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EXTRA-HEPATIC UTILIZATION OF ^{14}C-GLUCOSE AND ^{14}C-GLYCEROL IN THE EVISCERATED RAT

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SUMMARY :

Hepatectomy and nephrectomy in the rat produced an increase in blood glycerol levels which was observed before the fall in blood glucose. The disappearance of total radioactivity from plasma after the i.v. injection of either (U-^{14}C)-glycerol or (U-^{14}C)-glucose in these animals was slower than in their sham operated controls. Total radioactivity in plasma was always lower after (U-^{14}C)-glycerol administration than after (U-^{14}C)-glucose. The loss of either tracer from plasma in the eviscerated animals was followed by the appearance of ^{14}C-lactate, demonstrating their rapid metabolism. The radioactivity appearing in the water soluble fraction of lumbar fat pads was increased in eviscerated animals while in the lipid fraction it was reduced from both tracers. These results show that adipose tissue is able to metabolize small quantities of glycerol directly *in vivo*. The use of both (^{14}C)-glycerol and (^{14}C)-glucose by skeletal and heart muscles was enhanced in hepatectomized rats but the effect on synthesis of labelled glyceride glycerol was greater for (^{14}C)-glycerol, suggesting its important role in the esterification of fatty acids in these tissues.

Key Words : Hepatectomy and nephrectomy. Glycerol *in vivo* utilization. Adipose tissue. Heart and skeletal muscles.

RÉSUMÉ :

Utilisation extra-hépatique du ^{14}C-glucose et du ^{14}C-glycerol chez le rat éviscéré.

L'hépatectomie et la néphrectomie produisaient chez le rat une élévation du taux du glycérol sanguin apparaissant avant la chute de la glycémie. Chez ces animaux la disparition totale de la radioactivité du plasma après injection I.V. de (U-^{14}C)-glycérol ou de (U-^{14}C)-glucose était plus lente que chez les animaux de contrôle ayant subi une intervention simulée. La radioactivité totale du plasma était toujours plus faible après l'administration de (U-^{14}C)-glycérol qu'après celle de (U-^{14}C)-glucose. La disparition du plasma de l'un ou de l'autre des traceurs chez les animaux éviscérés était suivie de l'apparition de ^{14}C-lactate, témoignant de la rapidité de leur métabolisme. Chez les animaux éviscérés avec les deux traceurs la radioactivité apparaissant dans la fraction aqueuse de la graisse lombaire était augmentée tandis qu'elle était réduite dans la fraction lipidique. Ces résultats montrent que, *in vivo*, le tissu adipeux est capable de métaboliser de faibles quantités de glycérol. L'utilisation du (U-^{14}C)-glycérol et du (U-^{14}C)-glucose par le muscle squelletique ou cardiaque était augmentée chez les rats hépatectomisés mais l'effet sur la synthèse des glycérides marqués était plus marqué avec le (U-^{14}C)-glycérol, ce qui suggère que son rôle soit important dans ces tissus pour l'estérification des acides gras.

Mots Clés : Hépatectomie et néphrectomie. Utilisation *in vivo* du glycérol. Tissu adipeux. Muscle cardiaque et squelettique.

Studies of the *in vivo* utilization of labelled glucose and glycerol have demonstrated that the liver rapidly takes up and metabolizes these substrates which return to the circulation in altered form (1-5). Thus their utilization by extrahepatic tissues is masked by the earlier liver transformation. The use of hepatectomized animals is therefore necessary to determine the comparative utilization of these metabolites by peripheral tissues. The kidney cortex may actively contribute to the net production of endogenous glucose in totally (6) or partially hepatectomized animals (7), demonstrating that, to avoid the use of glycerol for gluconeogenesis and/or the recycling of glucose by different tissues, nephrectomy is also necessary for investigation of the direct utilization of these metabolites.

On the basis of previous studies in intact animals (5), *in vivo* utilization of (U-^{14}C)-glycerol and (U-^{14}C)-glucose by extrahepatic tissues in hepatectomized, nephrectomized rats has been determined in this study.

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MATERIALS AND METHODS

Female Wistar rats, fasted for 24 h, were anesthetized with sodium pentobarbital (40 mg/kg), the blood vessels supplying the liver (the coeliac axis and portal vein just above the first division to the hepatic lobes) were tied (6), and median and left lateral lobes were ligated (8) to ensure total hepatectomy. The renal arteries and veins were also tied leaving the circulation to the adrenals intact. The entire surgical procedure took under 15 min. In sham operated controls studied in parallel, laparotomy and handling of liver and kidneys were performed as in the experimental animals but without tying any vessel. In all animals, blood was collected from the inferior cava into heparinized syringes and aliquots of blood were deproteinized with Ba $(OH)_2$-Zn SO_4 (9) for glucose (10) and glycerol (11) assay in the supernatants. Samples of plasma were assayed for insulin (12) with a radioimmunoassay kit for rat insulin supplied by Novo Industri A/S (Denmark).

In another series of experiments, immediately after surgery the rats received through the inferior cava a single dose of 15 uCi of either (U-^{14}C)-glucose (3.8 mCi/mmol) or (U-^{14}C)-glycerol (46 mCi/mmol) dissolved in saline. Blood samples were collected at 5, 10 and 30 min after the injection of each tracer and plasma aliquots were used for counting radioactivity and for purification of lipids (13) and further fractionation (14). Other aliquots of plasma were deproteinized with acetone (15) and supernatants were subjected to ascending chromatography on Whatman 3MM paper in n-butanol-water-metanol-formic acid (320:320:81:1, by vol.) (16). Unlabelled glucose, glycerol and lactate were used as carriers, spots were identified by autoradiography and purified standards run in parallel. Recovery of tracer added to initial plasma was very reproducible : 95.3 ± 1.1 for (U-^{14}C)-glucose, 86 3 ± 1.2 for (U-^{14}C)-glycerol, and 78.5 ± 2.1 for (U-^{14}C)-lactate, and final values were corrected accordingly.

After the 30 min blood collection, the organs and carcass (muscle and skeleton without skin and viscera) of the animals were dissected and rapidly placed in ice cold saline for lipid extraction (13) and fractionation (14).

Radioactive measurements were performed in a PPO/POPOP based scintillation cocktail dissolved in xylene and Triton-X-100 and the samples were counted in a Nuclear Chicago (Isocap 300) counter provided with an external standard device. Quenched standards (NEN chemicals GmbH) were always counted with the samples to determine the channels-ratio to convert CPM into DPM. Total radioactivity administered to each animal was always adjusted to 10^6 DPM.

RESULTS

As shown in *Fig. 1,* blood glucose levels were unchanged 5 min after hepatectomy while circulating glycerol was significantly increased compared with pre-hepatectomy values. During the following 30 min period, levels of blood glucose decreased and glycerol levels progressively increased in hepatectomized animals while neither changed in the sham operated controls (*Fig. 1*). In another series of experiments, both hepatectomized and sham operated control animals were injected i.v. immediately after surgery with trace amounts of either (U-^{14}C)-glycerol or (U-^{14}C)-glucose. As shown in *Table I,* 5 and 10 min after the injection of either tracer

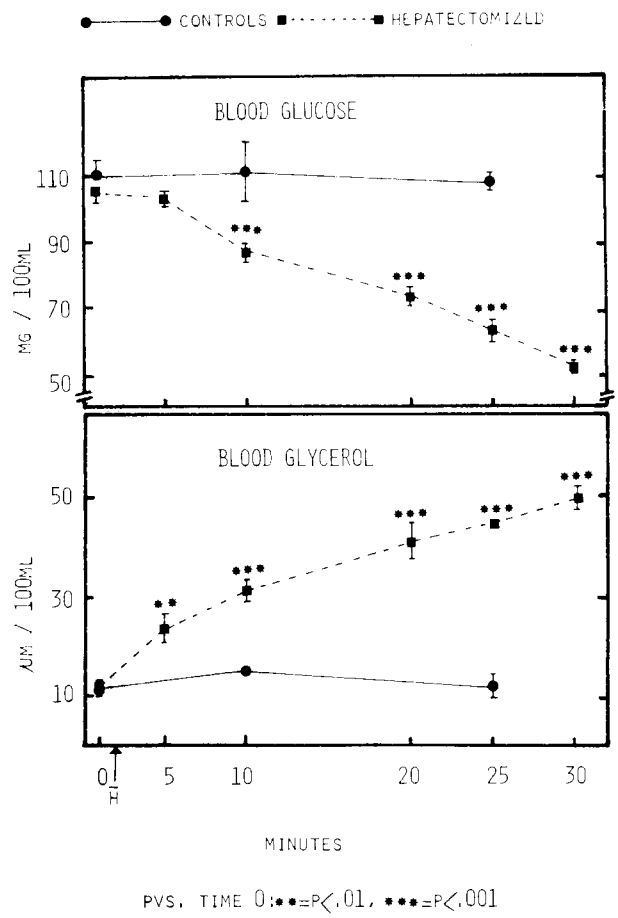

FIG. 1. — *Effect of hepatectomy and nephrectomy on blood glucose and glycerol in the rat.*

the total radioactivity remaining in plasma was higher in hepatectomized rats than controls. In both hepatectomized and control animals the disappearance of radioactivity from blood was faster in animals receiving (U-^{14}C)-glycerol than in those injected with (U-^{14}C)-glucose. In hepatectomized rats receiving (U-^{14}C)-glycerol, the percentage of labelled glycerol in blood decreased slowly after injection of the tracer, while the appearance of ^{14}C-lactate increased progressively (*Table I);* in this preparation, the amount of ^{14}C-glucose in blood was negligible. In contrast, 5 min after injection of (U-^{14}C)-glycerol in control animals, almost all the label in plasma appeared in the form of glucose with a negligible percentage remaining in glycerol or converted to lactate. With (U-^{14}C)-glucose, the percentage of labelled glucose diminished progressively in the blood of hepatectomized animals while labelled lactate increased, whereas in controls most radioactivity was maintained as glucose during the 30 min period (*Table I*).

Radioactivity in water soluble material was enhanced in the lumbar fat pads of hepatectomized animals 30 min after injection of either (U-^{14}C)-glycerol or (U-^{14}C)-glucose, although the increase in the latter tracer was not

statistically significant compared with controls, due to the great variability of the data (*Table II*). Hepatectomy produced a significant reduction in the radioactivity incorporated into the lipid fraction in the lumbar fat pads from both substrates. Thus the total radioactivity incorporated into this tissue (water soluble plus lipid radioactivity) did not differ between hepatectomized animals and the controls. The percentage distribution of lipid fractions between the groups shows that hepatectomy caused reduction in esterified fatty acids and enhancement in glyceride glycerol formed from (U-^{14}C)-glycerol, while a reduction in glyceride glycerol from (U-^{14}C)-glucose occurred.

The amount of radioactivity formed in water soluble material and total lipids was enhanced in the heart with hepatectomy when either tracer was administered (*Table II*), the greatest effect being observed in the lipids formed from (U-^{14}C)-glycerol with values more than three times higher in hepatectomized rats than in controls. With either substrate, all the labelled lipids synthesized in heart were in the form of glyceride glycerol. This fraction, as well as the FFA fraction, was proportionally augmented in the hearts of hepatectomized animals treated with (U-^{14}C)-glycerol but not in those treated with labelled glucose.

The highest amount of radioactivity from either substrate was taken up by the carcass and this parameter was greatly augmented by hepatectomy, especially when (U-^{14}C)-glycerol was the injected tracer, this enhancement corresponding to both water soluble and lipid fractions (*Table II*). The percentage distribution of labelled lipids decreased in FFA and esterified fatty acids and increased in glyceride glycerol in the (U-^{14}C)-glycerol treated hepatectomized animals compared to their controls. No percentage distribution of lipids was determined in the (U-^{14}C)-glucose injected animals as their net amount of radioactivity in total lipid extracts per unit of weight was very low.

The differences observed between hepatectomized-nephrectomized rats and their controls do not appear to be the result of changes in circulating insulin concentration. In both groups the level was very low appropriate to their fasted state, and no significant differences were found between the groups in hepatectomized - nephrectomized rats and controls (13.3 $\pm$ 3.7 (5) and 18.8 $\pm$ 3.8 (6)/μU/ml respectively).

DISCUSSION

The enhanced levels of circulating glycerol first observed after hepatectomy-nephrectomy may be the result of either augmented lipolysis from adipose tissue, reduced consumption of this metabolite, or both. The first possibility has been well documented by different authors (17, 18) in situations of reduced hepatic function in the rat. The second possibility is also supported by the slower disappearance of the administered (U-^{14}C)-glycerol from plasma observed here in the hepatectomized rats. The ability to convert administered ^{14}C-glycerol (and presumably other gluconeogenic substrates) to ^{14}C-glucose was abolished in hepatectomized-nephrectomized rats and, as these animals have no compensatory gluconeogenic capacity in the kidney (7), their blood glucose decreased to levels of intense hypoglycemia.

The present observations are based on tracer radioactivity incorporated into tissues and quantitative aspects of glycerol and glucose utilization remain to be evaluated. In these experiments it is difficult to make precise inter-group comparisons of the use of either (U-^{14}C)-glycerol or (U-^{14}C)-glucose by the different tissues due to the variable dilution of the tracer. Skeletal and heart muscles are the tissues that most effectively use these substrates in the hepatectomized-nephrectomized animals. Relative utilization of the two substrates differs, however, because with glycerol, the effect of hepatectomy is observed in the amount of label appearing both in lipid and water soluble fractions while the effect of glucose is seen mainly in the water soluble fraction.

In either case, almost all the label in lipid fractions of tissues from hepatectomized animals appeared in the glyceride glycerol form, showing that lipogenesis was negligible. The augmented formation of glyceride glycerol from glycerol in the heart of hepatectomized animals may be related to that formed in hypertrophied myocardium during experimental hypoxia (19) indicating the important role of glycerol as a source of α-glycerolphosphate in these tissues when more active and free fatty acids released from adipose tissue must be temporarily retained in the heart for later use as extra fuel or as building blocks for new membrane formation. The incorporation of label in adipose tissue and its relative distribution in lipid and water soluble fractions in hepatectomized rats and controls did not differ for glucose and glycerol although, during the experimental period, the specific activity of labelled glycerol decreased while that of labelled glucose was enhanced due to their differing concentrations in blood. These findings demonstrate that adipose tissue is able to metabolize glycerol directly *in vivo* in small quantities, without the need for its previous conversion to glucose, a fact that has been amply demonstrated *in vitro* (20-23) but never in an *in vivo* preparation.

The reduced conversion of either substrate to adipose tissue lipids in the hepatectomized animal is not surprising as it is known that adipose tissue lipogenesis is decreased in fasting animals (24-25); reduced hepatic function (18) and enhanced endogenous lipid breakdown further limit storage of these substrates as lipid products.

Although glycerol utilization by a tissue would imply its phosphorylation by glycerol kinase action, the activity of this enzyme is high in liver and kidney cortex but low in muscle and adipose tissue (26). In conditions when the utilization of glycerol by these extrahepatic tissue is increased, as in the hepatectomized rat, other mechanisms of glycerol utilization are also working. Among these mechanisms it may be quoted the conversion of glycerol into glyceraldehyde by reversal of

TABLE I. — *Radioactivity in plasma after the i.v. injection of either* (U-^{14}C) *-glycerol or*

Administered Tracer	*Group*	Total radioactivity (DPM/ml)			^{14}C-glucose (%) +		
		5 min	10 min	30 min	5 min	10 min	30 min
(U-^{14}C) glycerol	H̄ (n = 7)	12512 ± 577	10706 ± 353	8464 ± 309	4,95 ± 0,34	5,83 ± 0.42	7,80 ± 0,86
	C (n = 5)	10179 ± 264	9186 ± 225	7353 ± 519	58,2 ± 5,13	66,5 ± 4.58	56,9 ± 4,91
	p	< 0,05	< 0,05	N.S.	< 0,001	< 0,001	< 0,001
(U-^{14}C) glucose	H̄ (n = 5)	27388 ± 888***	23940 ± 639***	13283 ± 595***	69,8 ± 3,37***	67,0 ± 2,17***	42,1 ± 4,07***
	C (n = 5)	18550 ± 1368***	16069 ± 1300***	11948 ± 1060**	75,9 ± 3,46**	71,6 ± 2,49	69,2 ± 2,91
	p	< 0,01	< 0,01	N.S.	N.S.	N.S.	< 0,001

The radioactive values were adjusted by standardising the administered tracer to each animal to 10^6 DPM. Blood samples were collected from the inferior cava vein at the times indicated after the injection of each tracer.

p values relate to the statistical comparison between hepatectomized and sham operated controls.
Asterisks correspond to the statistical comparison between (U-^{14}C) glucose and (U-^{14}C) glycerol treated animals :
* = p < .05 ** = p < .01 *** = p < .001

n = number of animals/group.
+ Percentage values are refered to plasma total radioactivity considered as 100 % at each time point.

TABLE II. — *Distribution of radioactivity in tissues at 30min after the i.v. injection of either* (U-^{14}C)

Administered Tracer	*Group*	LUMBAR FAT PADS				
		Water soluble material (DPM+)	LIPIDS			
			Total DPM	FFA (%)	+Esterified FA (%)	Glyceride Glycerol (%)
(U-^{14}C) glycerol	H̄ (n = 7)	294±± 32,6	101±± 33,4	4,15±± 0,77	14,54± 2,44	81,10± 2,04
	C (n = 5)	129± 23,8	336± 14,8	5,98± 0,74	28,67± 5,02	64,91± 5,14
	p	< 0,001	< 0,001	N.S.	< 0,05	< 0,05
(U-^{14}C) glucose	H̄ (n = 5)	561± 225	298±* 69,5	3,54± 0,62	16,48± 1,96	79,75± 1,39
	C (n = 5)	140± 22,5	817±* 153	3,37±* 0,71	9,69±** 2,74	88,5±** 2,67
	p	N.S.	< 0,05	N.S.	N.S.	< 0,05

The radioactive values were adjusted by standardising the administered tracer to each animal to 10^6 DPM. At 30 min after the injection of either tracer the organs and carcass were dissected and processed for lipid extraction and fractionation as indicated in the text.
p values relate to the statistical comparison between H̄ and sham operated controls.
Asterisks correspond to the statistical comparison between (U-^{14}C) glucose and (U-^{14}C) glycerol treated animals :
* = p < 0,05 ** = p < 0,01 *** = p < 0.001
n = number of animal/group.
+ DPM values are expressed per whole tissue.
++ % values are refered to the total lipid DPM in each tissue, considered as 100 %.

NADP-dependent glycerol dehydrogenase, known to be present in heart and skeletal muscle (27) and in adipose tissue (28). The relative contributions of these pathways remain to be established.

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(U-^{14}C) - *glucose in hepatectomized-nephrectomized* (H̃) *rats and sham operated controls* (C)

^{14}C-glycerol (%)			^{14}C-lactate (%)		
5 min	10 min	30 min	5 min	10 min	30 min
60,4 ± 2,36	56,3 ± 1,97	46,4 ± 2,90	20,5 ± 0,99	26,9 ± 1,44	35,6 ± 1,10
12,2 ± 1,57	8,8 ± 0,93	5,4 ± 0,68	11,6 ± 1,94	10,3 ± 1,60	8,95 ± 1,44
< 0,001	< 0,001	< 0,001	< 0,01	< 0,001	< 0,001
—	—	—	8,48 ± 2,72***	13,0 ± 2,40***	31,4 ± 3,75
—	—	—	3,62 ± 0,37**	4,09 ± 0,64**	6,04 ± 0,85
—	—	—	N.S.	< 0,01	< 0,001

glycerol or (U-^{14}C) *glucose in hepatectomized-nephrectomized* (H̃) *rats and sham operated controls* (C)

HEART					CARCASS				
Water soluble material (DPM+)	LIPIDS				Water soluble material (DPM+)	LIPIDS			
	Total DPM	FFA (%)	++Esterified FA (%)	Glyceride Glycerol (%)		Total DPM	FFA (%)	++Esterified FA (%)	Glyceride Glycerol (%)
4269± 180	6032± 738	0,57± 0,11	0,30± 0,03	99,09± 0,11	229009± 25146	26576± 2925	0,43± 0,10	1,04± 0,20	98,15± 0,28
3288± 257	1209± 227	1,31± 0,24	1,31± 0,28	97,28± 0,41	79716± 6411	7608± 543	3,29± 0,19	6,12± 1,76	88,12± 3,19
< 0,01	< 0,001	< 0,01	< 0,01	< 0,001	< 0,001	< 0,001	< 0,001	< 0,01	< 0,01
9665± 1287***	457± 76,5***	1,88± 0,005***	1,13 ± 0,18***	96,80 ± 0,67**	93461 ± 7377***	654 ± 133***	—	—	100 ± 0***
4203 ± 473	209 ± 16,2**	2,08 ± 0,47	3,01 ± 0,74	94,52 ± 1,05**	50965 ± 2294**	1178 ± 177**	2,73 ± 0,58	9,21 ± 2,63	87,82 ± 2,98
< 0,01	< 0,01	N.S.	N.S.	N.S.	< 0,001	< 0,05	< 0,001	< 0,001	< 0,001

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