logic inactivation did not result from nonspecific destruction of all types of implanted cells.

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Effect of Epinephrine on Glucose Uptake and Glycerol Release by Adipose Tissue *in vitro*.* (25306)

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(Introduced by George W. Thorn)

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Available information suggests an inverse relationship between carbohydrate utilization and release of free fatty‖ acids by adipose tissue. Decreased glucose metabolism (fasting, diabetes mellitus) is generally accompanied by increased free fatty acid release, and conversely, increased glucose metabolism (glucose feeding, insulin administration) by decreased release(1-5). *In vitro* addition of epinephrine to adipose tissue, however, results in accelerated free fatty acid release concomitant with increased rate of glucose assimilation. In addition, there is increased release of free glycerol from adipose tissue under these conditions. This report demonstrates that carbohydrate utilization and free fatty acid release are not necessarily linked, and suggests that rate of lipolysis may be regulated independently.

Materials and methods. Adipose tissue fragments from epididymal fat pads of normal fed rats (Wistar strain obtained from Albino Farms or Harvard Biological Labs) were incubated in Krebs-Ringer bicarbonate buffer containing 4% human serum albumin (obtained from Protein Fn., through courtesy of Dr. R. J. Pennell). Glucose concentration was 5 mM/L and that of free fatty acids bound to the albumin approximately 0.8 mM/L. For analysis of hormonal effects, tissues from the same animal were always used as controls. Incubations were otherwise carried out as previously described(6,7,8). At the end of incubation, medium glucose was determined by method of Nelson(9), after precipitation of proteins with $ZnSO_4$ and $Ba(OH)_2$. For glycerol determinations, an aliquot of the supernatant fluid was oxidized with periodic acid(10), and the formaldehyde thus generated was determined with chromotropic acid(11). That the formaldehyde formed in excess of amount derived from glucose could be taken to measure glycerol was shown by submitting an aliquot of supernatant fluid to paper chromatography. Using n-butanol-acetic acid-water (4:1:5) and n-butanol-acetic acid-water-conc. HCl (20:5:25:1) as solvents and lead tetraacetate as location reagent, 3 spots could be identified, cor-

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‖ Free fatty acid = nonesterified fatty acid (NEFA) or unesterified fatty acid (UFA).

TABLE I. Effects of Insulin and Epinephrine on Adipose Tissue Metabolism *In Vitro*. Values in μmoles/100 mg wet weight adipose tissue/3 hours incubation. Mean of 9 experiments (standard errors in parentheses).

	Glucose uptake	Free fatty acid release*	Glycerol release
Control	.47 (.07)	−.31 (.10)	.49 (.13)
Insulin (.1 unit/ml)	1.99 (.13)	−.43 (.10)	.35 (.13)
Epinephrine (10^{-4}M)	1.05 (.09)	1.34 (.32)	2.21 (.21)

* Minus sign signifies uptake.

responding to mobilities of glucose, free glycerol and lactic acid(12). In experiments with labeled glucose, chromatography of a deproteinized aliquot of the medium after incubation, and scanning of strips with an open window proportional flow counter (Baird Atomic), demonstrated radioactivity peaks associated with the same spots. Free fatty acids in the medium were determined according to Gordon's method(13).

Results. Nine experiments are summarized in the Table, showing effects of insulin (0.1 unit/ml, using a highly purified crystalline preparation obtained through the courtesy of Dr. W. R. Kirtley) and epinephrine (10^{-4} M) on glucose uptake and on exchange of free fatty acids between tissue and incubating medium. Also shown is the amount of free glycerol released by tissue under same conditions. Whereas insulin and epinephrine both increase glucose uptake, their effects on release of fatty acids are opposite. Insulin favors uptake of fatty acids, epinephrine favors their release. Likewise, insulin slightly decreases amount of free glycerol released, whereas epinephrine has a strong stimulatory effect upon this release.

Discussion. The effect of epinephrine on glucose uptake by adipose tissue was unexpected. One might rather have anticipated that epinephrine increases release of free fatty acids by diminishing availability of exogenous glucose to the tissue. The inhibitory effect of epinephrine on peripheral glucose utilization *in vivo*(14) and on glucose uptake of diaphragm muscle incubated *in vitro*(15) is indeed well documented and has been attributed to its glycogenolytic effect, with secondary accumulation of glucose-6-phosphate (16), a known inhibitor of hexokinase(17,18). Absence of an inhibitory effect of epinephrine on glucose uptake by adipose tissue may therefore be related to the barely detectable concentrations of glycogen found in adipose tissue from normal fed animals(19). Even so, the only well demonstrated effect of epinephrine at the cellular level, increased phosphorylase activity(20,21), provides no adequate explanation for epinephrine-induced increase in glucose uptake in adipose tissue. It is interesting that under certain conditions epinephrine can also increase glucose uptake of muscle(22).

Whatever the mechanism of this effect of epinephrine on adipose tissue, the fact that insulin and epinephrine both increase glucose uptake, yet have opposite effects on release of free fatty acids, has to be explained. Recently, epinephrine has been shown to stimulate several-fold the incorporation of glucose-C^{14} into glyceride-glycerol by adipose tissue *in vitro*(23). Since net synthesis of glycerides presumably does not occur under these conditions, these findings suggest accelerated breakdown and resynthesis of glycerides, *i.e.* accelerated turnover. Furthermore, it would appear that glycerolphosphate newly synthesized from glucose is utilized for the esterification reactions, rather than free (unlabeled) glycerol resulting from the complete breakdown of triglycerides. Experiments by Shapiro(24), confirmed in our laboratory, have shown little utilization of exogenous labeled free glycerol, when compared to utilization of other substrates such as glucose or pyruvate. It is therefore not surprising to find a release of free glycerol by the control tissue, presumably reflecting lipolytic activity, even in the absence of hormonal stimulation. The increased release of free glycerol resulting from presence of epinephrine suggests an acceleration of this process. Furthermore, it is conceivable that accelerated lipolysis calls for accelerated re-esterification and thus for increased glycerolphosphate synthesis from glucose.

Summary. Epinephrine increases glucose uptake of adipose tissue incubated *in vitro*. This effect is common to both insulin and epinephrine. However, epinephrine increases and insulin decreases release of free fatty

acids. It would appear that the effect of epinephrine on mobilization of free fatty acids is not mediated by a decrease in carbohydrate availability, but possibly by increased rate of glyceride breakdown.

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Antibody Response to Heterologous Protein in Rabbits of Varying Maturity.* (25307)

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Injection of a variety of heterologous proteins into the neonatal animal induces a state of immunological tolerance(1,2) which is of finite duration related to the amount injected at birth. The period of susceptibility to induction of tolerance to 100 mg bovine serum albumin in the rabbit has been found to end by 15-17 days of age(1). However, a state of immunological unresponsiveness similar in many respects to that induced at birth has been produced by repeated injections of very large amounts of heterologous proteins into adult rabbits(3). Calculations from available data do not entirely rule out the possibility that the difference in the adult and newborn response to heterologous protein may be a quantitative rather than a qualitative one. The 5-7 fold increase in body weight of rabbits during the first three weeks of age could conceivably reduce the concentration of injected antigen below a critical level. These experiments were designed to re-examine the immunological response of rabbits of varying maturity to weight-adjusted amounts of a relatively pure heterologous protein. The data add further evidence that a qualitative defect in immunological capacity exists in the neonatal rabbit.

Procedure and methods. New Zealand

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