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Metabolic Effects of Actinomycin in Rats.* (32579)

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Largely because of its rather specific ability to block the synthesis of messenger RNA (1,2), the antibiotic, actinomycin D has been used widely by endocrinologists seeking to determine the modes of action of various hormones. Experiments in this laboratory revealed that the late, inhibitory effects of growth hormone on carbohydrate utilization in adipose tissue were blocked by pretreatment of the rats with actinomycin D(3). Further experiments initiated to determine what other effects of growth hormone might also be blocked by treatment with actinomycin D failed to yield useful information as far as growth hormone was concerned, but provided intriguing observations on the effects of actinomycin. For these studies, fasting rats were used, and it was found that actinomycin reversed many of the metabolic changes caused by fasting.

Materials and methods. Experiments were conducted using hypophysectomized rats (120 g) obtained from the Charles River Breeding Laboratories. They were fed a high carbohydrate, fat-free diet‡ until studied 2 weeks post-operatively. Fasting rats were deprived of food 18-24 hours before sacrifice. Actinomycin D§ (50 µg/rat) was injected intraperitoneally at the beginning of the fasting period. No attempt was made to study fed rats after actinomycin treatment because these animals spontaneously stopped eating several

hours after injection, at which time the gastrointestinal tract was quite hyperemic and distended with fluid.

The rats were anesthetized with pentobarbital and blood was drawn from the abdominal aorta into heparinized syringes. The hearts and samples of liver were rapidly excised, weighed and digested in hot 30% potassium hydroxide. The diaphragms were dissected free of the ribs, soaked for 3 minutes in cold Krebs-Ringer bicarbonate buffer containing 1 mg/ml of glucose and then incubated for 2 hours at 37° in Krebs-Ringer bicarbonate buffer containing 1 mg/ml of glucose. Epididymal adipose tissue was excised and a sample immediately placed in Dole's extraction mixture(4) for determination of initial content of free fatty acids (FFA). The remaining fat was dissected into several segments and incubated as previously described (5) with randomly labeled glucose for the determination of incorporation of carbon-14 into long chain fatty acids and CO₂. To measure lipolysis, adipose tissue segments were incubated in bicarbonate buffer to which was added 1 mg/ml of glucose and 40 mg/ml of bovine serum albumin (Fraction V, Armour). Chemical analyses were made as described in previous publications(5-8).

Results and discussion. When the animals were deprived of dietary glucose, the concentration of glucose in the blood fell significantly below the fed level (Table I). The decline in blood glucose was less precipitous, but nevertheless present when actinomycin was given at the start of the fasting period. Even in the absence of the pituitary gland the concentration of FFA in the plasma increased more than twofold, in agreement with our earlier conclusions that the pituitary gland is not required for the mobilization of fatty acids

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TABLE I. Effects of Fasting and Actinomycin Treatment on Various Aspects of Carbohydrate and Lipid Metabolism in Hypophysectomized Rats.

	Fed	Fasting	Fasting + actinomycin
Blood glucose (mg/100 ml)	122.4 ± 3.1 *	50.4 ± 2.3 †	75.5 ± 9.3 ††
Plasma FFA (mEq/l)	0.34 ± 0.02	0.72 ± 0.04†	0.48 ± 0.04††
Hepatic glycogen (mg/g)	62.4 ± 5.2	25.8 ± 2.0 †	50.4 ± 3.1 †
Cardiac glycogen (mg/g)	6.91 ± 0.44	4.31 ± 0.18†	8.08 ± 0.36††
FFA in adipose tissue (μEq/g)	3.11 ± 0.27	8.78 ± 0.26†	5.23 ± 0.35††

* Mean ± S.E.M., at least 7 observations/group.

† Significantly different from corresponding value in fed rats ($p < .05$).

†† Significantly different from corresponding value in fasting rats ($p < .05$).

TABLE II. Effects of Fasting and Actinomycin Treatment on the Metabolism of Diaphragm Muscle and Adipose Tissue Isolated from Hypophysectomized Rats.

	Fed	Fasting	Fasting + actinomycin
Diaphragm muscle:*			
Glucose uptake (mg/g/hr)	1.69 ± 0.10†	1.25 ± 0.09‡	1.77 ± 0.21§
Adipose tissue:			
¹⁴ C ₂ production (cpm/mg/hr)	18.6 ± 2.1	10.8 ± 2.0 †	59.5 ± 14.0 §
¹⁴ C incorporation into fatty acids (cpm/mg/hr)	15.7 ± 3.2	1.7 ± 0.6 †	47.3 ± 15.4 §
FFA production (μEq/g/hr)	-0.04 ± 0.08	0.45 ± 0.13‡	-0.05 ± 0.07§
Glycerol production (μmoles/g/hr)	1.27 ± 0.11	1.73 ± 0.07‡	1.18 ± 0.12§

* 15 observations/group.

† Mean ± S.E.M.

‡ Significantly different from corresponding value in fed rats ($p < .05$).

§ Significantly different from corresponding value in fasting rats ($p < .05$).

|| 8 observations/group.

induced by fasting(9). This response, although present, was markedly reduced in the actinomycin-treated rats. Measurement of FFA content in adipose tissue yielded parallel results, indicating that fat mobilization was indeed in progress, although to a significantly lesser extent in the actinomycin-treated rats.

As already noted elsewhere by others(10), fasting reduced the concentration of glycogen in the hearts of hypophysectomized rats (Table I). In the present studies, actinomycin completely prevented the decline in cardiac glycogen. Actinomycin also largely prevented the decrease in hepatic glycogen attendant upon fasting. A completely satisfactory explanation for this glycogen-sparing effect of actinomycin is not at hand, although it is possible that the antibiotic brought on a generalized depression of physical and metabolic activity. A recent study(11) indicated that actinomycin reduced blood pressure in rats.

Although fasting caused a small but significant decrease in the uptake of glucose by isolated diaphragm muscle, pretreatment

with actinomycin restored glucose uptake to fed values (Table II). Similarly, fasting reduced glucose utilization in epididymal fat. Pretreatment of the fasting rats with actinomycin D not only prevented the decline in glucose utilization, but actually increased, by at least threefold, the incorporation of the label into fatty acids and CO₂. Thus actinomycin D prevented the inhibition of glucose utilization by muscle and adipose tissue that usually results from fasting. Actinomycin D also prevented the appearance of increased lipolysis in response to fasting (Table II). The adipose tissue obtained from fasting rats produced more FFA and glycerol than did comparable tissue obtained from fed rats. These observations on the activity of adipose tissue *in vitro* thus confirm conclusions drawn from the data on plasma and tissue contents of FFA shown in Table I.

The effects of fasting and actinomycin are not limited to the tissues of hypophysectomized rats. *In vitro* production of FFA by tissues obtained from normal rats was increased 15 fold when the donor rats were

TABLE III. Effects of Pretreatment with Actinomycin D on the Response of Adipose Tissues to Various Lipolytic Stimuli.

	Saline-treated	Actinomycin-treated*
Fed rats	0.21 ± 0.07†	—
Fasting 18 hr	3.10 ± 0.29‡	1.40 ± 0.15‡§
+ ACTH (1 µg/ml)	6.46 ± 0.61	4.40 ± 0.56
+ epinephrine (0.5 µg/ml)	6.58 ± 0.18	4.92 ± 0.39

* 25 µg/rat at beginning of fast and 25 µg/rat 9 hr later.

† Mean ± S.E., 7 observations/group.

‡ Significantly greater than fed rats ($p < .001$).

§ Significantly less than saline-treated fasting rats ($p < .001$).

|| Significant increase ($p < .001$) compared with fasting controls.

deprived of food 18 hours previously (Table II). Again, 50 µg of actinomycin significantly reduced, but did not abolish the increase in FFA production brought on by fasting. Although this dose of actinomycin partially blocked the lipolysis which occurred in response to fasting, it was without effect on the lipolysis induced by epinephrine or ACTH. Fain *et al*(12) have also found that actinomycin failed to block the lipolytic response to epinephrine.

These results indicate that even in the absence of the pituitary gland, rats can shift their pattern of metabolism to that which is typical of the fasting state; *viz.* decreased utilization of glucose and increased mobilization of fatty acids. Although the physiological signal for such a shift in metabolism is still unknown, obviously the conversion of the metabolic pattern from the fed to the fasting state cannot be initiated by any pituitary hormone. As suggested previously(13), this

change may result from decreased levels of insulin in the blood of fasting animals. It is well known that the secretion of insulin is greatest immediately after a meal, and decreases to very low levels in fasting(14). It is also well known that in the absence of insulin the utilization of glucose by muscle and adipose tissue is reduced and fatty acid mobilization is accelerated.

Actinomycin prevented or reduced all of the effects of fasting measured in these studies. It is possible, but deemed unlikely, that these effects of actinomycin stem from the small differences in glycogen and blood glucose concentrations caused by treatment with actinomycin. Although fasting substantially lowered the blood sugar concentration even in actinomycin treated rats, the slightly higher blood glucose concentrations may have caused slightly higher concentrations of insulin in the plasma, which in turn may have prevented the response to fasting. Alternatively, these effects of actinomycin may stem from the well known action of the antibiotic in blocking RNA synthesis(1,2). In support of the latter hypothesis, it was observed that cycloheximide, an inhibitor of protein synthesis(15), also prevented the increase in lipolysis and the decrease in cardiac glycogen characteristic of the fasting state (Table IV). In these experiments hypophysectomized rats were injected intraperitoneally with 50 µg of cycloheximide at the onset of the fasting period. Cycloheximide did not affect all of the aspects of fasting metabolism altered by actinomycin, but because of its great toxicity the dose of cycloheximide used was only 1/200 of the amount used to block completely

TABLE IV. Effects of Fasting and Cycloheximide Treatment on Various Aspects of Metabolism.

	Fed	Fasting	Fasting + cycloheximide
Blood glucose	112.8 ± 12.4 *	79.3 ± 1.0 †	60.3 ± 3.8 †‡
Hepatic glycogen (mg/g)	88.3 ± 1.18	10.3 ± 1.6 †	7.3 ± 0.5 †
Cardiac glycogen (mg/g)	10.5 ± 1.5	6.6 ± 0.9	8.6 ± 0.3 ‡
FFA in adipose tissue (mEq/g)	3.30 ± 0.26	6.29 ± 0.32†	5.11 ± 0.32†‡
¹⁴ C incorporation into fatty acids (cpm/mg/hr)	12.6 ± 3.5	3.6 ± 1.1 †	2.8 ± 0.8 †
FFA production (µEq/hr)	0.12 ± 0.22	1.45 ± 0.28†	0.47 ± 0.26‡
Glycerol production (µmoles/g/hr)	1.78 ± 0.21	2.88 ± 0.14	1.66 ± 0.16‡

* Mean ± S.E.M., 7 observations/group.

† Significantly different from corresponding values in fed rats ($p < .05$).

‡ Significantly different from corresponding values in fasting rats ($p < .05$).

protein synthesis in rat liver (16). Inadequate blockade of protein synthesis may account for the differences between the effects of cycloheximide and actinomycin.

Although alternative explanations for these observations are by no means ruled out, it is suggested that actinomycin prevented the appearance of the metabolic changes characteristic of fasting by blocking the synthesis of messenger RNA. A corollary to this hypothesis is that in the conversion to a fasting-type metabolism different genes are expressed and certain new proteins are synthesized. Actinomycin D may have blocked the expression of these "fasting" genes by preventing the synthesis of messenger RNA. Under normal physiological circumstances, it may be that insulin, which is abundant in fed animals and present only in very low concentrations in fasting animals also regulates the expression of "fasting" genes, although activation of "fasting" genes by some extra-pituitary humoral agent is not ruled out.

Summary. In hypophysectomized rats fasting reduced the metabolism of glucose in adipose tissue and diaphragm muscle *in vitro* and increased the mobilization of free fatty acids and glycerol. Hepatic and cardiac glycogen were also reduced. Treatment with actinomycin D reversed these effects, and caused a substantial increase in glucose utilization by adipose tissue. Actinomycin partially prevented the increased lipolysis caused by

fasting in normal rats, but failed to reduce the lipolysis evoked by epinephrine or corticotropin.

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RNA and Protein Synthesis in the Response of Pigeon Crop-Sac to Prolactin.* (32580)

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The importance of RNA and protein synthesis in hormone-induced tissue responses has been underlined by a number of studies in recent years (1-3). The metabolic inhibitors, actinomycin D (AMD), and puromycin (P),

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which inhibit RNA and protein syntheses, respectively, have been of great utility in analyzing the action of hormones at the cellular level. AMD has been shown to block the response of several tissues to steroid hormones (4-8) and a similar effect has been observed with P (6,9). Response of tissues to stimulation by protein hormones is also blocked by AMD (10,11) and P (12-17) and they inhibit the action of thyroid hormones (18,19) and block hormone-induction of casein syn-