genic mechanism in autoregulation of renal blood flow.

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Plasma Free Fatty Acids after 2-Deoxy-D-Glucose in Intact, Adrenalectomized, Spinal and Hypophysectomized Rat.* (29503)

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Administration of 2-Deoxy-D-Glucose (2-DG) is followed by marked hyperglycemia in rat(1), dog(2) and man(3) and also a striking increase in secretion of adrenaline and noradrenaline from the adrenal medulla (4). The primary mechanism underlying these effects seems to be inhibition of intracellular glucose utilization(1). It is probable that 2-DG induced cellular glucopenia within the central nervous system, which triggers the release of catecholamines, is largely responsible for the hyperglycemia(4). Since sympathomimetic amines as well as glucose influence the release of free fatty acids (FFA) both *in vivo* and *in vitro* (see 5 for review), we investigated the effect of 2-DG on plasma FFA and its relation to the release of adrenal medullary hormones. In addition, studies were performed to explore the role of the adrenal cortex and the pituitary in this connection.

Materials and methods. Inbred, albino rats of both sexes, 200-300 g, maintained on a standard diet, were used. All experiments were started AM, at which time food was removed but water allowed *ad libitum.* Adrenalectomy was performed *via* 2 lumbar incisions 3 days before administration of 2-DG and the rats were maintained on 0.9% saline.. Parapharyngeal hypophysectomy was performed 6 days prior to 2-DG and adrenal denervation by spinal cord transsection at level C-7, about one hour before an experiment. Ether anesthesia was used for all operations. 2-DG (Aldrich Chemical Co., Milwaukee, Wisc.) was obtained through courtesy of Cancer Chemotherapy National Service Center, NIH, Bethesda. In all instances 2-DG (100 mg/100 g b.w.) was administered subcutaneously (s.c.) in water solution (100

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TABLE I. Changes in Plasma Level of FFA Following Subcutaneous Administration of 2-DG (100 mg/100 g Body Wt) in the Intact, Adrenalectomized and Spinal Rat.

	Intact		Adrenalectomy saline		Adrenalectomy cortisone		Transsection of spinal cord	
	No. rats	FFA, μEq/l*	No. rats	FFA, μEq/l	No. rats	FFA, μEq/l	No. rats	FFA, μEq/l
Control: 0 hr	19	377 ± 24	11	458 ± 41	9	418 ± 43		
2-DG: ¼ hr	9	676 ± 47						
Control: ½ hr							5	327 ± 35
2-DG: ½ hr	15	737 ± 34	7	302 ± 28	5	297 ± 25	5	317 ± 62
Control: 1 hr	6	389 ± 25					7	373 ± 31
2-DG: 1 hr	6	634 ± 26	7	235 ± 29	6	279 ± 28	7	199 ± 11
Control: 2 hr	5	409 ± 39	6	444 ± 37	5	433 ± 34	5	435 ±114
2-DG: 2 hr	11	564 ±116	7	241 ± 20	9	351 ± 23	7	161 ± 12
2-DG: 4 hr	10	496 ± 54	6	239 ± 33	6	224 ± 22		
Control: 6 hr	9	447 ± 27	4	427 ± 40	4	456 ± 71		
2-DG: 6 hr	11	583 ± 48	7	233 ± 25	5	214 ± 32		
Control: 11 hr	6	781 ± 64	5	542 ± 18	7	501 ± 23		
2-DG: 11 hr	6	743 ± 23	6	334 ± 37	7	356 ± 32		

* ± S.E. of mean.

mg/ml). In control animals a corresponding quantity of distilled water was injected. Cortisone acetate (1 mg) was given s.c. once daily. At various times after 2-DG the rats were decapitated and bled into heparinized beakers. Plasma was analyzed for FFA(6) and corticosterone (fluorometric technique, 7). Glucose concentrations in tail vein blood were determined colorimetrically (condensation with O-toluidine, 8). Adrenaline and noradrenaline in urine and adrenals were estimated fluorometrically(9), as described previously(4).

Results. 2-DG induced elevation in FFA in intact rats (Table I), with a maximum increase of 360 μEq/l at 30 minutes, then a gradual return to control levels by 11 hours. In intact fasted controls, FFA rose continuously, in accord with earlier observations (10). In adrenalectomized rats on saline, instead of an increase, 2-DG evoked a decrease in FFA of 220 μEq/l, with minimum one hour after injection, and no return toward control levels at 11 hours. Adrenalectomized rats on cortisone for 3 days prior to the experiment in general showed the same response as adrenalectomized rats on saline. Adrenal denervation was about equivalent to adrenalectomy. In hypophysectomized rats (without cortisone or on cortisone for 6 days), 2-DG had no effect on FFA (Table II). Blood glucose, on the other hand, increased after 2-DG in hypophysectomized rats without cortisone, although the response was less than that in intact rats(4); cortisone pretreatment of hypophysectomized rats normalized blood glucose response to 2-DG. Lack of FFA increase after 2-DG in hypophysectomized rats was not due to inability to increase catecholamine secretion, as indicated by marked elevation of urinary adrenaline and noradrenaline, concomitant with depletion of medullary stores (Table III). Adrenalectomy as such caused elevation of FFA as compared to normal controls (Table I), whereas hypophysectomy resulted in decreased FFA (Table II). Cortisone normalized FFA levels in both adrenalectomized and hypophysectomized control rats. In intact rats the rise in plasma FFA after 2-DG was accompanied by a rise in plasma corticosterone (Table IV). In adrenalectomized or hypophysectomized rats, there was practically no measurable plasma corticosterone before or after 2-DG (Tables II and IV). After spinal cord transsection, corticosterone was high in both control and 2-DG treated rats. Thus, there was no correlation between plasma corticosterone and plasma FFA levels.

Discussion. The present studies confirm that 2-DG causes elevation of plasma FFA in the intact organism(11). As this response

is not seen either in cortisone treated adrenalectomized or adrenal denervated rats, it seems to depend on release of adrenal medullary hormones. This concept is supported by the fact that 2-DG evokes increased catecholamine secretion in the intact rat but not

TABLE II. Changes in Plasma FFA and Blood Glucose Following Subcutaneous Injection of 2-DG (100 mg/100 g Body Wt) in Untreated and Cortisone Injected, Hypophysectomized Rat. Levels of corticosterone in peripheral plasma are also given.

Treatment	No. rats	FFA, μEq/l	Blood glucose, mg %*	Corticosterone, μg/100 ml†
Hypophysectomized, untreated				
Control: 0 hr	9	259 ± 29	69 ± 7	8–11
2-DG: ½ hr	9	244 ± 30	137 ± 6	
2-DG: 1 hr	9	256 ± 38	169 ± 6	
Control: 2 hr	7	419 ± 52	49 ± 3	
2-DG: 2 hr	7	362 ± 41	218 ± 7	8– 9
Hypophysectomized, 1 mg cortisone acetate/day				
Control: 1 hr	6	336 ± 38	76 ± 2	8–10
2-DG: 1 hr	6	346 ± 26	238 ± 5	7–10

* S.E. of mean. † Range.

TABLE III. Effect of Subcutaneous Administration of 2-DG (100 mg/100 g Body Wt) on Catecholamines in Urine, Collected for 24 Hours, and Adrenals 24 Hours After Injection in Hypophysectomized Rat.

		Adrenals		Urine	
	No. rats*	Noradrenaline, μg/kg b.w.	Adrenaline, μg/kg b.w.	Noradrenaline, ng/kg/hr†	Adrenaline, ng/kg/hr
Hypophysectomized, controls	4	38 ± 4.2‡	132 ± 9.4	148 ± 6.2	46 ± 6.3
Hypophysectomized, 2-DG treated	5	27 ± 3.2	17 ± 2.4	226 ± 25.8	480 ± 62.8

* Both adrenals analyzed together. † ng = .001 μg. ‡ S.E. of mean.

TABLE IV. Concentration of Corticosterone in Peripheral Plasma at Various Times Following Subcutaneous Administration of 2-DG (100 mg/100 g Body Wt) in Intact, Adrenalectomized and Spinal Rat.

	Intact		Adrenalectomy saline		Transsection of spinal cord	
Treatment	No. rats	Corticosterone, μg/100 ml*	No. rats	Corticosterone, μg/100 ml	No. rats	Corticosterone, μg/100 ml
Control: 0 hr	13	30 ± 3.3	11	6 ± .5		
2-DG: ¼ hr	6	64 ±11.1				
Control: ½ hr					6	51 ± 3.9
2-DG: ½ hr	9	44 ± 2.1	7	4 ± .3	5	41 ± 2.6
Control: 1 hr					7	39 ± 2.1
2-DG: 1 hr					7	40 ± 2.0
Control: 2 hr					7	38 ± 1.7
2-DG: 2 hr	8	48 ± .4	7	5 ±1.0	7	38 ± 1.7
2-DG: 4 hr	7	49 ± 2.6	6	6 ± .5		
Control: 6 hr	6	27 ± .9	4	7 ± .6		
2-DG: 6 hr	9	63 ± 3.1	7	7 ±1.0		
Control: 11 hr	6	25 ± 3.0				
2-DG: 11 hr	6	24 ± 3.9				

* ± S.E. of mean.

in the adrenal denervated animal(4). In hypophysectomized animals, 2-DG induced catecholamine release but no increase in FFA. This confirms that some pituitary factor (or factors) is necessary for the lipolytic action of catecholamines(12). Our experiments do not reveal the nature of this pituitary factor(s), but do show that the pituitary-adrenocortical system is either of no importance or requires some additional pituitary factor. In view of earlier findings(13, 14) it seems likely that lack of thyroid hormone(s) is responsible for the altered response to 2-DG in the hypophysectomized rat.

The reason for the decreased FFA after 2-DG in the adrenalectomized, cortisone treated rat is not clear. A possible explanation would be the increased insulin release occurring after 2-DG(15); furthermore, injection of insulin has been found to reverse the effect of 2-DG on FFA in humans(11). Another alternative would be that 2-DG as such affects specific reactions involved in FFA metabolism within the adipose tissue or elsewhere and that this effect of 2-DG *in vivo* becomes evident only in the absence of the adrenal medulla.

Summary. Plasma free fatty acids (FFA) were followed in the rat after injection of 2-deoxy-D-glucose (2-DG). In the intact animal 2-DG caused elevation of FFA, probably due to release of adrenal medullary hormones. In the hypophysectomized rat there was no change in FFA after 2-DG, in spite of pronounced secretion of catecholamines and independent of cortisone substitution. In the adrenalectomized rat 2-DG caused a marked decrease of FFA, likewise independent of cortisone medication.

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Connective Tissue XII. Stimulating Effects of Estrogens on Collagen Synthesis in Rat Uterine Slices.* (29504)

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The uterotrophic effect of estrogen and other steroids(1-6) has been ascribed partly to water inhibition(1,2,7) and partly to uterine growth(3,7). While studying the nature of these changes, Harkness and Harkness (8) and Woessner(9) observed in rats and humans, respectively, that during pregnancy, total uterine collagen increased steadily.

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