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Table 1. *Effect of 48 h of starvation on maternal and foetal plasma radioactivity after the infusion through the left uterine artery with either* L-|U-^{14}C|*alanine or* |U-^{14}C|*glycerol in the 21-day-pregnant rat*

Infusions were performed for 20 min under pentobarbital anaesthesia with the tracers diluted in 0.9% NaCl, at the rate of 12.5 μl/min. Values were always corrected by considering 1×10^6 c.p.m. as the total infused radioactivity per rat, and are expressed as means ± s.e.m. for five rats per group. Statistical significance between fed and 48 h starved rats is shown by the row of *p* values, and for those between a mother and her foetuses by asterisks, and those between the left and the right horns by crosses: *,† = $P < 0.05$; **,†† = $P < 0.01$; ***,††† = $P < 0.001$ (NS, not significant).

| | Total radioactivity in plasma (c.p.m./ml) | | | |
	Mother	Right-uterine-horn foetuses	Mother/right-uterine-horn-foetus ratio	Left-uterine-horn foetuses
|U-^{14}C|Alanine				
Fed	2323 ± 230	1251 ± 70**	1.85 ± 0.14	12223 ± 2097***†††
48 h starved	3830 ± 159	1826 ± 99***	2.12 ± 0.14	10627 ± 496***†††
P	<0.001	<0.001	NS	NS
|U-^{14}C|Glycerol				
Fed	4798 ± 287	1650 ± 57***	2.94 ± 0.25	3594 ± 407*†††
48 h starved	6111 ± 225	1965 ± 107***	3.14 ± 0.14	3990 ± 213***†††
P	<0.01	<0.05	NS	NS

to foetuses of starved mothers is an indirect consequence of its changes in the maternal circulation.

When [U-^{14}C]glycerol was infused through the left uterine artery, radioactivity present in plasma of foetuses from that side was lower than in maternal plasma and much lower than when L-|U-^{14}C|alanine was infused (Table 1). This result suggests that transfer of glycerol from mother to foetus is quantitatively less important than that of L-alanine and agrees with the finding that, unlike that of amino acids, glycerol transfer through the placenta is a passive process which depends on differences in maternal/foetal concentration. In any case, plasma radioactivity in foetuses from the left side was significantly higher than in those from the right side (Table 1) and, although maternal starvation did not affect that parameter in plasma of foetuses from the left side, it significantly enhanced plasma radioactivity in both mother and foetuses from the right side (Table 1). As occurred with L-|U-^{14}C|alanine, maternal/right-side-foetal ratios were unaffected by maternal starvation, Owing to the known rapid conversion of administered [^{14}C]glycerol into glucose in the rat (Carmaniu & Herrera, 1980), enhanced radioactivity values in maternal plasma with starvation after the infusion with |U-^{14}C|glycerol could well be the result of enhanced gluconeogenesis. Changes found in plasma of foetuses from the right side probably correspond to placental transfer of newly formed glucose in the mother.

In summary, the present results indicate that the actual placental transfer of L-alanine and glycerol is unaffected by maternal food deprivation, and consequently changes in concentrations of these metabolites in maternal circulation parallel their actual availability to the foetus.

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Method for the infusion of periuterine adipose tissue *in situ* in the rat

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White-adipose-tissue metabolism is normally studied in preparations 'in vitro', as most tracers administered to intact animals are rapidly transformed by other organs, mainly the liver, before they are taken up by that tissue. Studies 'in vivo' with adipose tissue are, however, required to determine whether findings from experiments 'in vitro' may be extrapolated to the intact animal, and how endogenous events (e.g. changes in the availability of substrates, in nervous activity etc.) affect its metabolism. In the present work we have developed a technique for the study *in situ* of periuterine adipose tissue metabolism in

rats, based on our method for placental metabolite transfer in the late-pregnant rat (Lasunción *et al.*, 1983). This technique consists of the infusion of ^{14}C-labelled substrates through the left uterine artery and comparison of the radioactivity present in lipids of periuterine adipose tissue in the left side with that on the right side. With this method, the periuterine adipose tissue in the left side received the infused substrate directly, whereas in the right side it was received after being diluted in the intact animal.

Female Wistar rats fed *ad libitum* were anaesthetized with sodium pentobarbital (33 mg/kg body wt., administered intravenously). After laparatomy, the hypogastric trunk, superior gluteal, superior external pudendal and deep circumflex arteries of the left side were clamped. A cannula (PE-10; Intramedic, U.S.A.) was introduced through the left external iliac artery and placed at the beginning of the left uterine artery so that medium

Table 1. ^{14}C-labelled lipids in periuterine adipose tissue after 20min infusion of ^{14}C-labelled substrates through the left uterine artery in the fed rat

^{14}C-labelled substrates were diluted in tracer amounts in 0.9% NaCl and infused for 20min through a cannula placed at the beginning of the left uterine artery. Lipids were purified separately from the left- and right-side periuterine adipose tissues, and values were corrected by considering 1×10^6c.p.m. as the total radioactivity infused per rat. Values are means ± s.e.m. for five rats per group. Statistical significance between (II) and (III) versus (I) is shown by the rows of P values, $(t_{Independent})$, and those between the left and right sides by asterisks $(t_{dependent})$: $*P < 0.05$, $**P < 0.01$ (NS, not significant).

| | Radioactivity (c.p.m./g fresh wt. of tissue) | | | |
| | Adipose tissue from the left side | | Adipose tissue from the right side | |
Infused substrate	^{14}C-labelled fatty acids	^{14}C-labelled fatty acids as % of ^{14}C-labelled total lipids	^{14}C-labelled fatty acids	^{14}C-labelled fatty acids as % of ^{14}C-labelled total lipids
(I) L-[U-^{14}C]Alanine	35680 ± 8012	92 ± 3	1587 ± 622**	92 ± 1[NS]
(II) D-[U-^{14}C]Glucose	1974 ± 643	41 ± 8	287 ± 124*	36 ± 10[NS]
P	<0.01	<0.001	NS	<0.001
(III) [U-^{14}C]Glycerol	1697 ± 624	32 ± 8	284 ± 131*	41 ± 11[NS]
P	<0.01	<0.001	NS	<0.01

infused through the cannula combines with blood circulating through the left uterine artery. After surgery, abdominal viscerae were conveniently placed in the abdominal cavity and the abdomen was closed for the rest of the experiment. The tracer, diluted in 0.9% NaCl, was infused with a Gilson peristaltic pump at the rate of 12.5μl/min. After 20min of infusion, the experiment was terminated by separate dissection of the left and right periuterine adipose tissues, which were then thoroughly rinsed with 0.9% NaCl and placed in chloroform/methanol (2:1, v/v) for lipid extraction (Folch et al., 1957) and fractionation as previously described (Dominguez & Herrera, 1976). Radioactivity measurements were made in a xylene/Triton X-100-based scintillation 'cocktail', and values were always corrected by considering 1×10^6c.p.m. as the total amount of ^{14}C-labelled substrate infused in each rat.

After 20min of infusion of D-[U-^{14}C]glucose (257mCi/mmol), [U-^{14}C]glycerol (171mCi/mmol) or L-[U-^{14}C]alanine (10mCi/mmol) (from The Radiochemical Centre, Amersham, Bucks., U.K.) in fed rats through the left uterine artery, much more radioactivity was incorporated into lipids of the left than of the right periuterine adipose tissue (Table 1). These results indicate the greater specific radioactivity of the labelled substrates reaching the left than the right periuterine adipose tissue, and values for ^{14}C-labelled lipids in the left-side tissue give an index of its direct utilization of these substrates for lipid synthesis.

As shown in Table 1, the formation of ^{14}C-labelled fatty acids from L-[U-^{14}C]alanine in periuterine adipose tissue in the left side was greater than that from D-[U-^{14}C]glucose. The plasma concentration of L-alanine is, however, lower than that of glucose (Metzger & Freinkel, 1975), showing that the specific radioactivity of labelled L-alanine reaching the tissue was higher than that of glucose, and the actual contribution of either substrate to lipogenesis was probably of similar magnitude. Comparison of percentages of ^{14}C-labelled fatty acid with ^{14}C-labelled total lipids demonstrated that values were much higher for alanine than for glucose (Table 1), so that, although most of the L-[U-^{14}C]alanine was incorporated into adipose-tissue lipids through lipogenesis, a considerable proportion of [U-^{14}C]glucose was used for glyceride glycerol formation. The present findings are consonant with the comparative utilization of labelled L-alanine (Bellido & Herrera, 1978) and glucose (Dominguez & Herrera, 1975) for the formation of fatty acids and glyceride glycerol in experiments in vitro with rat adipose tissue from other sources, validating the present technique.

This technique was also applied to determine whether adipose tissue infused in situ uses labelled glycerol for lipid synthesis, as reported for preparations in vitro (Herrera & Ayanz, 1972;

Dominguez & Herrera, 1976). As shown in Table 1, after the infusion of [U-^{14}C]glycerol through the left uterine artery, a greater amount of ^{14}C-labelled fatty acids appeared in the periuterine adipose tissue of the left as compared with the right side, as occurred with the other substrates utilized. As also shown in Table 1, the percentage contribution of the ^{14}C-labelled fatty acid fraction to the ^{14}C-labelled total lipids was much lower when labelled glycerol was used than with labelled alanine, indicating that a higher proportion of the lipids formed from glycerol corresponded to the glyceride glycerol fraction, as found with labelled glucose (Table 1). Present results demonstrate that white adipose tissue in vivo utilizes glycerol for the synthesis of lipids, confirming previous findings from experiments in vitro (Herrera & Ayanz, 1972; Dominguez & Herrera, 1976). Although the plasma concentration of glycerol is low (Carmaniu & Herrera, 1980) compared with that of L-alanine and glucose, it should be emphasized that the specific radioactivity of glycerol reaching the infused adipose tissue is greater than that of the other substrates, and consequently the contribution of circulating glycerol to lipid synthesis in adipose tissue in vivo is much smaller than that of the other substrates studied, a finding which coincides with the low glycerol kinase activity found in adipose tissue (Robinson & Newsholme, 1967). This activity is augmented in some conditions such as obesity (Trebel & Mayer, 1963) or prolonged insulin treatment (Koschinsky et al., 1971), and the present technique would make it possible to establish whether this activity results in a greater contribution of circulating glycerol to the deposition of lipids.

In summary, infusion of ^{14}C-labelled substrates through the left uterine artery is a useful technique for the study of adipose-tissue metabolism in situ, allowing determination of how endogenous factors modulate the actual contribution of different substrates to lipid synthesis in the tissue. Combined infusion of the ^{14}C-labelled substrates with other components (hormones, drugs, etc.) would permit evaluation of their acute effects on this preparation.

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Electron-microscopic immunolocalization of proteoglycan in articular cartilage

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Proteoglycans together with collagen are the major components of the extracellular matrix of articular cartilage, and confer on the cartilage stiffness in compression and elasticity (Muir, 1979). The present paper reports an electron-microscopic immunolocalization study using antibodies specific to the hyaluronate-binding region of proteoglycan to show the variation of aggregating proteoglycan through the depth of articular cartilage. The distribution of the immunolabel has been quantified and compared with the results obtained by using the same antiserum in a radioimmunoassay to measure aggregating proteoglycan in extracts from serial sections of the same tissue.

Full-depth slices of pig articular cartilage were cut into serial sections (20 μm) from the articular surface down to the subchondrial bone; the proteoglycans were extracted with 4 M-guanidinium chloride (Hardingham, 1979) and the binding-region content was analysed by radioimmunoassay (Ratcliffe & Hardingham, 1983). For immuno-electron microscopy the cartilage was fixed and embedded in Lowicryl K4M low-temperature resin (Fryer et al., 1983). The immunolocalization studies were performed on the surface of ultrathin sections mounted on nickel grids. Each section was incubated with chondroitinase ABC (0.5 unit/ml) for 45 min at 37°C, and washed twice by incubation for 5 min each in phosphate-buffered saline, pH 7.3, containing 1% (w/v) bovine serum albumin. The sections were then incubated with the anti-(binding region) antiserum diluted in phosphate-buffered saline/1% albumin for 2 h, washed in the same medium, and incubated for 1 h in a solution of protein-A-coated gold particles (12 nm diameter). The grids were washed with phosphate-buffered saline and finally in distilled water. The sections were stained and viewed in a Philips 300 electron microscope. The intensity of the protein-A–gold labelling was measured by using the Magiscan image-analysis system.

The density of the protein-A–gold labelling in the pericellular and intercellular matrix of the cartilage sections has been quantified in the superficial zone (extending 30 μm from the surface of the cartilage), and in the middle (80–260 μm) and deep (280–480 μm) zones (Fig. 1). Within each zone a significant proportion of the densities of staining in the pericellular matrix was above the range for the intercellular matrix, and the average pericellular-matrix staining was higher than that for the intercellular-matrix staining by 30–40%. In the intercellular matrix the highest amount of labelling for binding region was in the middle zone, whereas the extent of staining in the superficial zone was 54% of that in the middle zone, and that in the deep zone was 84%.

A similar distribution of binding region was found by radioimmunoassay. The middle zone was found to contain the highest amount of binding region, the deep zone having only 79% that of the middle zone, and the superficial zone containing the last amount, 61%.

The results of the electron-microscopic immunolocalization showed a good correlation with the radioimmunoassay of extracted proteoglycans from the same tissue and thus offered a technique for the quantitative assessment of the distribution of components in cartilage.
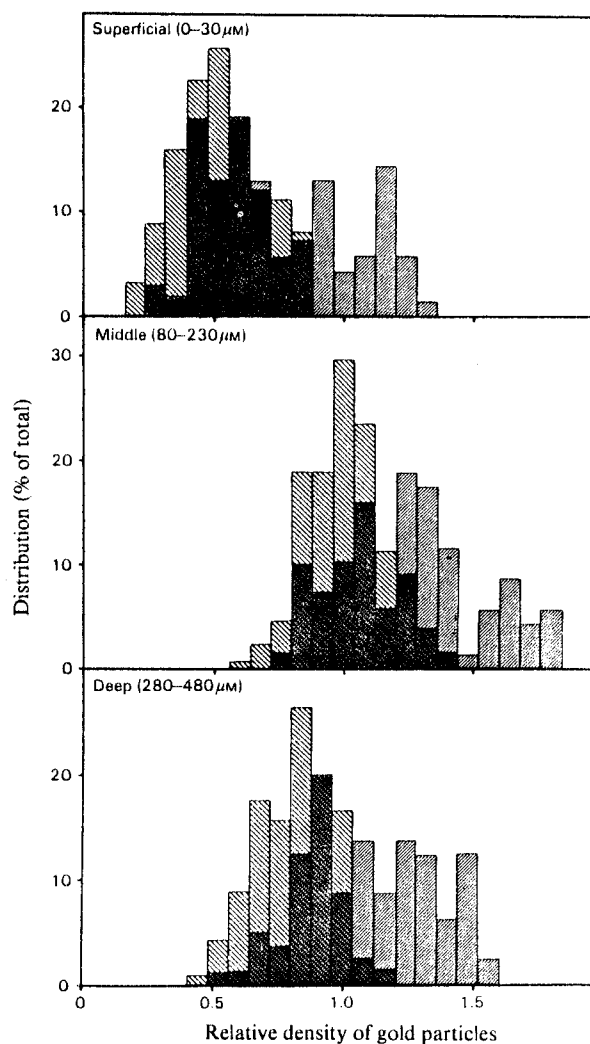

Fig. 1. *Distribution of protein-A–gold labelling of proteo glycan-binding region in articular cartilage*

The extent of protein-A–gold labelling was measured in the superficial, middle and deep zones, and within each zone in the pericellular (▨) and intercellular (▩) matrix. To allow for the variation between experiments in the overall intensity of labelling, the results of each experiment have been calculated relative to the extent of staining in the intercellular matrix of the middle zone.