that seen in the untreated hypophysectomized dog. Furthermore, the secondary hypoglycemia seen in the glucose tolerance of the untreated dog was completely eliminated. In some of the animals, no further change was evident but in a few dogs a tendency toward a diabetic-type of glucose tolerance was present in that the peak of the blood sugar curve at the end of the infusion was elevated far more and the slope of the declining blood sugar curve was shifted to the right (Fig. 3).

Before ACTH administration, all the hypophysectomized dogs exhibited only slight rises in blood sugar (maximum increase: 14 to 20 mg %) after the intravenous administration of adrenaline. These results agree with previous

work reported on the response to adrenaline infusion of untreated hypophysectomized dogs (8,9). During the ACTH regimen, the blood sugar rise in response to adrenaline increased (maximum rise: 65 to 94 mg %); this was greater than that seen in normal dogs—average rise: 49 mg % (8,9). In 2 dogs, the rises were within normal range (33 and 43 mg %, respectively). Only one dog had an exceptional rise of 36 mg % before ACTH, but during ACTH regimen this dog exhibited a rise of 88 mg %.

The weights of the 2 adrenal glands were within the normal range (1.5 to 3.0 g) in 4 ACTH-treated hypophysectomized dogs(9); of the remaining dogs in this series, 2 others thus far studied had adrenal weights which were not yet within the normal range.

Discussion. It is obvious from the above results that cortisone and the adrenal corticosteroids secreted under the influence of ACTH administration exert a potent influence in rectifying the defects in carbohydrate metabolism of the hypophysectomized dog. These hormones elevated the postabsorptive blood sugar and abolished the insulin hypersensitivity,¶ the secondary hypoglycemia following the intravenous administration of glucose and the adrenaline resistance of these dogs. ACE produced the same effects qualitatively but to a much lesser degree; this may be attributed to the smaller quantities of steroid present in the dosage schedule employed. However, in the experiments with cortisone and ACTH, effects of overdosage or oversecretion were observed, namely, 1) resistance to the test dose of insulin, 2) a tendency towards the production of a diabetic postabsorptive blood sugar level and glucose tolerance, and 3) a greater glycemic response to adrenaline than seen in normal dogs.

There is no doubt that the adrenocortical hormones can produce a significant reversal in the many abnormalities of carbohydrate metabolism outlined above. It is most probable, therefore, that the adrenal cortical atrophy of the hypophysectomized dog is of great importance in the production of these defects. The exact part played by the adrenal cortical atrophy in the disturbed carbohydrate metabolism of hypophysectomized animals and, consequently, the exact role of the adrenal cortex in the regulation of normal carbohydrate metabolism cannot be accurately evaluated from the results of the above experiments. This would have been possible only if physiological hormone levels had been obtained by the replacement therapy used in these experiments. In view of this, a comparison of the carbohydrate metabolism of the adrenalectomized dog with that of the hypophysectomized dog may further clarify the importance of the adrenocortical hormones in the regulation of carbohydrate metabolism.

Summary and conclusions. Prolonged administration of ACTH and cortisone diminished and eventually abolished the insulin hypersensitivity of the hypophysectomized dog. Concomitant with these effects, ACTH and cortisone also abolished the secondary hypoglycemia of the intravenous glucose tolerance test and produced a marked blood sugar rise in response to adrenaline. In some animals, however, there appeared some degree of insulin resistance together with a diabetic-type of glucose tolerance and an increased response above normal to adrenaline. It is concluded, therefore, that the adrenal cortical atrophy of the hypophysectomized dog exerts a significant part in the production of the insulin hypersensitivity of this animal. Although the adrenal cortical steroids are potent anti-insulin agents, their exact role as anti-insulin agents cannot be delineated in view of the evidence that abnormal amounts of adrenocortical steroids were present in the above studies.

¶ With regard to the abolition of the insulin hypersensitivity, the adrenocortical steroids may be aided, in part, by the glycogen-mobilizing action of adrenaline secreted by the adrenal medulla of the hypophysectomized dog. In the untreated hypophysectomized dog, the blood sugar-raising effect of intravenously administered adrenaline is minimal (8,9).

1. Thorn, G. W., Koepf, G. F., Lewis, R. A., and Olsen, E. F., *J. Clin. Invest.*, 1940, v19, 813.

2. Grattan, J. F., Jensen, H., and Ingle, D. J., *Am. J. Physiol.*, 1941, v134, 8.

3. Long, C. N. H., *Endocrinology*, 1942, v30, 870.

4. Ingle, D. J., *Ann. New York Acad. Sc.*, 1949, v50, 576.

5. Houssay, B. A., and Magenta, M. A., *Compt. rend. Soc. de biol.*, 1925, v92, 822; 1927, v97, 596; 1929, v102, 429.

6. Slater, I. H., de Bodo, R. C., Weisberg, H. F., and Kiang, S. P., *Fed. Proc.*, 1948, v7, 116.

7. de Bodo, R. C., Kurtz, M., Ancowitz, A., and Kiang, S. P., *Am. J. Physiol.*, 1950, v163, 310.

8. de Bodo, R. C., Bloch, H. I., and Gross, I. H., *Am. J. Physiol.*, 1942, v137, 124.

9. Lane, N., and de Bodo, R. C., *Am. J. Physiol.*, 1952, v168, 1.

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Comparison of Insulin Hypersensitivity of Adrenalectomized and of Hypophysectomized Dogs.*† (19619)

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In a previous publication it was shown that the prolonged administration of ACTH or cortisone abolished the insulin hypersensitivity of the hypophysectomized dog(1). However, as an accompaniment of this effect, a tendency towards diabetes appeared which was strongly suggestive of either excessive adrenal steroid secretion or replacement, respectively, in these animals.

Since physiological hormone levels were not obtained in the above experiments, a comparison of the insulin hypersensitivity of the adrenalectomized animal with that of the hypophysectomized animal was made in order to determine to what extent the adrenal cortical atrophy of the hypophysectomized dog is responsible for its insulin hypersensitivity. A report of these studies is presented in this paper.

Methods. In this investigation 7 trained, unanesthetized, adrenalectomized‡ female dogs were used. All the animals were fed a mixed diet and maintained in good condition with minimal doses (1.5 to 2.5 mg daily) of desoxycorticosterone acetate (DCA) in oil (Schering)‡ administered intramuscularly. All experiments, unless otherwise stated, were done in the postabsorptive state, 17 to 18 hours after the last feeding. The insulin sensitivity of these dogs was determined 7 to 323 days postoperatively and tested with insulin (Lilly),‡ 0.025 u/kg, i.v., (referred to as the test dose), and 0.25 u/kg, i.v. In all the adrenalectomized dogs the sensitivity was tested with both doses of insulin while the animals were maintained on DCA. With the exception of one dog, all the others were then taken off maintenance DCA and tested repeatedly during a DCA-free period (lasting 8 to 14 days), until signs of adrenocortical insufficiency appeared. Some of these dogs were subjected several times to such DCA-free periods. Serum sodium concentration (measured on an internal standard flame photometer) was determined while each animal was on DCA and then was followed during the DCA-free period. No remedial measures were undertaken to alleviate the signs of severe adrenocortical insufficiency until the last insulin sensitivity experiment had been performed. Only then were the animals given adequate intravenous infusions of dextrose and saline several times daily, in addition to intravenous injections of adrenal cortical extract and intramuscular injections of large doses of cortisone as well as the maintenance dose of DCA. The insulin sensitivity of the hypophysectomized dogs used as a basis for comparison in this paper was compiled from a series of 38 tests

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