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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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TABLE I. Blood Sugar Changes Produced by Insulin (Test Dose) in Adrenalectomized (Adrex) Dog A-8 Compared with Normal and Hypophysectomized (Hyphex) Dogs.

		Adrex A-8			
		On	Off DCA		
	Normal*	DCA	6 days	9 days	Hyphex†
		Serum Na in mEq.			
		156	144	116	
Time, min		Blood sugar in mg %			
0	78	84	79	81	66
Insulin .025 u/kg given intrav. at 0 time					
10	67	77	71	68	53
20	66	75	71	65	38
30	74	75	77	73	36
40	75	80	78	73	35
50	77	81	75	73	36
60		83	76	79	37
75		81	78	78	38
90		79	78	76	42
120		85	77	76	47
150		83	74	73	51
180		84	73	76	54
210		83	75	73	56
240		79	74		58

* Values obtained from 37 experiments on 18 normal dogs.

† Values obtained from 38 experiments on 12 hypophysectomized dogs.

TABLE II. Blood Sugar Changes Produced by Insulin (Test Dose) in Adrenalectomized (Adrex) Dog A-2 Compared with Normal and Hypophysectomized (Hyphex) Dogs.

		Adrex A-2			
		On	Off DCA		
	Normal*	DCA	3 days	6 days	Hyphex†
		Serum Na in mEq.			
		141	—	127	
Time, min		Blood sugar in mg %			
0	78	69	65	70	66
Insulin .025 u/kg given intrav. at 0 time					
10	67	55	52	40	53
20	66	42	43	38	38
30	74	52	44	40	36
40	75	47	47	44	35
50	77	43	48	42	36
60		47	48	46	37
75		49	54	51	38
90		52	56	55	42
120			58	53	47
150			59	51	51
180			63	56	54
210			59	51	56
240			61	53	58

* Values obtained from 37 experiments on 18 normal dogs.

† Values obtained from 38 experiments on 12 hypophysectomized dogs.

performed on 12 animals and reported in other publications. This group of animals includes only those dogs which were found to be "completely" hypophysectomized upon histological examination of the pituitary region utilizing a serial section technic reported elsewhere(2).

Results. I. *Response to the test dose of insulin (0.025 u/kg, i.v.).* Table I records the experimental data obtained on dog A-8, which is representative of a group of 5 of the 7 adrenalectomized animals studied. Table II records the results of the insulin response of dog A-2, which is typical of those exhibited by the 2 remaining dogs in this series. As seen in Table I, dog A-8 exhibited an insulin response similar to that seen in normal dogs. This was true of the animal's response while on DCA maintenance, or even without DCA maintenance for a period of 9 days, although the animal developed signs of adrenal insufficiency, *viz.*, anorexia, weakness, apathy, and had a progressively declining serum sodium level. These results are in striking contrast to the marked insulin sensitivity of the hypophysectomized dogs recorded in Table I. The insulin sensitivity of dog A-8 and of the other 4 dogs in this group increased markedly only when their adrenal insufficiency became severe enough to cause the animals to vomit even force-fed food; thus, the insulin response obtained was that of animals fasted more than 40 hours.

Dog A-2 (Table II), on the other hand, showed an insulin response greater than that of normal dogs but less than that of hypophysectomized dogs. Here, too, the response obtained during a test period of 8 days without DCA maintenance showed little change from that obtained while the dog was on DCA maintenance. This was true, although the dog began to exhibit signs of adrenal insufficiency and had a lowering of the serum sodium level. Once again, the onset of vomiting, signifying a more severe state of insufficiency, introduced prolonged fasting (40 hours or more) into the experimental conditions, and the insulin response of the animal increased over that seen in all the experiments performed in the postabsorptive state (17 to 18 hours fasted).

Fig. 1 is a graphic presentation of the insulin responses to the test dose of normal and hypophysectomized dogs compared with the composite response obtained on the 7 ad-

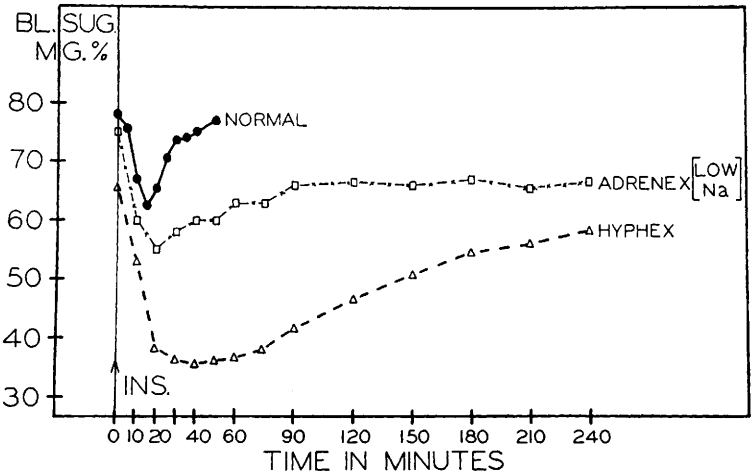

FIG. 1. Composite blood sugar curves produced by insulin (.025 u/kg i.v.) in normal, hypophysectomized (Hyphex) and adrenalectomized (Adrenex) dogs.

renalectomized dogs during DCA-free periods, when all 7 animals exhibited decreases in their serum sodium concentrations which were significantly below normal.

II. *Response to 10X test dose of insulin (0.25 u/kg, i.v.).* Since 5 of the 7 adrenalectomized dogs exhibited an insulin response to the test dose similar to that of normal dogs, the response to a larger dose of insulin (10X the test dose = 0.25 u/kg, i.v.) was examined.

TABLE III. Blood Sugar Changes Produced by Insulin (10 × Test Dose) in Normal Dogs and Those Adrenalectomized (Adrex) Dogs Not Sensitive to Test Dose of Insulin.

Time, min	Normal*	Adrex dogs A-8‡	Adrex dogs Comp.†‡
	Blood sugar in mg %		
0	78	70	74
Insulin .25 u/kg given intrav. at 0 time			
10	61	57	60
20	46	36	38
30	—	24	31
40	45	24	25
50	—	21§	29
60	52		32
75	—		36
90	66		48
120	76		54
150			55
180			62
210			61
240			64

* Values obtained from 17 exp. on 8 normal dogs.
† " " " experiments on 4 adrenalectomized dogs.
‡ Dose of DCA: 2-2.5 mg i.m. daily.
§ Convulsions; experiments discontinued.

Table III records the response to 10X the test dose of insulin of dog A-8, one of these 5 adrenalectomized dogs. Included, too, in Table III is the composite response of the remaining 4 dogs which were similar to A-8 in their response to the test dose of insulin. These are then contrasted with the response of normal dogs to 10X the test dose. As can be seen, all the adrenalectomized animals manifested a greater sensitivity than normal dogs. The hypophysectomized dog's response to the larger dose of insulin is not recorded, since the response to the test dose, 0.025 u/kg, is so marked and at times accompanied by convulsions that this consideration militates against the use of 10X the test dose.

Discussion. It is evident from the work presented that the adrenalectomized dog is more sensitive to insulin than the normal dog but less sensitive than the hypophysectomized dog. The experiments with 0.25 u/kg (10X the test dose) clearly demonstrate the difference in insulin response of normal and adrenalectomized dogs, whereas the experiments with the test dose (0.025 u/kg) reveal the differences obtained in the insulin response of normal, hypophysectomized and adrenalectomized dogs. Most of the adrenalectomized dogs had insulin responses with the test dose similar to those of normal dogs, whereas 2 reacted to insulin to a greater degree than do normal animals. However, all the adrenalec-

tomized dogs were less sensitive to insulin than the hypophysectomized dogs, even when the former were without DCA maintenance for 8 to 14 days and showed evidence of being steroid-free by virtue of their low serum sodium level. The fact that the adrenalectomized animal in the pre-mortal state, lacking the ability to retain food and thereby fasted for more than 40 hours, has a marked increase in insulin sensitivity does not contribute to the evaluation of this particular problem since comparison of the insulin response is being made to the postabsorptive hypophysectomized animal.

These results are not in contradiction with the earlier studies of other investigators who demonstrated the insulin sensitivity of adrenalectomized animals. Rather, these experiments can be considered as an extension of those studies in order to evaluate more accurately the degree of sensitivity of the adrenalectomized dog, utilizing a more quantitative approach to the problem. Grattan, Jensen, and Ingle(3) obtained convulsions in adrenalectomized mice with doses of insulin (0.4 u/kg) which were much larger than our test dose; no smaller dose of insulin was used in their study. This is true, too, of other workers (Zucker and Berg(4)), who studied the insulin sensitivity of the adrenalectomized dog but, again, used doses which were larger than the test dose employed in the current study.

The insulin sensitivity of the adrenalectomized dog is not entirely due to the lack of the adrenal cortical hormones, since it lacks adrenaline, too, which exerts an anti-insulin action although this is not as potent as that of the corticosteroids. The hypophysectomized dog has a diminished secretion of adrenocortical steroids due to the adrenal cortical atrophy, and furthermore, the adrenaline from its adrenal medulla, if secreted, exerts little, if any, blood sugar raising action(2,7). Undoubtedly, the adrenal cortical steroids are effective anti-insulin agents in the hypophysectomized dog as seen from the previously reported work on the effects of ACTH and cortisone on the insulin hypersensitivity of the hypophysectomized dog(1). Nevertheless, the adrenal cortical atrophy of the hypophysectomized dog cannot wholly explain its marked hypersensitivity. It follows, therefore, that in the production of the insulin hypersensitivity of the hypophysectomized animal, the absence of anti-insulin agents of pituitary origin must be implicated. In view of previous work demonstrating the anti-insulin and diabetogenic effects of growth hormone (Armour) in the hypophysectomized dog(5), it would appear that growth hormone is the absent anterior pituitary anti-insulin agent in these animals. In more recent work, however, a growth hormone preparation without any diabetogenic effect seems to have been isolated(6). Whether the anterior pituitary hormone exerting the marked anti-insulin effect in the hypophysectomized dog is the growth-promoting factor itself or another anterior pituitary factor intimately associated with it is of no consequence to this discussion. The fact remains that there is an anterior pituitary factor (in addition to ACTH) that is an effective anti-insulin agent and the lack of this agent is important in the production of the marked insulin hypersensitivity of the hypophysectomized dog.§

Summary and conclusions. The adrenalectomized dog is more sensitive to insulin than the normal dog but less sensitive than the hypophysectomized dog. This is true so long as the adrenalectomized dog, whether with or without DCA maintenance, is still well-nourished and tested only in the post-absorptive state. It is concluded that the adrenal cortical atrophy is an important factor in the production of the insulin hypersensitivity of the hypophysectomized dog. However, absence of an anterior pituitary factor (other than ACTH) is also of great importance in the production of this metabolic abnormality.

§ It is possible that an over-secretion of this anterior pituitary factor (growth hormone?) accompanies the over-secretion of ACTH in the adrenalectomized animal and exerts an anti-insulin action. However, the glucose tolerance of the adrenalectomized dog does not reveal any evidence for the over-secretion of such a factor.

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Effects of C_{17}-Hydroxy-Steroids on Urinary 17-Ketosteroids in the Rat.* (19620)

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Because all the urinary 17-ketosteroids (17-KS) of females and some part of those excreted by males originate in the adrenal cortex, their determination is one of the means of evaluating adrenocortical function. The association of 17-KS content with androgenic properties, the presence of adrenosterone in the adrenal cortex and the masculinization elicited by some forms of adrenal cortical hyperfunction have supported the concept of a specific androgenic secretion of the adrenal cortex. However, recent evidence(1-6) indicates that 17-KS are formed and excreted during metabolism of corticosteroids, particularly of those hydroxylated at C 17. Relevant studies in human subjects are scanty. Those here reported were undertaken in rats, in which relatively large doses of potential 17-KS precursors could be readily administered.

Methods. Female albino rats (Sprague-Dawley) of 150-250 g body weight were separated into 7 groups, 5 experimental and 2 control; each group contained at least 4 rats. The animals were placed separately in metabolism cages for a period of 13 days; in order to accustom the animals to this environment, 4 days elapsed before real or simulated treatment was begun; during the treatment period, 10 mg of each steroid tested was given by stomach tube daily for 5 days; 4 days were allowed for post-treatment observation. Drinking water was substituted for with 1% NaCl in order to obtain a diuresis; commercial fox chow was given *ad libitum*. A few drops of Merthiolate solution were placed in the collection bottles; the urine was collected and measured daily. The steroids administered were as follows: 11-desoxycorticosterone acetate (DCA); 17-hydroxy-11-desoxycorticosterone acetate (compound S); progesterone; 17-hydroxyprogesterone and Δ^4-pregnene-17 α, 20, 21-triol-3-one diacetate (pregnenetrio-

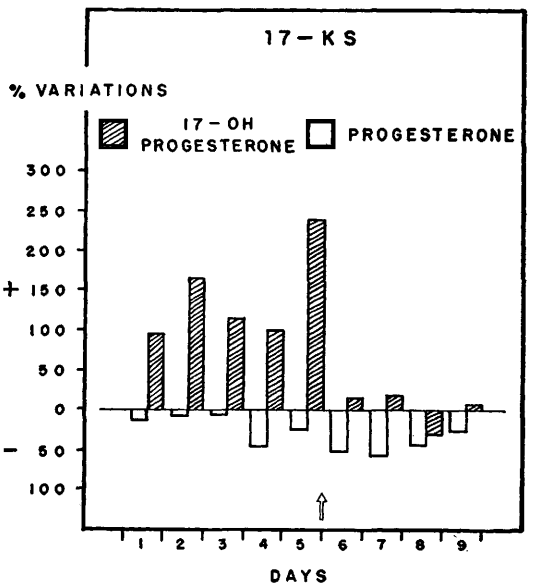

FIG. 1. % variations from control mean of urinary 17-ketosteroids following administration of progesterone and 17-hydroxyprogesterone. Arrow indicates end of treatment.

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