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Comparative utilization *in vivo* of [U-^{14}C] glycerol, [2-^{3}H] glycerol, [U-^{14}C] glucose and [1-C^{14}] palmitate in the rat

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Female rats were injected i.v. with comparable trace amounts of [U-^{14}C] glycerol, [2-^{3}H] glycerol, [U-^{14}C] glucose, or [1-^{14}C] palmitate, and killed 30 min afterwards. The radioactivity remaining in plasma at that time was maximal in animals receiving [U-^{14}C] glucose while the appearance of radioactive lipids was higher in the [U-^{14}C] glycerol animals than in other groups receiving hydrosoluble substrates. The carcass, more than the liver, was the tissue where the greatest proportion of radioactivity was recovered, while the greatest percentage of radioactivity appeared in the liver in the form of lipids. The values of total radioactivity found in different tissues were very similar when using either labelled glucose or glycerol but the amount recovered as lipids was much greater in the latter. The maximal proportion of radioactive lipids appeared in the fatty-acid form in the liver, carcass, and lumbar fat pads when using [U-^{14}C] glycerol as a hydrosoluble substrate, and the highest lipidic fraction appeared in adipose tissue as labelled, esterified fatty acids. In the spleen, heart, and kidney, most of the lipidic radioactivity from any of the hydrosoluble substrates appeared as glyceride glycerol. The highest proportion of radioactivity from [1-^{14}C] palmitate appeared in the esterified fatty acid in adipose tissue, being followed in decreasing proportion by the heart, carcass, liver, kidney, and spleen. Thus at least in part, both labelled glucose and glycerol are used throughout different routes for their conversion *in vivo* to lipids. A certain proportion of glycerol is directly utilized by adipose tissue. The fatty acids esterification ability differs among the tissues and does not correspond directly with the reported activities of glycerokinase, suggesting that the α-glycerophosphate for esterification comes mainly from glucose and not from glycerol.

It is well known that injected glycerol disappears rapidly from blood and is transformed mainly by the liver into glucose (GIDEZ & KARNOVSKY, 1954; HAGEN, 1963; NIKKILA & OJALA, 1963; WINKLER *et al.*, 1970; HALL *et al.*, 1976; GILBERT & RICQUIER, 1977) and lipids (GIDEZ & KARNOVSKY, 1954; ABELIN *et al.*, 1966; SHREEVE *et al.*, 1967; CLOUET *et al.*, 1974; CHANUSSOT & DEBRY, 1977). This makes it difficult to determine the actual utilization of glycerol by extrahepatic tissues in physiological conditions. Glycerokinase (EN 2.7.1.30), the enzyme which catalyses the

conversion of glycerol to its active metabolizable form (α-glycerophosphate) is widely distributed. Actually it is known that glycerol may be utilized by different tissues (see LIN, 1977), including adipose tissue *in vitro* (CAHILL *et al.*, 1960; HERRERA & LAMAS, 1970; HERRERA & AYANZ, 1972; PERSICO *et al.*, 1975; DOMÍNGUEZ & HERRERA, 1976*a*) in spite of the fact that glycerokinase activity has been reported to be very low (WIELAND & SUYTER, 1957). The aim of the present study was to determine the comparative utilization *in vivo* of glycerol as a lipogenetic substrate among different tissues. Due to differences in the use of carbon and hydrogen atoms from glycerol to lipid synthesis (CHEN, 1967), the work was performed using both [U-^{14}C] and [2-H^3] labelled glycerol as tracer. In order to determine whether the observed results were a consequence of the previous conversion of glycerol to glucose, the utilization *in vivo* of [U-^{14}C] glucose was also determined in a parallel experiment. As both glycerol and glucose are partially used for the synthesis of glyceride glycerol, the work was extended to study the utilization of [1-^{14}C] palmitate in order to determine the fatty-acid esterification ability of the different tissues.

Materials and Methods

Female virgin Wistar rats were used, weighing 170-190 g and fed standard rat chow and water *ad libitum*. The rats were housed in collective cages in a temperature (23 ± 2 °C) and light cycle (12 h on - 12 h off) controlled room. They were injected i.v. through a tail vein, without anaesthesia at the beginning of the light cycle with 15 μCi of [U-^{14}C] glycerol (46 mCi/mol), or 30 μCi of [2-^{3}H] glycerol (200 mCi/mmol), or 18 μCi of [U-^{14}C] glucose (3.8 mCi/mmol) per rat, dissolved in 0.5 ml saline; or with 15 μCi of [1-^{14}C] palmitate (sodium salt) (51.8 mCi/mmol) dissolved in 0.5 ml of 8 % bovine albumin purified by the method of CHEN (1967). All radioactive material was obtained from the Radiochemical Center, Amersham. Animals were killed by decapitation 30 min after the injections, blood was collected from the neck into heparinized beakers, and the different organs were immediately placed in ice-cold saline. The rat carcasses were dissected, including the skeleton and muscles but without the skin and viscera, and they were mechanically ground. After being weighed, aliquots of each organ and carcass and aliquots of plasma were placed in chloroform-methanol for lipid extraction (FOLCH *et al.*, 1957) and fractionation, following some modifications of the method of KERPEL *et al.* (1961). An aliquot of the lipid extract was blown to dryness at 37 °C under N_2 suspended with 0.05 N NaOH in 40 % isopropylalcohol and extracted with heptane. The heptane washes were used for saponification to determine the radioactivity in the esterified fatty acids and glycerid glycerol, as described previously (DOMINGUEZ & HERRERA, 1976*a*), while the NaOH isopropylalcohol infranatants were acidified with 1N HCl and further extracted with heptane for evaluation of the radioactivity in the free fatty-acid fraction. Recovery experiments were carried out with non-radioactive lipid extracts from both liver and adipose tissue supplemented with either glycerol-tri [1-^{14}C] palmitate or [1-^{14}C] palmitate. More than 96 % of the radioactivity from [1-^{14}C] palmitate was recovered in the free fatty-acid fraction and more than 92.2 % from glycerol tri [1-^{14}C] palmitate was recovered in the esterified fatty-acid fraction.

Other aliquots of blood were used for the enzymatic evaluation of glucose (HUGGETT & NIXON, 1957) and glycerol (GARLAND & RANDLE, 1962) after protein precipitation (SOMOGYI, 1945) and for plasma free fatty-acid determination (FALHOLT *et al.*, 1973).

Analysis of the data was performed using an Ataio electronic calculator (Compucorp 445). Radioactive measurements were expressed either as a percentage of the

injected dpm or as absolute dpm per unit of volume or weight, considering that all animals were treated with 10^6 dpm of each tracer.

Results

The plasma concentration of glucose, glycerol and free fatty acids in fed rats were 5.96 ± 0.12, 0.115 ± 0.012 and 0.405 ± 0.038 mM respectively. The animals were injected i.v. with comparative amounts of different radioactive metabolites in trace concentrations and sacrificed 30 min afterwards. As shown in Table I, the radioactivity remaining in plasma at that time was maximal when the animals received [U-^{14}C] glucose, followed by those receiving [2-^{3}H] glycerol, [1-^{14}C] palmitate and [U-^{14}C] glycerol. The appearance of radioactive lipids in plasma did not follow the same trend seen in groups receiving hydrosoluble substrate. The highest values were observed in the [U-^{14}C] glycerol-treated group and the lowest in that of [U-^{14}C] glucose, the [2-^{3}H] group having less than half of the plasma labelled lipids found in the [U-^{14}C] glycerol group.

The distribution of radioactivity in the plasma lipidic fractions indicated that the highest percentage of glyceride glycerol was in the [2-^{3}H] glycerol-treated animals, followed by those with [U-^{14}C] glucose and [U-^{14}C] glycerol. More than 50 % of the labelled fatty acids in plasma appeared in esterified form in all groups including rats injected with [1-^{14}C] palmitate (Table I).

The distribution of radioactivity in different tissues is shown in Table II. The carcass, more than the liver, was the tissue where the highest proportion of radioactivity was recovered from all four substrates utilized, although the liver contained the greatest percentage of radioactivity in the form of lipids. With the exception of a high amount of radioactivity in the carcass when using [2-^{3}H] glycerol as a tracer, most of the values of total radioactivity found in the different tissues were similar when using either labelled glucose or glycerol. In spite of this, the amount of radioactivity recovered as lipids was much greater in the latter. This is especially true in the liver where 70-59 % of the radioactivity was in the lipidic form when [U-^{14}C] or [2-^{3}H] glycerol respectively were used, while the amount was only 14.4 % with glucose.

The distribution of lipidic fractions also differed between the groups. In the liver, half of the radioactivity in the form of lipids in the animals receiving [U-^{14}C] glycerol appeared in glyceride glycerol while the other half was found in the fatty-acid fraction, in equal parts in the free and esterified form. When either [2-^{3}H] glycerol or [U-^{14}C] glucose was the substrate, most of the lipid radioactivity in the liver appeared in the glyceride-glycerol fraction while when using [1-^{14}C] palmitate, the lipid radioactivity was distributed in equal parts between the free fatty acids and esterified fatty acids. In the other tissues studied, with the exception of the lumbar fat pads, most of the radioactivity coming from [U-^{14}C] glycerol, [2-^{3}H] glycerol or [U-^{14}C] glucose incorporated to lipids appeared in the glyceride-glycerol fraction. [1-^{14}C] palmitate is converted half to free fatty acids and half to esterified fatty acids in the carcass and heart muscle while a maximal percentage remains in the free fatty-acid form in both kidney and spleen. The distribution of labelled lipids in adipose tissue differed substantially from that in other tissues : when [2-^{3}H] glycerol or [U-^{14}C] glucose were the tracers, the greatest percentage of labelled lipids appeared in the glyceride-glycerol fraction followed by that in the esterified fatty acids with a minimal proportion in the free fatty acids. When [U-^{14}C] glycerol was the injected tracer, most radioactivity appeared in the adipose tissue lipids in the form of esterified fatty acids, followed by the glyceride glycerol and a minimum amount in the free fatty-

TABLE I. *Plasma radioactivity 30 min after the i.v. injection of different tracers to fed rats.*

Tracer	Total radioactivity dpm × 10^{-3}/ml	Total lipids dpm × 10^{-2}/ml	% Distribution of lipid fractions		
			FFA	Esterified fatty acids	Glyceride glycerol
[U-^{14}C] glycerol	3.39 ± 0.35	7.70 ± 0.80	9.34 ± 4.72	19.8 ± 7.41	61.4 ± 1.1
[2-^{3}H] glycerol	6.72 ± 0.28	3.97 ± 0.59	2.62 ± 0.68	5.65 ± 1.01	90.2 ± 1.2
[U-^{14}C] glucose	11.95 ± 1.06	0.41 ± 0.12	−19.2 ± 3.28−		77.7 ± 4.1
[1-^{14}C] palmitate	4.39 ± 0.68	38.6 ± 8.7	36.9 ± 6.9	55.3 ± 6.26	—

Values are mean ± SEM of 5 animals/group.

Radioactivity values have been adjusted to 1 × 10^{6} dpm for the injected tracer.

In Tables I & II "Total radioactivity" corresponds to the amount of label found in the water-soluble fraction (aqueous washes of the lipid extract : FOLCH *et al.*, 1957) plus that in the lipid extract, while "total lipids" corresponds to the label found in aliquots of the liquid extract (FOLCH *et al.*, 1957). Values are expressed as absolute dpm of each tracer per ml of plasma (Table I) or as the percentage of the injected dpm of each tracer per whole tissue (Table II).

acids fraction. By using [1-^{14}C] palmitate as a tracer, adipose tissue was found to be the tissue where the highest percentage of labelled lipids appeared in the esterified fatty acids, the proportion found in free fatty acids being very low.

Discussion

Although the present study is based on the protocols used by others (ABELIN *et al.*, 1966; SHREEVE *et al.*, 1967; CLOUET *et al.*, 1974; GILBERT & RICQUIER, 1977), the data may not be corrected by the different isotopic dilution of the administered tracers as it is not possible to know the actual specific activity of intracellular precursor pools. The results may, however, be discussed in terms of the interorgan utilization of each tracer and their proportional conversion into lipidic fractions. Some qualitative comparison in the use of the different substrates may also be inferred.

In spite of the well known, rapid conversion *in vivo* of glycerol into glucose (GIDEZ & KARNOVSKY, 1954; HAGEN, 1963; NIKKILA & OJALA, 1963; WINKLER *et al.*, 1970; HALL *et al.*, 1976; GILBERT & RICQUIER, 1977), the present study demonstrates that 30 min after their i.v. administration, the conversion of labelled glycerol into lipids and the percentual distribution of radioactivity found in lipidic fractions in different tissues differ substantially from those found with labelled glucose, which indicates that, at least in part, both substrates are used throughout different routes for their conversion to lipids.

The comparison of absolute values among the groups is difficult due to the different isotopic dilution of the administered substrates with the endogenous metabolites. Thus the [^{14}C] glucose is more diluted than glycerol because of the higher concentration of blood glucose than glycerol. It was evident, however, in all tissues studied that although the total amount of radioactivity was similar when using either [U-^{14}C] glucose or [U-^{14}C] glycerol, a much greater proportion of the total radioactivity appeared in the lipidic fraction with glycerol as a substrate than with glucose, showing the more rapid and efficient use of the former as a lipogenetic substrate. The liver is the organ where this transformation is more effective although the carcass takes up the greatest proportion of any of the substrates utilized.

The lumbar fat pads constitutes the tissue where the amount of radioactivity was found to be the lowest but it is where the greatest proportion of the tracer appeared as lipids. The greater proportion of radioactivity appeared in the fatty-acid fraction when using [U-^{14}C] glycerol as a substrate, contrasts with that found when using [U-^{14}C] glucose, and does not appear to be direct consequence of the nature of the labelled lipids that are taken up by the tissue from the circulation, as the lipidic distribution in plasma did not differ greatly between either substrate. The difference may, however, reflect the direct utilization of glycerol by the adipose tissue, a phenomenon that has been amply demonstrated *in vitro* (CAHILL *et al.*, 1960; HERRERA & LAMAS, 1970; HERRERA & AYANZ, 1972; PERSICO *et al.*, 1975; DOMÍNGUEZ & HERRERA, 1976*a*) but not *in vivo*. The situation *in vivo* would correspond to the tissues incubated *in vitro* in the presence of both [^{14}C] glycerol and glucose where a considerable proportion of the label taken up by the tissue appears in the fatty-acid fraction (DOMÍNGUEZ & HERRERA, 1976*a* & *b*).

It is not possible to extrapolate the present data on lumbar fat pads to the other adipose tissue sights as it must be taken into account that the ability to utilize glycerol differs among them according to their diverse glycerokinase activity, as has already been shown *in vitro* (PERSICO *et al.*, 1975). In any event, it is important to consider that the lumbar fat pads only represent a minimum proportion of the body

TABLE II. *Tissue distribution of radioactivity 30 min after the i.v. injection of different tracers to fed rats.*

Tracer	Total radioactivity % of injected dpm/ whole tissue	Total lipids % of injected dpm/ whole tissue	% Distribution of lipid fractions		
			FFA	Esterified fatty acids	Glyceride glycerol
			Liver		
[U-^{14}C] glycerol	5.05 ± 0.67	3.57 ± 0.58	23.4 ± 1.0	29.1 ± 3.4	45.8 ± 3.7
[2-^{3}H] glycerol	5.26 ± 0.51	3.11 ± 0.17	3.18 ± 0.7	2.94 ± 0.8	92.7 ± 1.6
[U-^{14}C] glucose	4.58 ± 0.23	0.66 ± 0.14	6.55 ± 1.1	5.92 ± 1.2	82.3 ± 2.5
[1-^{14}C] palmitate	26.67 ± 3.02	26.44 ± 2.93	54.0 ± 3.0	39.9 ± 3.8	—
			Carcass		
[U-^{14}C] glycerol	7.01 ± 0.87	0.56 ± 0.05	7.84 ± 1.4	20.0 ± 6.1	69.7 ± 7.6
[2-^{3}H] glycerol	14.89 ± 1.75	0.83 ± 0.01	8.37 ± 2.2	10.4 ± 3.3	80.3 ± 4.5
[U-^{14}C] glucose	5.21 ± 0.24	0.12 ± 0.01	2.73 ± 0.6	9.21 ± 2.6	87.8 ± 2.9
[1-^{14}C] palmitate	34.05 ± 0.69	33.64 ± 7.30	49.4 ± 4.7	54.4 ± 4.8	—
			Adipose tissue (Lumbar fat pads)		
[U-^{14}C] glycerol	0.092 ± 0.025	0.080 ± 0.02	2.87 ± 0.2	75.5 ± 3.5	20.6 ± 3.5
[2-^{3}H] glycerol	0.034 ± 0.006	0.015 ± 0.001	3.78 ± 0.7	38.6 ± 5.8	53.1 ± 6.2
[U-^{14}C] glucose	0.095 ± 0.010	0.081 ± 0.01	3.37 ± 0.7	9.69 ± 2.7	88.5 ± 2.6
[1-^{14}C] palmitate	0.257 ± 0.074	0.255 ± 0.06	9.54 ± 2.9	83.7 ± 3.4	—

			Spleen		
[U-^{14}C] glycerol	0.091 ± 0.006	0.025 ± 0.003	7.90 ± 1.3	2.99 ± 0.4	87.4 ± 1.7
[2-^{3}H] glycerol	0.120 ± 0.021	0.025 ± 0.006	2.96 ± 1.1	3.0 ± 0.9	92.0 ± 1.5
[U-^{14}C] glucose	0.076 ± 0.007	0.004 ± 0.0003	16.9 ± 2.3	7.95 ± 0.7	78.8 ± 2.6
[1-^{14}C] palmitate	0.283 ± 0.030	0.270 ± 0.018	78.6 ± 2.0	10.6 ± 1.2	—
			Heart		
[U-^{14}C] glycerol	0.148 ± 0.014	0.031 ± 0.003	4.92 ± 0.9	7.54 ± 1.9	86.8 ± 2.9
[2-^{3}H] glycerol	0.170 ± 0.018	0.050 ± 0.008	2.79 ± 0.8	3.51 ± 0.9	93.2 ± 1.3
[U-^{14}C] glucose	0.441 ± 0.004	0.021 ± 0.001	2.08 ± 0.4	3.01 ± 0.7	94.5 ± 1.0
[1-^{14}C] palmitate	1.450 ± 0.300	1.425 ± 0.244	31.5 ± 2.8	62.3 ± 2.6	—
			Kidney		
[U-^{14}C] glycerol	0.341 ± 0.044	0.15 ± 0.02	3.26 ± 0.4	1.96 ± 0.6	94.5 ± 1.0
[2-^{3}H] glycerol	0.483 ± 0.020	0.21 ± 0.01	1.44 ± 0.1	0.78 ± 0.1	97.4 ± 0.3
[U-^{14}C] glucose	0.515 ± 0.050	0.015 ± 0.001	5.68 ± 0.6	4.03 ± 0.9	90.2 ± 2.0
[1-^{14}C] palmitate	1.260 ± 0.090	0.615 ± 0.03	81.6 ± 4.4	17.40 ± 1.9	—

Values are mean ± SEM of 5 animals/group.
Radioactivity values have been adjusted to 1×10^6 dpm for the injected tracer.

adipose tissue and thus the total amount of glycerol being used by the whole body adipose mass must be much greater than the one here reported. Although the amount of label remaining in plasma and that taken up by the different tissues was greater when using [2-^{3}H] glycerol than when the [U-^{14}C] glycerol was the tracer, its proportional lipidic fraction was much lower in the former which would partially correspond to the loss of ^{3}H from the glycerol molecule due to interchange with water protons (ABELIN *et al.*, 1966; SHREEVE *et al.*, 1967). A certain amount of the ^{3}H from [2-^{3}H] glycerol may also be used for hydrogenation of NAD^{+} in the oxidation of α-glycerophosphate to dihydroxyacetonephosphate. The latter possibility seems to be effective in adipose tissue where an appreciable amount of the ^{3}H label appeared as fatty acids. In this case, the ^{3}H moiety gets to the fatty acids *via* the transhydrogenation through the NAD^{+}-linked malic dehydrogenase and $NADP^{+}$-linked "malic enzyme" (YOUNG *et al.*, 1964; LEVEILLÉ & HANSON, 1966), allowing the radioactive protons to be used for lipogenesis.

After the administration of [1-^{14}C] palmitate, the label passes throughout the liver where it is partially converted to glycerides (CLOUET *et al.*, 1974) to return to the blood mainly in the form of very low-density-lipoprotein triglycerides to be processed by extrahepatic tissues (see review in MAZORO, 1977). The present data agree with this concept as the proportional distribution of esterified and free fatty acids in the liver is very similar to that found in plasma. To be taken up by the different tissues, all the fatty acids must be converted into their free fatty-acid form by the action of lipoprotein lipase, thus the proportional distribution of esterified *vs.* free fatty acid into the tissue represents its esterificating ability. The present data, by using either [1-^{14}C] palmitate or any of the other substrates, show that this ability is maximal in adipose tissue followed by the carcass, heart, kidney, and spleen. This sequence does not correspond to that of the respective reported activities of glycerokinase in these tissues (LIN, 1977), suggesting that most of the α-glycerophosphate utilized for the esterification of fatty acids is not derived from glycerol but from glucose, while the former substrate is mainly used for lipogenesis in those tissues where this pathway is especially active, as in the liver and adipose tissue.

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