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Short Communications

Utilization of Glycerol by Rat Adipose Tissue *in vitro*

By EMILIO HERRERA and LUIS LAMAS
Instituto G. Marañón, Consejo Superior de Investigaciones Científicas, Velázquez 144, *Madrid*-6, *Spain*

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Since 1957 it has been considered that adipose tissue incubated *in vitro* has a very low capacity for the utilization of glycerol from the medium (Shapiro, Chowers & Rose, 1957) owing to a lack of glycerokinase (ATP–glycerol phosphotransferase, EC 2.7.1.30) (Wieland & Suyter, 1957). On the basis of this assumption, the production of glycerol has been used as an index of lipolysis and thrice this value minus the production of free fatty acids has been taken as an index of esterification. However, Robinson & Newsholme (1967), using a sensitive radiochemical method, have described the presence of glycerokinase in the rat epididymal fat-pad. We have investigated whether or not this glycerokinase activity is enough to cause a meaningful utilization of glycerol in adipose tissue when incubated *in vitro* under conditions used by others to determine lipolysis and esterification.

Materials and methods. Male Wistar rats (202–228g) were killed by cervical fracture and 61–70mg pieces of epididymal fat-pad were placed in rubber-sealed 20ml vials each containing 1ml of Krebs–Ringer bicarbonate buffer, pH 7.4 (Umbreit, Burris & Stauffer, 1964). At zero time 1ml of the same buffer containing 0.5μCi of [1-^{14}C]glycerol (15.4mCi/mmol) was injected into each of the vials and these were gassed for 5min with O_2+CO_2 (95:5) and incubated with agitation (100cycles/min) at 37°C. The incubation was stopped by injecting 250μl of Hyamine 10X hydroxide into small polyethylene cups suspended from the cover of the vial and 1ml of 10% (w/v) $HClO_4$ into the medium. The $^{14}CO_2$ evolved was trapped in the Hyamine by gentle shaking at room temperature for 60min. Lipids were extracted from the incubated tissue (Folch, Lees & Sloane-Stanley, 1957) and portions of the total lipid extracts were saponified in 5M-KOH in 95% (v/v) ethanol for 2h at 100°C. Fatty acids and non-saponified lipids were extracted with heptane after acidification, and radioactivity in the glyceride glycerol was calculated from the difference between that in the total lipid and that in the heptane layer. The medium was neutralized with $KHCO_3$ and centrifuged to remove $KClO_4$, and portions of the supernatant were taken for measurement of radioactivity, for determination of glycerol (Garland & Randle, 1962) and for the isolation of [^{14}C]glycerol by ascending paper chromatography in the upper phase of butan-1-ol–water–methanol–90.7% (w/v) formic acid (320:320:80:1, by vol.).

The purity of the [1-^{14}C]glycerol used was determined in two ways. (*a*) Portions of the radioactive preparation were passed through Dowex 1 (X2-400)–Duolite A-4 (OH^- form)–Dowex 1 (X2-400) micro-columns, prepared and processed as described previously (Herrera, Knopp & Freinkel, 1969). It was found that 99.8–100.9% of the [1-^{14}C]glycerol added was recovered in the eluates. On the other hand, when the portions were incubated with sufficient glycerokinase and cofactors to convert all the glycerol into glycerol 1-phosphate before passage of the mixture through the columns less than 0.3% of the radioactivity present was recovered in the eluates. (*b*) When the radioactive preparation was chromatographed on paper as indicated above, more than 99.7% of the radioactivity recovered on the strip was found in the glycerol spot.

Results and discussion. Pieces of epididymal fat-pad from fed and 48h-starved rats were incubated with [1-^{14}C]glycerol. After 180min of incubation the radioactivity remaining in the medium was practically all in glycerol (99.1$\pm$0.4 and 98.8$\pm$0.5% for the fed and starved animals respectively), and consequently the difference between the initial and the final radioactivity in the medium was assumed to be the amount of [^{14}C]glycerol taken up by the tissue. This uptake was not different for fed and starved rats and in both cases increased with time (Table 1), being 158% and 160% higher after 180min than after 60min of incubation for fed and starved animals respectively. Approx. 75% of the [^{14}C]-glycerol taken up by the tissue was found as radioactivity incorporated into CO_2 and total lipids (Table 1). Significant differences between the groups were found only in the formation of ^{14}C-labelled total lipid after 60min, which was smaller in starved than in fed rats (Table 1). Of the radioactivity incorporated into total lipid, 85.6–96.7% was in the glyceride glycerol fraction and, as was the case for the other parameters studied, the amount incorporated increased with the time of incubation.

The amount of glycerol present in the tissue at zero time was significatively higher in starved than in fed animals (Table 1). After 60min and 180min of incubation the glycerol produced by the tissues

Table 1. *Utilization of* [1-^{14}C] *glycerol and formation of glycerol by rat epididymal fat-pad incubated* in vitro

The incubation procedure is described in the text under 'Materials and methods'. The results are given as means ± S.E.M. of seven rats/group. P corresponds to the differences between fed and 48h-starved groups and P' to the differences between 60min and 180min (N.S., not significant, i.e. P or $P'>0.05$).

			Utilization of [^{14}C]glycerol (% of initial radioactivity/100mg of tissue)		
	Status of rats	Incubation time ...	60min	180min	P'
Uptake	Fed		6.0 ± 0.5	15.4 ± 2.9	<0.01
	Starved		5.3 ± 0.9	13.8 ± 1.8	<0.01
	P		N.S.	N.S.	
$^{14}CO_2$	Fed		0.5 ± 0.1	2.0 ± 0.4	<0.01
	Starved		0.3 ± 0.1	1.2 ± 0.3	<0.05
	P		N.S.	N.S.	
^{14}C-labelled total lipid	Fed		4.1 ± 0.2	11.9 ± 1.4	<0.001
	Starved		3.0 ± 0.6	9.8 ± 1.7	<0.01
	P		<0.05	N.S.	
^{14}C-labelled glyceride glycerol	Fed		3.5 ± 0.6	11.5 ± 1.4	<0.001
	Starved		2.6 ± 0.7	9.0 ± 1.9	<0.01
	P		N.S.	N.S.	

		Formation of glycerol (μmol in whole system/100mg of tissue)			
Status of rats	Incubation time ...	0min	60min	180min	P'
Fed		0.013 ± 0.001	0.072 ± 0.007	0.109 ± 0.016	N.S.
Starved		0.028 ± 0.004	0.178 ± 0.012	0.180 ± 0.024	N.S.
P		<0.01	<0.001	<0.05	

from starved rats continued to be higher than in tissue from fed rats. In contrast with the amount of [^{14}C]glycerol taken up by the tissue, the amount of glycerol produced after 180min was only slightly greater than that formed during the first 60min of incubation (51.4% and 1.1% higher in fed and starved rats respectively).

Epididymal fat-pads from fed and starved rats utilize up to 14–15% of [^{14}C]glycerol after 3h of incubation. At first sight it might be concluded that this percentage does not represent an appreciable error when lipolysis is being calculated from the net glycerol production. However, consideration of the mass of glycerol formed leads to quite a different conclusion. The very small increase in the amount of glycerol between 60min and 180min of incubation can be explained in one of two ways: (*a*) lipolysis stopped at 60min of incubation; (*b*) the tissue continued to hydrolyse glycerides, but part of the glycerol that was coming out into the medium was being reutilized. Current studies in our laboratory suggest that the second explanation is more likely: the radioactive glycerol in the medium is being continuously diluted with the glycerol coming out from the tissue and thus its specific radioactivity decreases progressively. This means that the radioactivity measurements underestimate the actual percentage of glycerol that has been reutilized by the tissue. It is most likely that in adipose tissue, as in other tissues, the glycerol must undergo phosphorylation (Antony, White & Landau, 1969) before it can form $^{14}CO_2$ and ^{14}C-labelled glyceride glycerol from [1-^{14}C]glycerol. We conclude that glycerokinase activity in adipose tissue is sufficient to result in a glycerol utilization that is considerably higher than previously thought and is likely to affect calculations of lipolysis and esterification that omit to take account of this factor.

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