circulation, and this process seems to be independent of the administered substrate. Since glycogen stores remain low in spite of parallel enhancement of liver glycogenolysis and gluconeogenesis with starvation, it may be proposed that in this condition a considerable proportion of gluconeogenic substrates is initially transformed to liver glycogen, which becomes a direct precursor of circulating glucose. Enzymic conditions for maintaining simultaneous enhancement of liver glycogenesis and glycogenolysis have been identified in the starved rat liver (Roach & Larner, 1976; Hems & Whitton, 1980). This persistent futile cycle in the presence of increased gluconeogenesis may have two main functions: (i) to ensure maintenance of a permanent minimum glycogen content in liver, which is required as primer, and (ii) to allow rapid recovery of glycogen stores whenever food is available after starvation while gluconeogenesis is still augmented.

Formation of glyceride glycerol from gluconeogenic substrates may be facilitated in the starved condition by the cytosolic redox state of the liver (Lindall & Lazarow, 1964; Williamson *et al.*, 1967; Berdanier *et al.*, 1979) facilitating esterification of incoming fatty acids. This hypothesis is supported by the augmented proportional formation of [^{14}C]-glyceride glycerol from the administration tracers in the starved late-pregnant rats (Table 1), where it is known that lipolysis is greatly enhanced (Leat & Ford, 1966; Knopp *et al.*, 1970), inducing the liver to increase its contribution of gluconeogenetic substrates for the synthesis of glyceride glycerol. This explanation also coincides with the fact that liver is the main receptor site of unesterified fatty acids released from adipose tissue by lipolysis (Mampel *et al.*, 1981), which is known to be enhanced in the starved condition (Schimmel & Knobil, 1970). Esterification of fatty acids in liver also changes proportionally according to fatty acids availability (Debeer *et al.*, 1981); thus deviation of gluconeogenic intermediates to glyceride glycerol formation in the starved condition accounts for the temporary esterification of the incoming fatty acids in the liver.

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Transfer from mother to foetus of L-alanine and glycerol in fed and 48 h-starved pregnant rats

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Maternal malnutrition or starvation in the rat at late gestation causes intrauterine growth retardation (Rosso, 1977; Anderson *et al.*, 1980; Ahokas *et al.*, 1981). Although it has been hypothesized that decreased maternal–foetal nutrient transfer may occur during periods of food restriction, this has not been directly tested in the rat. Placental transfer in this species has only been examined indirectly by giving a radioactive substrate to the mother and determining its appearance in the foetus. With this method, the administered tracer is diluted in the maternal circulation before it becomes available to the placenta. Metabolic adaptation occurring in the late-pregnant rat is augmented in the starved condition (Freinkel, 1965), thus differences in the availability of substrates to the foetus when the mother is deprived of food may be an indirect consequence of changes in maternal metabolism, and/or an alteration in the actual placental transfer activity. To determine which of these possibilities is correct, in the present study our method (Lasunción *et al.*, 1983) for determination of the metabolite transfer from rat mother to her foetuses was used in fed and 48 h-starved 21-day-pregnant rats. The method consists basically of a 20 min infusion through the left uterine artery of a medium containing a ^{14}C-labelled metabolite. In this way, the left uterine horn received the tracer directly, whereas it reached the right horn after dilution in the mother's circulation. Therefore analysis of radioactivity present in foetal blood from both uterine sides and in the maternal circulation permits estimation of actual nutrient transfer.

In the present study, L-[U-^{14}C]alanine and [U-^{14}C]glycerol were used as tracers, since they cross the placenta by different mechanisms: the first by active transport against a gradient (Hill & Young, 1973), and the second by diffusion in favour of the gradient (Gilbert, 1977). It is also known that circulating concentrations of these maternal metabolites are affected conversely by starvation: L-alanine decreases (Girard *et al.*, 1977), glycerol increases (Girard *et al.*, 1977). As shown in Table 1, 20 min after infusion of L-[U-^{14}C]alanine through the left uterine artery, total radioactivity in plasma of foetuses from the left uterine horn was much greater than in their mothers, whereas radioactivity in plasma of foetuses from the right side was significantly lower than in the mother's plasma or in foetuses from the left side. These results confirm previous findings (Hill & Young, 1973) and indicate that, whereas plasma radioactivity in foetuses from the left side corresponds to the direct transfer of L-[U-^{14}C]alanine infused through the left uterine artery, plasma radioactivity present in the foetuses from the right side results from dilution of the tracer in the maternal circulation. When the same experiment was conducted in pregnant rats after 48 h of starvation, the radioactivity present in plasma of foetuses from the left uterine horn did not differ from that in foetuses from the same side in fed mothers (Table 1). There was, however, a significant increase in total plasma radioactivity in foetuses from the right side in starved as compared with fed mothers. This difference parallels the increased plasma radioactivity in the 48 h-starved as compared with the fed mothers (Table 1); thus the ratio of plasma radioactivity in mothers and their foetuses remained unchanged in both fed and starved conditions. The present results indicate that maternal-foetal transfer of L-alanine is not affected by starvation, and any change in the availability of this amino acid

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
L-[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