substance LRF is not active in hypophysectomized animals in conditions in which the sensitivity to LH is known to be adequate (9). Similarly we have shown earlier that acid extracts of the brain cortex or of the cerebellum, *i.e.*, control tissues not containing hypothalamus, do not stimulate endogenous secretion of LH(2). Thus the results observed here with the most specific criteria for evidence of secretion of LH (*i.e.*, ovulation and formation of corpora lutea) and in animals with a hypothalamic lesion blocking spontaneous release of LH, confirm our earlier conclusions based on the ovarian ascorbic acid depletion test, which had led us to conclude that we had purified in hypothalamic extracts a substance stimulating the secretion of luteinizing hormone.

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Failure of Cortisol to Reduce Glucose Uptake in Granuloma Tissue.* (28697)

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Inhibitory effects of corticoids on glucose uptake have been reported for several tissues, including rat granuloma pouch(1), rat epididymal fat pad(1,2), lymph nodes, thymus, lymphosarcoma(3), rat diaphragm muscle(4, 5,6), mouse ear skin(7), and human white blood cells(8). These studies have served to renew interest in the view that corticoid action at a cellular level may involve inhibition of glucose metabolism(9,10).

Since formation of granuloma tissue, as stimulated by foreign body implants, is inhibited by adrenocortical hormones(11), this tissue is especially suitable for examination of this hypothesis. Granuloma tissue, as used here, provides a conveniently excised sample of connective tissue elements, consisting of two principal components: a thick, well-organized, fibrillar outer zone, containing fibroblasts, monocytes, and giant cells; and a central zone of acute inflammation(12). Growth of the tissue, *in vivo*, is influenced by insulin (14), and growth hormone(15), as well as by corticoids(11,12,15,16). Accordingly, studies were undertaken to determine the effects of cortisol upon glucose uptake in granuloma tissue formed by subcutaneous implantation of cotton pellets. The results indicate that cortisol, whether administered *in vivo* or *in vitro*, does not decrease glucose uptake.

Methods. Male rats of the Sprague-Dawley strain (Charles River) weighing 135 to 170 g were adrenalectomized, at which time cotton pellets were inserted into subcutaneous tissue at sites about 1 inch lateral to the incision. Two pellets of unbleached cotton (Pride, Lockport Mills) weighing 6 mg each were implanted per rat. Saline (0.9%) was supplied for drinking water. Groups of rats were injected with medium or cortisol on the 6th day or 7th day after implantation as noted below and in Table I. Granulomas were excised on the 7th day after implantation and incubated in 2 ml of one of the following media: (a) Krebs-Ringer bicarbonate (KRB); (b) Krebs-Ringer phosphate ($KRPO_4$); (c) Eagles' L-amino acid medium supplemented with glutamine, and bicarbon-

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TABLE I. *In vitro* Glucose Uptake of Cotton Granuloma Under Various Cortisol Treatments in Several Media.

Exp No.	Medium	Interval (hr)	Cortisol*	Glucose uptake†		
				mg glucose per total granuloma	μg glucose per mg wet granuloma	μg glucose per mg granuloma protein
I	KRB		0	.61 ± .10 (7)	2.6 ± .4	
		2	+	.58 ± .07 (7)	2.7 ± .5	
II	"		0	.73 ± .13 (6)	4.1 ± .4	
		2	+	.73 ± .11 (6)	3.6 ± .6	
III	"		0	.47 ± .03 (11)	3.9 ± .2	68 ± 5
		24	+	.41 ± .02 (12)	4.0 ± .3	78 ± 6
IV	"		0	.54 ± .05 (8)	3.4 ± .2	63 ± 5
		24	+	.43 ± .03 (11)	3.9 ± .3	82 ± 3
V	"		0	.46 ± .06 (6)	4.6 ± .7	81 ± 1
		24	+	.47 ± .05 (6)	5.2 ± .7	83 ± 1
VI	$KRPO_4$	—	0	.30 ± .02 (8)	1.7 ± .1	75 ± 7
		2	+	.29 ± .01 (5)	1.6 ± .1	65 ± 3
		24	+	.30 ± .01 (5)	2.1 ± .1	79 ± 3
		Insulin	0	.31 ± .04 (8)	1.8 ± .1	67 ± 3
VII	Eagle L	—	0	.69 ± .07 (9)	3.5 ± .2	77 ± 1
		5	+	.69 ± .10 (6)	3.6 ± .2	97 ± 1
		24	+	.66 ± .02 (8)	4.9 ± .2	121 ±12
VIII	Plasma		0	.58 ± .02 (9)	4.2 ± .2	129 ± 5
		24	+	.54 ± .03 (9)	6.2 ± .4	176 ±11
IX	"		0	.54 ± .07 (6)	5.3 ± .8	83 ±14
		24	+	.61 ± .04 (6)	6.5 ± .8	92 ±10
X	KRB	Cortisol *in vitro*	0	.44 ± .05 (8)	3.1 ± .3	88 ±10
			+	.48 ± .06 (7)	3.1 ± .3	96 ±11

* Cortisol: Exp 1 & II, 3 mg/rat given intraperitoneally 2 hr before sacrifice; III-IX, 4.8 mg/rat given subcutaneously in 3 equally divided doses spaced throughout the day preceding sacrifice, in VI & VII, the 2 or 5 hr treatments, 4.8 mg in one injection; X, 72 μg dried in vessel before medium added. Controls received equivalent injections of vehicle without cortisol. In VI, 0.3 U/ml insulin.

† Mean, standard error, and No. of observations. Glucose uptake is for 3 hr period of incubation, 37° using 2 ml medium, KRB gassed with O_2:CO_2 (95:5%), $KRPO_4$ with O_2.

ate(17); or (d) rat plasma from either control or cortisol-treated rats obtained immediately from blood drawn into heparin under avertin anesthesia and diluted with one part Krebs-Ringer bicarbonate per 7 parts of plasma. Rats donating plasma had been adrenalectomized and pretreated with or without cortisol in the same manner as those donating granulomas. In Experiments I through IX, cortisol (Merck microfine) was suspended in Steroid Diluent (National Service Center 17874), and injected either intraperitoneally, or subcutaneously at sites distant from the pellet implants. Dosage is given in Table I. In Exp. X, cortisol was added *in vitro* by depositing a thin layer of hormone (72 μg) in dried state in the incubation vessel before addition of medium. In VI, 0.3 U/ml of glucagon-free insulin (Lilly #499667) was added to the incubation medium of a group of otherwise untreated granulomas. Initial glucose concentration was 1 mg/ml for KRB, $KRPO_4$, Eagles' L-media, and 1.5 mg/ml for the experiments with plasma. Glucose was determined on the media after 3 hours' incubation by the direct glucose oxidase-peroxidase method(18). Protein determinations were by the Lowry *et al* method(19) on an alkaline digest of defatted, hot TCA-washed tissue. Deoxyribonucleic acid (DNA) was determined by the method of Webb and Levy (20) on granulomas from control or cortisol-treated rats which were processed according to recommendations comprehensively described by Hutchinson and Munro(21).

Results. Table I shows the lack of effect

TABLE II. Effect of Cortisol* Treatment on Wet Weight, Protein, and DNA Content of Total Granuloma.

	Controls	Cortisol-treated	Δ
Wet weight (mg/granuloma)	129 ± 5 (12)	100 ± 3 (10)	22%
Protein (mg/granuloma)	6.3 ± .3 (12)	5.5 ± .1 (10)	13%
DNA (μg/granuloma)	527 ±23 (12)	439 ±23 (10)	17%

* Condition of rats and schedule of cortisol administration identical to 24 hr treatment of Table I.

of cortisol administered under the various conditions described. The glucose data is expressed in terms of absolute uptake per total granuloma, and also as uptake per mg granuloma weight or per mg granuloma protein. The rate of glucose utilization found for the 7-day granuloma is roughly 2-4 times that of the fat pad, about equal to mouse skin, and half or less that of diaphragm. Cortisol did not reduce glucose uptake under any of the conditions examined. No change is observed whether the hormone is administered *in vivo* 2 or 5 hours before excision (Exp. I, II, VI, and VII), over a longer period beginning 24 hours prior to sacrifice (III to IX), or added *in vitro* (X). Use of $KRPO_4$ (VI), a supplemented media (VII), or of plasma from cortisol-treated rats (VIII and IX) likewise failed to reveal a significant inhibitory effect of cortisol. A tendency for the absolute uptake of glucose per total granuloma to decline with cortisol treatment beginning 24 hours before excision is related to a reduction in wet weight and protein content of the granuloma. When results of these experiments are expressed in terms of glucose used per unit wet weight or protein, cortisol appears to increase rather than inhibit glucose uptake. To assess this relationship further the effect of cortisol on cell content, as indicated by DNA, was determined on control and cortisol-treated granulomas. Table II shows that cortisol treatment reduces the DNA content of the granuloma an average of 17%, which is about equal to the average decline of 13% in protein content. Presumably then, since these two parameters decline in a similar fashion, the results indicate that cortisol may actually cause an increase in glucose uptake per unit mass of cells in VII and VIII ($P = < .01$, in both experiments). However, since this apparent stimulation is not uniformly observed, it can only be concluded at this time that an inhibition by cortisol does not occur in this tissue.

Insulin, *in vitro,* in amounts effective in diaphragm(24), had no effect on the glucose uptake of the granuloma (VI).

Discussion. The present work indicates that, under the conditions studied, corticoid does not reduce glucose uptake by cells of the granuloma, and no evidence was found to suggest that cortisol inhibits granuloma growth by reducing local utilization of glucose. Thus, this study does not support the concept that corticoid action upon responsive cells involves an inhibitory effect on glucose metabolism despite the fact that in certain systems inhibitory effects have been obtained. While glucose uptake in fat pad, diaphragm, and skin is reduced under corticoid influence, granuloma tissue is of special interest, since it is considered to be a particular target of these hormones. Details on the studies of Glenn *et al*(1) reporting that corticoid inhibits glucose uptake in a Tocopherol granuloma are not yet available; hence these studies cannot be compared. Perhaps it is not to be expected that *all* tissues will respond to corticoids in a similar fashion, but rather that certain specific tissues might benefit from the elevated blood glucose resulting from corticoid-induced gluconeogenesis, and also possibly, in part from the asceticism of other tissues.

In any case, one cannot yet generalize on the information available, since a significant lack of agreement exists in reported findings, which may be related to experimental conditions employed. Herman & Ramey report that glucose uptake by rat diaphragm is inhibited by cortisol in Krebs-Ringer-phosphate medium, but not in Krebs-Ringer-bicarbonate(4,22). Although many of these studies

reporting inhibition by corticoid used a phosphate medium(3,4,5,6,7,8) variability in reported results cannot be accounted for solely on this basis. In the present work, moreover, no inhibition by corticoid was seen in either KRB or $KRPO_4$, or in a more physiological medium, rat plasma.

Several studies suggest a role of insulin in the effect of corticoid to reduce glucose uptake in other tissues studied. Correa *et al* (23) using fat pad found an inhibition by cortisone of the stimulatory effect of insulin. Manchester *et al* report a similar effect in diaphragm(24), while Munck(2), and Jeanrenaud and Renold(25) achieved inhibitory effects with corticoid in fat pad without insulin. Other workers suggest involvement of a blood factor(26,27). Insulin had no effect on glucose uptake by granuloma tissue under the conditions tested in the present study.

It is noteworthy, considering variability in reported results, that corticoids have a lipolytic action(1,25,29,30) and that Verner *et al* report that both inhibitory and stimulatory effects of certain lipolytic hormones may be obtained on glucose uptake of the fat pad depending on the level of protein added to the medium(28). Several factors require further study before a definitive conclusion regarding the effect of corticoid on glucose uptake in the total organism is resolved.

Summary. The influence of cortisol on glucose uptake by rat granuloma tissue was examined in various media. No inhibitory effects were observed, whether the hormone was administered to the granuloma donors 2 or 24 hours before sacrifice, or was added *in vitro*. No support was obtained for the concept that corticoids may retard granuloma growth by inhibiting glucose uptake.

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