Evidence for the Presence of a Pool of Glycerides with a Rapid Rate of Turnover in Brown Fat from Newborn Rabbits

By B. L. KNIGHT

Medical Research Council Lipid Metabolism Unit,
Hammersmith Hospital, London W12 0HS, U.K.

(Received 25 February 1971)

1. The specific radioactivity of [^{14}C]glycerol released during the incubation of brown fat with [^{14}C]glucose is much greater than that of the tissue lipid glycerol.
2. From a study of the release of [^{14}C]glycerol from pre-labelled brown fat, it is concluded that the tissue contains a pool of glycerides with a higher rate of turnover than those in the main lipid store.
3. This pool contains newly synthesized glycerides, has a half-life of 25–30 min and supplies about 25% of the glycerol liberated by brown fat.
4. Thus, a significant fraction of the total ^{14}C incorporated from glucose into brown-fat lipids is released as [^{14}C]glycerol during an incubation.

The existence of more than one metabolic pool of glycerides in white adipose tissue has been deduced from studies on the incorporation of fatty acids into glycerides. Kerpel, Shafir & Shapiro (1961) found that the incorporation of [^{14}C]palmitate into glycerol esters by white fat ceased after 3–6 h of incubation, although the specific radioactivity of the esters was still much below that of the tissue or medium-free fatty acids. It was proposed that isotopic equilibrium had been reached between an 'active' compartment of the tissue esters and the fatty acids in the medium. Further, centrifugation of homogenates of white fat that had been incubated with [^{14}C]palmitate reveals that the specific radioactivity of the glycerides which precipitate with the cellular particles is far higher than that of the glycerides in the floating fat layer (Kerpel *et al.* 1961; Stein & Stein, 1961). Compartmentation of the glycerides in white fat was also proposed by Hirsch (1965) to explain the very slow change in the composition of the fatty acids in adipose tissue of patients who received 40% of their calorific intake as corn oil.

In the present paper evidence is presented which suggests that there is more than one metabolic pool of glyceride in brown adipose tissue.

MATERIALS AND METHODS

Treatment of animals. Two-day-old Dutch rabbits were used for all experiments. They were left with their mother until the morning of the experiment.

Experiments were performed either with unlabelled brown fat or with brown fat that had been labelled *in vivo* with ^{14}C before removal from the rabbits. Two methods were used to label the brown fat. In the first, newborn rabbits were given an intraperitoneal injection of 0.4 ml of 10 mM-[U- ^{14}C]glucose (20 $\mu\text{Ci/ml}$) and were then kept

at 36°C. After 3 h they were given a second, similar injection. After a further hour at 36°C the rabbits were killed by breaking their necks and the brown fat was removed. In the second method, rabbits were kept at 36°C and given three intraperitoneal injections of 0.3 ml of 10 mM-[U- ^{14}C]glucose (20 $\mu\text{Ci/ml}$) with 3 h between each injection. The rabbits were killed after a further 20 h at 36°C. Blood was obtained by heart puncture just before the rabbits were killed.

Incubation of brown fat. Brown fat was placed in Krebs–Ringer bicarbonate buffer, pH 7.4, and connective tissue was quickly removed. The brown fat was cut with a tissue chopper (McIlwain & Buddle, 1953) into pieces weighing 3–5 mg each, which were then dispersed in fresh buffer and transferred to weighed, siliconized conical flasks containing 6 ml of incubation medium. The flasks were reweighed. Each flask contained approx. 300 mg of brown fat. The contents of the flasks were gassed for 5 min with $\text{O}_2 + \text{CO}_2$ (95:5) before incubation at 37°C. Noradrenaline, if required, was added just before the incubation was started. The composition of the incubation medium is given in the legends to the tables and figures. At the end of the incubation the tissue pieces were separated from the medium by rapid filtration under suction. Tissue that was not to be fractionated was quickly placed in 10 ml of chloroform–methanol (2:1, v/v) at the temperature of solid CO_2 –acetone. The medium was kept frozen until analysed.

Fractionation of brown fat. After the removal of the medium, brown fat was immediately homogenized in 5 ml of ice-cold 10% (w/v) sucrose. Subcellular particles and the floating fat layer were isolated essentially by the method described by Stein & Stein (1961).

Analysis of tissue lipids. The tissue or tissue fraction was homogenized in 10 ml of chloroform–methanol (2:1, v/v) in a glass tube with a motor-driven glass plunger. The homogenate was filtered, rehomogenized in another 10 ml of chloroform–methanol and the residue rinsed with a further 10 ml of chloroform–methanol. The combined extracts were washed with 7 ml of 4 mM- MgCl_2 and then

three times with the 'upper phase' solvent of Folch, Lees & Sloane-Stanley (1957). The lipid extract was evaporated to dryness under reduced pressure and redissolved in chloroform.

Triglycerides were isolated from the lipid extract by submitting a portion of the chloroform solution to t.l.c. on Kieselgel H. The plates were developed by the two-stage method described by Skipski, Smolowe, Sullivan & Barclay (1965) with a marker of glyceryl tripalmitate run on an adjacent lane. After the lipids had been stained with iodine vapour the band corresponding to the triglyceride marker was scraped from the plate and eluted with chloroform. For isolation of monoglycerides, the lipid sample, dissolved in benzene, was applied to a column (5 cm x 1 cm) of silicic acid (100 mesh) and the triglycerides were eluted with 50 ml of benzene. Mono- and diglycerides were then eluted with chloroform and separated by t.l.c. as described above. There was evidence of breakdown and rearrangement of diglycerides during the silicic acid treatment, so these compounds were not isolated. Phospholipids were removed from the particulate lipid extract by shaking the lipids, dissolved in 5 ml of light petroleum (b.p. 40–60°C), with 0.2 g of silicic acid (100 mesh, fine particles). The suspension was filtered and the glycerides eluted from the silicic acid with ether (40 ml).

Lipids were hydrolysed by heating for 1 h at 60°C in 4.5 ml of 0.3% KOH in ethanol-water (17:3, v/v). The hydrolysate was neutralized with 0.5 ml of 6% (v/v) acetic acid and evaporated to dryness. After the addition of 2 ml of 0.67 M-H₂SO₄ to the residue, the fatty acids were extracted three times with hexane. The extract was washed with 0.67 M-H₂SO₄ and assayed for radioactivity. Samples from the aqueous phase were assayed for glycerol by the method of Garland & Randle (1962) and for radioactive glycerol.

Analysis of the incubation medium. The incubation medium was acidified with HClO₄ (final concentration 50 g/l) and the precipitated protein was removed after centrifugation. Perchlorate was removed from the supernatant by centrifugation at 0°C after neutralizing with ice-cold saturated KHCO₃. Samples of the deproteinized medium were assayed for glucose with hexokinase and glucose 6-phosphate dehydrogenase (Bergmeyer, 1965) and for radioactivity.

Glycerol was assayed in the deproteinized medium and isolated by the method described by Denton & Randle (1967). To check that the L-glycerol 3-phosphate formed from the medium glycerol by the above method was free from any major radioactive contaminants, crystalline glyceryl tribenzoate was prepared from the final eluate. The eluates were applied to a column (40 cm x 3 cm) of Sephadex G-15 and the compounds were eluted with water. L-Glycerol 3-phosphate (elution volume 110–120 ml) was well separated from formate (elution volume 200–210 ml). The Sephadex column also separated L-glycerol 3-phosphate from any residual glucose 6-phosphate (elution volume 90–100 ml) or lactate (elution volume 130 ml). The L-glycerol 3-phosphate was dephosphorylated with 8 mg of acid phosphatase at room temperature and pH 4.6. When no more L-glycerol 3-phosphate remained, the acid phosphatase was precipitated by heating and removed by filtration. Carrier glycerol was added, and glyceryl tribenzoate was prepared as described by Winkler, Steele, Bjerknes, Rathgeb & Altszuler (1967). The tribenzoate

was recrystallized three times from ethanol-water (2:1, v/v), and dried in a vacuum desiccator over P₂O₅. The colourless, needle-like crystals melted at 73–74°C. The specific radioactivity of the crystals, determined by dissolving a weighed sample in 10 ml of scintillation fluid and assaying the radioactivity, was never more than 3% below that of crystals which had been twice recrystallized.

Assay of radioactivity. The extracts containing fatty acids were evaporated to dryness in counting vials under a stream of warm air and the residues were each dissolved in 2 ml of ethanol and 10 ml of scintillation fluid. For radioassay of fatty acids and glyceryl tribenzoate the scintillation fluid was a solution of 0.3% of 2,5-diphenyloxazole and 0.01% of 1,4-bis-(5-phenyloxazol-2-yl)-benzene in toluene. For the assay of radioactivity in aqueous solutions the scintillator was a mixture of 1 part of Triton X-100 to 2 parts of a solution containing 0.6% 2,5-diphenyloxazole and 0.08% 1,4-bis-(5-phenyloxazol-2-yl)benzene in xylene (Smith, Easter & Dils, 1966). Up to 1 ml of the sample was added to 10 ml of the scintillation fluid.

Radioactivity was assayed in a Beckman LS-250 scintillation spectrometer. The energy spectrum was corrected for quenching by an automatic gain control. This correction was checked with known amounts of radioactivity in differently quenched samples and was found to be reliable over the range of quenching encountered in the present experiments. Counting rates were corrected for quenching by the external standard channels-ratio method.

Materials. Whenever possible, reagents were of analytical grade. Diethyl ether and di-isopropyl ether used for chromatography were purified by passing through a column of activated alumina (type H, 100–200 mesh, from Laporte Industries Ltd., Widnes, Lancs., U.K.). Pyridine nucleotides, ATP, L-arterenol bitartrate and bovine serum albumin (fraction V powder) were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Phosphoenolpyruvate (tricyclohexylammonium salt) and all enzymes were obtained from C. F. Boehringer und Soehne, Mannheim, Germany. Pig insulin (pro actrapid) which was free from glucagon (lot A62) was supplied by Novo Industries, London E.2, U.K. Kieselgel H for t.l.c. was obtained from E. Merck Laboratory Chemicals, Darmstadt, Germany, and silicic acid (100 mesh) from Mallinckrodt Chemical Works, St Louis, Mo., U.S.A. Dowex I ion-exchange resin (AGI-X4, Cl⁻ form, 200–400 mesh) was obtained from Bio-Rad Laboratories, Richmond, Calif., U.S.A. It was converted into the formate form with 3 M-sodium formate. Sephadex G-15 was obtained from Pharmacia, Uppsala, Sweden. [U-¹⁴C]Glucose and [6-³H]glucose were obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and Triton X-100 from Lennig Chemicals Ltd., London W.C.1, U.K. The radioactive glucose was purified by removal of traces of [¹⁴C]glycerol by the method of Denton & Randle (1967).

RESULTS

Release of radioactive glycerol by brown fat incubated with [U-¹⁴C]glucose. Table 1 shows the results of experiments in which the tissue glycerides and the glycerol in the medium were isolated after

Table 1. *Release of [^{14}C]glycerol from brown fat during incubation with [$\text{U-}^{14}\text{C}$]glucose and a comparison of the specific radioactivity of the glycerol released with that of triglyceride and monoglyceride glycerol*

Pieces of brown fat were incubated in Krebs-Ringer bicarbonate buffer, pH 7.4 (with half the recommended Ca^{2+}), containing 5% (w/v) bovine serum albumin (fraction V), 10 mM-glucose, [$\text{U-}^{14}\text{C}$]glucose (0.3 $\mu\text{Ci/ml}$) and insulin (0.1 unit/ml). Noradrenaline (1 $\mu\text{g/ml}$) was present where indicated. Results are given as means $\pm$ S.E.M. of three incubations. Radioactivity is expressed as nmol of [^{14}C]glucose.

Hormones	Time of incubation (min)	Glycerol released ($\mu\text{mol/g}$ wet wt.)	[^{14}C]Glycerol released (nmol of [^{14}C]glucose/ μg wet wt.)	Specific radioactivity (nmol of [^{14}C]glucose/ μmol of glycerol)		
				Glycerol released	Tri-glyceride glycerol	Mono-glyceride glycerol
Insulin + nor-adrenaline	70	9.7 $\pm$ 0.3	686 $\pm$ 65	72.9 $\pm$ 7.6	5.6 $\pm$ 0.5	6.2 $\pm$ 2.7
	90	11.4 $\pm$ 1.5	968 $\pm$ 180	86.9 $\pm$ 4.9	9.3 $\pm$ 0.5	7.3 $\pm$ 1.2
	120	15.7 $\pm$ 0.6	1552 $\pm$ 103	101.6 $\pm$ 4.7	10.0 $\pm$ 0.2	7.5 $\pm$ 1.6
Insulin	90	1.1 $\pm$ 0.1	211 $\pm$ 99	163.9 $\pm$ 54.3	3.3 $\pm$ 0.6	7.9 $\pm$ 2.3

incubation of brown fat with [$\text{U-}^{14}\text{C}$]glucose. In the presence of noradrenaline, glycerol was released by brown fat at a constant rate. [^{14}C]Glycerol was released at a much faster rate, so that the specific radioactivity of the glycerol in the medium rose as the incubation proceeded. The specific radioactivity of the tissue triglyceride glycerol was much lower than that of the glycerol released into the medium. The specific radioactivity of the monoglyceride glycerol resembled that of the triglycerides rather than that of the medium glycerol. In the absence of noradrenaline the release of both glycerol and [^{14}C]glycerol by brown fat was depressed, but the specific radioactivity of the glycerol liberated was again higher than that of the glycerides.

In the experiments shown in Table 1, the glycerol in the incubation medium was isolated by the method described by Denton & Randle (1967). In this method, radioactive glucose is enzymically phosphorylated to glucose 6-phosphate, which is then removed by an ion-exchange resin. The remaining metabolites are treated with glycerokinase and applied to a second column of ion-exchange resin. Because of the specificity of the enzymes employed, L-glycerol 3-phosphate derived from glycerol in the medium should be the only compound retained on the column, and this is eluted with formate ions. In view of the unexpectedly large amount of radioactivity that was recovered in the present experiments, the purity of L-glycerol 3-phosphate in the final eluate was checked by converting a part of it into glyceryl tribenzoate. The mean ($\pm$ S.E.M.) specific radioactivity of the glycerol in the medium from three incubations, calculated from the radioactivity isolated by the Denton & Randle (1967) method was 2140 ± 360 d.p.m./ μmol , whereas that calculated from the specific radioactivity of the tribenzoate was 2430 ± 30 d.p.m./ μmol . The specific radioactivity of

the brown-fat glyceride glycerol in these incubations was 395 ± 15 d.p.m./ μmol . It is clear, therefore, that the determinations of the specific radioactivity of the glycerol released from brown fat were not erroneously high because of contamination of the L-glycerol 3-phosphate with other radioactive compounds.

Loss of [^{14}C]glycerol from pre-labelled brown fat. The previous results indicate that the glycerol released from brown fat incubated with [^{14}C]glucose contains much more radioactivity than would be expected if it were all derived from the large lipid store of the cell. Some of the glycerol must, therefore, have been released from a pool, or pools, of a metabolite with a high specific radioactivity and a rapid rate of turnover. In an attempt to provide more information about this process, the loss of [^{14}C]glycerol from brown fat was measured when tissue that had been labelled with ^{14}C was incubated in medium containing no ^{14}C precursor.

In Expts. A and C, brown fat was removed shortly after a brief period of labelling (see the Materials and Methods section), when the specific radioactivity of a pool with rapid turnover would be expected to be relatively high. The specific radioactivity of the glycerol released during incubation *in vitro* should therefore fall rapidly as the ^{14}C in the pool with rapid turnover is replaced. Investigation of the radioactivity of the lipids in the brown fat should show whether or not the glycerol in this pool is present as glyceride. In Expts. B and D, brown fat was removed 20 h after the last injection of [^{14}C]glucose, when the amount of radioactivity in the blood of the rabbits had fallen to less than 10% of that in Expts. A and C. Under these conditions, a pool with rapid turnover would be expected to be poorly labelled in comparison with the bulk of the tissue lipid, and the specific radioactivity of the glycerol released during the subsequent incubation

Fig. 1. Specific radioactivity of lipid glycerol in brown fat which was pre-labelled with [^{14}C]glucose and incubated with [$6\text{-}^3\text{H}$]glucose. ^{14}C had previously been incorporated into brown-fat lipids by injecting newborn rabbits with [$\text{U-}^{14}\text{C}$]glucose. Tissue pieces were incubated in Krebs-Ringer bicarbonate buffer, pH 7.4 (with half the recommended Ca^{2+}), containing 5% (w/v) bovine serum albumin (fraction V), 10 mM-glucose, [$6\text{-}^3\text{H}$]glucose ($0.4\text{ }\mu\text{Ci/ml}$), insulin (0.1 unit/ml) and noradrenaline ($1\text{ }\mu\text{g/ml}$). Each point represents the mean of the results from three incubations. The results given are specific radioactivity of ^{14}C in total lipid ($\square$) and triglyceride ($\blacksquare$) glycerol, and the specific radioactivity of ^3H in total lipid ($\circ$) and triglyceride ($\bullet$) glycerol. (a) Expt. A, in which brown fat was removed from a rabbit with high plasma glucose specific radioactivity. (b) Expt. B, in which brown fat was removed from a rabbit with low plasma glucose specific radioactivity.

should be lower than that of the glycerol in the tissue lipids as a whole.

In Expts. A and B, triglycerides were isolated from the total brown-fat lipids. The pieces of brown fat were incubated in medium containing [$6\text{-}^3\text{H}$]glucose to check that glyceride synthesis was proceeding normally. As the incubation proceeded, brown fat incorporated [$6\text{-}^3\text{H}$]glucose into lipids. The rise in specific radioactivity of the glycerol in the triglyceride fraction lagged slightly behind that of the total lipid glycerol (Fig. 1). The specific radioactivity of the [^{14}C]glycerol of the triglycerides did not change greatly during either experiment (Fig. 1). However, in Expt. A (Fig. 1a), the specific radioactivity of the glycerol in the lipids, which was similar to that of the triglycerides at zero time, fell by 20% during the first hour of incubation. In Expt. B (Fig. 1b) the specific radioactivity of the glycerol in the total lipids was 80% of that of the

triglycerides at the beginning of the incubation, and did not fall during the next 2 h. The specific radioactivity of the [^{14}C]labelled fatty acids showed changes similar to those of the lipid glycerol in both experiments.

Fig. 2 shows the release of glycerol and [^{14}C]glycerol from the brown fat in Expts. A and B. As the incubations proceeded, the rate of release of [^{14}C]glycerol decreased more rapidly than that of the glycerol, so that the specific radioactivity of the glycerol in the medium fell. The curves of glycerol and [^{14}C]glycerol release were used to calculate the specific radioactivity of the glycerol liberated in successive periods of 0.25 h as shown in Fig. 3. The specific radioactivities of the glycerol in triglyceride and total lipid are included for comparison. In Expt. A (Fig. 3a), the specific radioactivity of the glycerol released in the first 0.25 h of incubation was about three times that of the tissue lipid glycerol. As the incubation proceeded, the specific radioactivity of the liberated glycerol gradually fell, eventually reaching a value below that of the triglyceride glycerol. In Expt. B (Fig. 3b), the specific radioactivity of the glycerol released during the first 0.25 h was slightly above that of the tissue triglyceride. Subsequently it fell to an amount comparable with that of the total lipid glycerol. If it is assumed that the pool with a rapid rate of turnover contained no radioactivity at the end of Expt. B, then it can be estimated that less than 75% of the glycerol released was derived from the large triglyceride pool.

Expts. C and D confirm that the rate of fall of the specific radioactivity of the glycerol released into the medium from pre-labelled brown fat depends on the method of labelling. Thus, when the specific radioactivity of a pool with rapid turnover is expected to be initially high (Expt. C), that of the glycerol released falls during the incubation (Fig. 4a), whereas when the pool with rapid turnover is poorly labelled (Expt. D), the specific radioactivity of the glycerol liberated remains relatively constant. In Expts. C and D the brown fat was homogenized after incubation and lipids were extracted from the subcellular particles and from the floating fat layer obtained by centrifugation of the homogenates (see the Materials and Methods section). The specific radioactivity of the fat-cake glyceride glycerol did not change greatly in either experiment (Fig. 4); that of the glycerol of the particulate glycerides in Expt. C, however, closely resembled that of the glycerol released (Fig. 4a). (The radioactivity present in the particulate glycerides in Expt. D was too low to give a significant result.) About 50% of the glycerol in the particulate fat is present in phospholipids. If the particulate lipid extract is not treated with silicic acid the radioactivity in the phospholipids overshadows that in the gly-

Fig. 2. Glycerol (○) and [^{14}C]glycerol (●) release from brown fat which had been pre-labelled with ^{14}C , and the specific radioactivity of the glycerol in the medium (△). See legend to Fig. 1 for details. (a) Expt. A; (b) Expt. B.

Fig. 3. Comparison between the specific radioactivities of brown-fat total lipid (□) and triglyceride (■) glycerol and glycerol released into the medium every 0.25 h (histogram). See legend to Fig. 1 for incubation conditions and text for details. The histogram values were calculated from the curves in Fig. 2. (a) Expt. A; (b) Expt. B.

cerides, and the specific radioactivity of the total particulate lipid glycerol is much higher than that of the glycerol released. It can be seen from Fig. 4(a) that there are at least two components of the particulate phospholipids, one with a high specific radioactivity but a slow rate of turnover, and another with a rapid rate of turnover (about 15 min). In Expt. D the component with the more rapid turnover was not labelled.

DISCUSSION

The specific radioactivity of the glycerol released by brown fat incubated with [$\text{U-}^{14}\text{C}$]glucose is much higher than that of the glycerol of the total tissue lipids. A similar conclusion can be drawn for white fat from observations of Denton & Randle (1967). Heart, however, which does not store droplets of lipid within the cell, releases glycerol of a specific radioactivity which is equal to, or lower than, that of the lipid glycerol (Denton & Randle, 1967).

It is clear, therefore, that some of the glycerol released by adipose tissue is derived not from the large lipid store within the cell, but from a compound on the pathway of triglyceride synthesis or from newly formed triglyceride before it has mixed with the main store of triglyceride. With white fat, the amount of ^{14}C released into the medium as glycerol is small when compared with that remaining in the

Fig. 4. Specific radioactivities of the glycerol in total particulate lipids (O), particulate glycerides (▲) and fat-cake (●), and that of glycerol released (histogram) from pre-labelled brown fat *in vitro*. See legend to Fig. 1 for incubation details. The histogram was calculated from curves of glycerol and [^{14}C]glycerol release. (a) Expt. C, in which brown fat was removed from a rabbit with high plasma glucose specific radioactivity. (b) Expt. D, in which brown fat was removed from a rabbit with low plasma glucose specific radioactivity.

lipid glycerol of the tissue at the end of the incubation (Denton & Randle, 1967). With brown fat, however, the amount of ^{14}C present in the medium glycerol at the end of the incubation is comparable with that in the lipid glycerol. If glyceride synthesis is to be measured, it is important to know whether the glycerol of high specific radioactivity is produced by dephosphorylation of L-glycerol 3-phosphate or by hydrolysis of some other esterified intermediate.

Homogenates of white fat have been reported to hydrolyse L-glycerol 3-phosphate at acid pH (Vaughan, 1961). However, there is no evidence for the presence in brown fat of a phosphatase capable of hydrolysing L-glycerol 3-phosphate. When Kornacker & Ball (1968) added L-glycerol 3-phosphate to homogenates of brown fat from adult rats they recovered over 95% as phosphorylated intermediates. This suggests that if a glycerophosphate phosphatase is present in brown fat, its activity is very low. Further, the more rapid fall

Fig. 5. Specific radioactivity of tissue particulate glyceride glycerol (▲) compared with that of a high-specific-radioactivity component of glycerol (Δ) released from pre-labelled brown fat *in vitro*. The values for the high-specific-radioactivity component were calculated from glycerol and [^{14}C]glycerol release, by assuming that 75% of the glycerol liberated is derived from fat-cake glycerides. The results were obtained from Expt. C (Fig. 4).

of the specific radioactivity of the total lipid glycerol compared with that of the triglyceride glycerol in pre-labelled brown fat (Fig. 1a) indicates that the tissue contains a lipid component with a rapid rate of turnover. The amount of ^{14}C lost from this component was more than enough to account for the ^{14}C found in medium glycerol and as CO_2 .

It was deduced from Fig. 3(b) that less than 75% of the glycerol released was derived from the large triglyceride pool. If it is assumed that 75% of the glycerol released in Expt. C is derived from the 'fat-cake', then the specific radioactivity of the remaining 25% of the glycerol declines exponentially with a half-life of about 30 min (Fig. 5). This is similar to the half-life of the 'active' compartment of glycerides described by Stein & Stein (1961), and is clearly longer than that of the phospholipid glycerol with a rapid rate of turnover in the particulate fraction (Fig. 4). Since these phospholipids cannot have a half-life shorter than that of their precursor L-glycerol 3-phosphate, it is unlikely that the high specific radioactivity glycerol is derived either directly from phospholipid or from L-glycerol 3-phosphate. Previous experiments, in which the specific radioactivity of L-glycerol 3-phosphate was measured directly, also indicate that the half-life of L-glycerol 3-phosphate in brown fat is much less than 30 min (B. L. Knight & N. B. Myant, un-

published work). The decay of the specific radioactivity of the glyceride glycerol in the particulate fraction (Fig. 5) indicates that at least two components are present. The least stable component has a high specific radioactivity and a half-life of between 30 and 45 min. This component, which must contain newly synthesized glycerides, could, therefore, be the source of the high-specific-radioactivity glycerol. In view of the similarity between the specific radioactivities of the glycerol released and the particulate glyceride glycerol, it is tempting to speculate that the glycerol in the medium is derived by the hydrolysis of particulate glycerides. This would, in turn, suggest that lipids are transported from the fat droplets and mixed with newly formed glycerides before they are hydrolysed.

Finally, the results of the present work indicate that a significant fraction of the ^{14}C of glucose incorporated into brown-fat lipids can be released as [^{14}C]glycerol during an incubation. This cannot be ignored in any estimate of glyceride synthesis based on the measurement of [^{14}C]glucose incorporation.

I thank Dr N. B. Myant for many helpful discussions.

REFERENCES

- Bergmeyer, H.-U. (Ed.) (1965). *Methods of Enzymatic Analysis*. New York: Academic Press Inc.
- Denton, R. M. & Randle, P. J. (1967). *Biochem. J.* **104**, 423.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
- Garland, P. B. & Randle, P. J. (1962). *Nature, Lond.*, **196**, 987.
- Hirsch, J. (1965). In *Handbook of Physiology, Section 5: Adipose Tissue*, pp. 181–189. Ed. by Renold, A. E. & Cahill, G. F., jun. Washington D.C.: American Physiological Society.
- Kerpel, S., Shafrir, E. & Shapiro, B. (1961). *Biochim. biophys. Acta*, **46**, 495.
- Kornacker, M. S. & Ball, E. G. (1968). *J. biol. Chem.* **243**, 1638.
- McIlwain, H. & Buddle, H. L. (1953). *Biochem. J.* **53**, 412.
- Skipiski, V. P., Smolowe, A. F., Sullivan, R. C. & Barclay, M. (1965). *Biochim. biophys. Acta*, **106**, 386.
- Smith, S., Easter, D. J. & Dils, R. (1966). *Biochim. biophys. Acta*, **126**, 445.
- Stein, Y. & Stein, O. (1961). *Biochim. biophys. Acta*, **54**, 555.
- Vaughan, M. (1961). *J. Lipid Res.* **2**, 293.
- Winkler, B., Steele, R., Bjerknes, C., Rathgeb, I. & Altszuler, N. (1967). *J. appl. Physiol.* **23**, 752.