Biochem. J. (1985) **228**, 103–110
Printed in Great Britain

A method to quantify glucose utilization *in vivo* in skeletal muscle and white adipose tissue of the anaesthetized rat

Pascal FERRÉ, Armelle LETURQUE, Anne-Françoise BURNOL, Luc PENICAUD and Jean GIRARD
Centre de Recherches sur la Nutrition, CNRS, 9 rue Jules Hetzel, 92190 Meudon-Bellevue, France

(*Received 25 October 1984/4 February 1985; accepted 13 February 1985*)

A quantitative method allowing determination of glucose metabolism *in vivo* in muscles and white adipose tissue of the anaesthetized rat is presented. A tracer dose of 2-deoxy[^{3}H]glucose was injected intravenously in an anaesthetized rat and the concentration of 2-deoxy[^{3}H]glucose was monitored in arterial blood. After 30–80 min, three muscles, the soleus, the extensor digitorum longus and the epitrochlearis, periovarian white adipose tissue and brain were sampled and analysed for their content of 2-deoxy[^{3}H]glucose 6-phosphate. This content could be related to glucose utilization during the same time period, since (1) the integral of the decrease of 2-deoxy[^{3}H]glucose in arterial blood was known and (2) correction factors for the analogue effect of 2-deoxyglucose compared with glucose in the transport and phosphorylation steps were determined from experiments *in vitro*. Glucose utilization was then measured by this technique in the tissues of post-absorptive rats in the basal state (0.1 munit of insulin/ml of plasma) or during euglycaemic–hyperinsulinaemic glucose clamp (8 munits of insulin/ml of plasma) and of 48 h-starved rats. Results corresponded qualitatively and quantitatively to the known physiological characteristics of the tissues studied.

The measurement of glucose turnover rate or glucose kinetics, coupled with the euglycaemic or hyperglycaemic glucose-clamp technique, provide quantitative information on whole-body glucose metabolism and its regulation *in vivo* in small laboratory animals. Quantitative measurements of glucose metabolism in individual tissues would allow further understanding of regulatory processes. A technique using 2-deoxy[^{14}C]glucose has been validated for the study of brain glucose utilization *in vivo* (Sokoloff *et al.*, 1977). More recently, two groups have proposed simplified 2-deoxy[^{3}H]glucose techniques to study insulin-sensitivity of glucose metabolism in various tissues of unanaesthetized rats (Hom *et al.*, 1984; Kraegen *et al.*, 1984). However, these methods do not allow quantification of glucose utilization by different tissues, since correction factors for the discrimination against 2-deoxyglucose metabolism in glucose pathways are not measured. Kraegen *et al.* (1984) have coupled the 2-deoxyglucose technique with the euglycaemic–hyperinsulinaemic clamp, which allows one to measure both glycaemia and glucose utilization in steady-state conditions, as required by the theoretical model (Sokoloff *et al.*, 1977). In contrast, Hom *et al.* (1984) have tested insulin-sensitivity in non-steady-state conditions (hypoglycaemia). Moreover, in this latter study, the radioactivity in tissues was not systematically corrected for free 2-deoxyglucose.

The aim of the present work was then to develop a quantitative method using labelled 2-deoxyglucose to study glucose metabolism *in vivo* in various tissues of the rat. This method satisfies the requirements of the theoretical model of Sokoloff *et al.* (1977): steady-state conditions and determination of correction factors for discrimination against 2-deoxyglucose in glucose pathways.

Materials and methods

Theoretical approach

The method used is derived from the technique of Sokoloff *et al.* (1977), developed for the brain. It is based on the biochemical properties of 2-deoxyglucose, which is transported in the brain by the same carrier as glucose and is phosphorylated by hexokinase. However, 2-deoxyglucose 6-phosphate cannot be metabolized further, and is trapped in the tissues if the hydrolysis of 2-deoxy-

glucose 6-phosphate by glucose-6-phosphatase is negligible. In skeletal muscle and adipose tissues 2-deoxyglucose and glucose are transported by the same carrier (Kipnis & Cori, 1959; Olefsky, 1975, 1976; Rennie *et al.*, 1983). On the other hand, glucose-6-phosphatase activity is very low both in skeletal muscles (Lackner *et al.*, 1984) and in white adipose tissue (Weber *et al.*, 1960).

After intravenous injection of a tracer dose of labelled 2-deoxyglucose in the animal, the arterial blood 2-deoxyglucose and glucose concentrations are recorded for a given length of time; after sampling the tissues, the quantity of glucose used per unit time (R_i) is determined from an equation set from a two-pool (plasma and tissue) mathematical model, derived from the equation described by Sokoloff *et al.* (1977) with the following modifications. (1) Since the content of 2-deoxyglucose 6-phosphate of each tissue at sampling time (τ) is measured directly by a chemical method, the numerator of the equation is [2-deoxyglucose 6-phosphate]τ. (2) We have not corrected for the lag in the equilibration of the tissue 2-deoxyglucose precursor pool with the plasma, since, if the experiment is kept long enough, this does not appear necessary (see the Results section). (3) We have determined 2-deoxyglucose and glucose concentrations in arterial blood, and we have checked that the integral thus obtained is similar to that obtained by measuring 2-deoxyglucose and glucose in plasma (results not shown). (4) The lumped constant (LC), which is a correction factor for the discrimination against 2-deoxyglucose in glucose transport and phosphorylation pathways, is determined *in vitro* by comparing glucose and 2-deoxyglucose fractional extraction by the different tissues. The equation we have finally used is then

$$R_i = \frac{[\text{2-deoxyglucose 6-phosphate}]\tau}{\text{LC} \int_0^{\tau} (C_B^*/C_B)dt} \quad (1)$$

where C_B^* is the blood 2-deoxyglucose expressed in term of radioactivity and C_B is the blood glucose concentration. The equation applies in steady-state conditions for both blood glucose and glucose utilization (Sokoloff *et al.*, 1977).

Animals

Female Wistar rats bred in our laboratory were used. They were housed at 24°C with light from 07:00 to 19:00 h. They had free access to water and chow pellets (carbohydrate 65%, fat 11% and protein 24% of total energy) until 08:00 h. Starved animals were studied after 48 h starvation.

Surgical procedures

All the experiments were performed between 09:00 h and 11:00 h, i.e. for fed animals in the post-absorptive state. The rats were anaesthetized with pentobarbitone (30 mg/kg body wt. intraperitoneally); one carotid artery was catheterized and a tracheotomy was performed. Experiments were started 30 min after the beginning of anaesthesia. Body temperature was maintained at 38°C.

Euglycaemic–hyperinsulinaemic-clamp studies

Euglycaemic–hyperinsulinaemic clamp was applied on post-absorptive animals as previously described (Leturque *et al.*, 1984). Usually, 40 min was necessary to reach a plateau for both glycaemia and exogenous glucose infusion rate.

2-Deoxyglucose injection and blood sampling

2-Deoxy[1-^{3}H]glucose (30 μCi; 20 Ci/mmol) (C.E.A., Saclay, France) was injected in 200 μl of 0.9% NaCl as a bolus through a saphenous vein. Blood (50 μl) was sampled via the arterial catheter. At time of tissue sampling, 2-deoxy[1-^{3}H]glucose concentration was 10% of the 1 min value in the different experimental groups (see Fig. 1). Blood was immediately deproteinized in $Ba(OH)_2/ZnSO_4$ as described by Somogyi (1945) and centrifuged (2 min, 16000 *g*). Supernatant was used for the determination of blood glucose by a glucose oxidase kit (Boehringer, Meylan, France) and for 2-deoxy[1-^{3}H]glucose with a liquid-scintillation counter (Kontron Betamatic, Vélizy, France).

At the end of each experiment, a larger blood sample was taken for plasma insulin determination by a radioimmunoassay previously described (Leturque *et al.*, 1984).

Tissue sampling

After the last blood sample, the rat was killed by cervical dislocation. Soleus and extensor digitorum longus of each hind-leg and epitrochlearis of each fore-leg, two pieces (about 200 mg each) of periovarian white adipose tissue and one piece (about 100 mg) of each cerebral hemisphere were removed and immediately immersed in plastic tubes containing 0.5 ml of 1 M-NaOH. This was accomplished in less than 5 min. By comparing paired fragments of tissues, we have checked that a delay of 5 min between death and sampling had no influence on the data obtained.

Determination of 2-deoxyglucose 6-phosphate tissue content

The principle of the method is that 2-deoxyglucose and 2-deoxyglucose 6-phosphate are soluble in 6% $HClO_4$, whereas only 2-deoxyglucose is soluble in the Somogyi reagent, since 2-deoxyglucose 6-phosphate is adsorbed on the $BaSO_4/Zn(OH)_2$ precipitate (Kipnis & Cori, 1959). Plastic

tubes containing the tissues in 1 M-NaOH were stoppered and heated at 60°C for 45 min until total digestion of the tissues; 0.5 ml of 1 M-HCl was then added. One sample of the neutralized solution (200 μl) was added to 1 ml of 6% $HClO_4$ and another sample (200 μl) to 1 ml of $Ba(OH)_2/ZnSO_4$. After centrifugation, samples (800 μl) of the $HClO_4$ and $Ba(OH)_2/ZnSO_4$ supernatants were used for determination of the radioisotope content after addition of 10 ml of a scintillation solution (Aqualuma Plus, Kontron) in a Kontron (Betamatic) liquid-scintillation counter. The content of 2-deoxy[1-^{3}H]glucose 6-phosphate in each tissue was the difference between the radioactivity (d.p.m.) in the $HClO_4$ and $Ba(OH)_2/ZnSO_4$ supernatants. We have checked that digestion of the tissue in 1 M-NaOH was not affecting 2-deoxyglucose 6-phosphate recovery (results not shown).

Calculation of glucose utilization rate per mg of tissue

This was done by using eqn. (1). The integral of arterial blood [2-deoxy[1-^{3}H]glucose]/[glucose] was determined from the experimental time points by using a desk calculator (Hewlett–Packard, HP 41CV).

Measurement in vitro of correction factor for 2-deoxyglucose analogue effect (lumped constants)

Adipose tissue. Rats were killed by cervical dislocation; four pieces (each 200 mg) of periovarian white adipose tissue were removed and incubated in 2 ml of bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), containing bovine serum albumin (1.5%) (Sigma), 5 mM-glucose, 1 μCi of [U-^{14}C]glucose (New England Nuclear, Boston, MA, U.S.A.), 2 μCi of 2-deoxy-D-[1-^{3}H]-glucose (C.E.A.) with or without 1 munit of insulin (Novo)/ml. Each flask was gassed with O_2/CO_2 (19:1) for 5 min, sealed with a rubber stopper and incubated for 1 h at 37°C. Before the incubation was stopped by adding 0.5 ml of 40% (w/v) $HClO_4$, pieces of tissue were removed, immersed in 1 ml of 1 M-NaOH and analysed for their 2-deoxyglucose 6-phosphate content as described above. Lipids were extracted as described by Dole & Meinertz (1960) and analysed for their ^{14}C content. The generated $^{14}CO_2$ was collected in Hyamine contained in a centre well; labelled lactate was determined as described by Ferré *et al.* (1978).

The correction factor for the discrimination against 2-deoxyglucose (lumped constant, LC) was calculated as follows:

$$LC = \frac{\text{tissue 2-deoxyglucose 6-phosphate/2-deoxyglucose in medium}}{\text{glucose utilization/glucose in medium}}$$

Glucose utilization was taken as the sum of [U-^{14}C]glucose converted into CO_2, lactate and lipids. This formula corresponds to the fractional extraction of 2-deoxyglucose by the tissue divided by the fractional extraction of glucose (Sokoloff *et al.*, 1977).

Muscles. Rats were killed by cervical dislocation and the two soleus, the two extensor digitorum longus and the two epitrochlearis muscles of each rat were dissected out. Skeletal muscles were stretched on a stainless-steel holder. Measurement of glucose utilization (glycolysis and glycogen synthesis) was performed in the presence or absence of 1 munit of insulin/ml with 5 mM-[5-^{3}H]-glucose (Amersham International, Amersham, Bucks., U.K.) as described by Leturque *et al.* (1981*b*), except that 2 μCi of 2-deoxy-D-[1-^{14}C]-glucose (New England Nuclear) was added per flask in the incubation medium. Muscle 2-deoxyglucose 6-phosphate was measured as described above. The correction factor for the discrimination against 2-deoxyglucose was calculated as for adipose tissue.

Measurement of whole-body glucose-utilization rate

In the hyperinsulinaemic–euglycaemic animals, the whole-body glucose-utilization rate was taken as the rate of exogenous glucose infusion, since at this plasma insulin concentration endogenous glucose production was totally suppressed (Leturque *et al.*, 1984). In other experimental groups, the animals used for the measurement of whole-body glucose-utilization rate were different from those used in the 2-deoxyglucose experiments, but were under similar nutritional and surgical conditions. Whole-body glucose-utilization rate was assessed by a primed-continuous infusion of [3-^{3}H]glucose (Amersham International) as described previously (Leturque *et al.*, 1981*a*).

Statistics

Results are presented as means ± S.E.M. Statistical significance of differences was assessed by Student's *t* test.

Results

Determination of lumped constants in vitro

The lumped constants in the absence or presence of 1 munit of insulin/ml are presented in Table 1. Even in the presence of insulin, the amount of glucose and 2-deoxyglucose utilized at the end of the incubation did not represent more than 3% of glucose and 2-deoxyglucose present at zero time. It can then be considered that the incubations were done under steady-state conditions for both 2-

Table 1. *Glucose-utilization rates in vitro and correction factor for the discrimination against 2-deoxyglucose (lumped constant) in skeletal muscles and white adipose tissue*

Results are means ± S.E.M., with the numbers of determinations in parentheses. For further details see the Materials and methods section. *, **, difference significant respectively for $P<0.05$ and $P<0.01$.

	Insulin	Soleus ($n=9$)	Extensor digitorum longus ($n=9$)	Epitrochlearis ($n=4$)	Periovarian adipose tissue ($n=23$)
Glucose utilization rate (ng/min per mg)	0	6±1	4.2±0.6	7.4±0.6	3.7±0.3
	1 munit/ml	14±1**	12±2**	15±0.9**	8±1**
2-Deoxyglucose lumped constant	0	0.89±0.09	0.91±0.06	0.76±0.03	0.67±0.03
	1 munit/ml	1.00±0.10	1.20±0.06	0.95±0.10	0.55±0.02*
Value of the lumped constant used *in vivo*		0.95	1.05	0.86	0.61

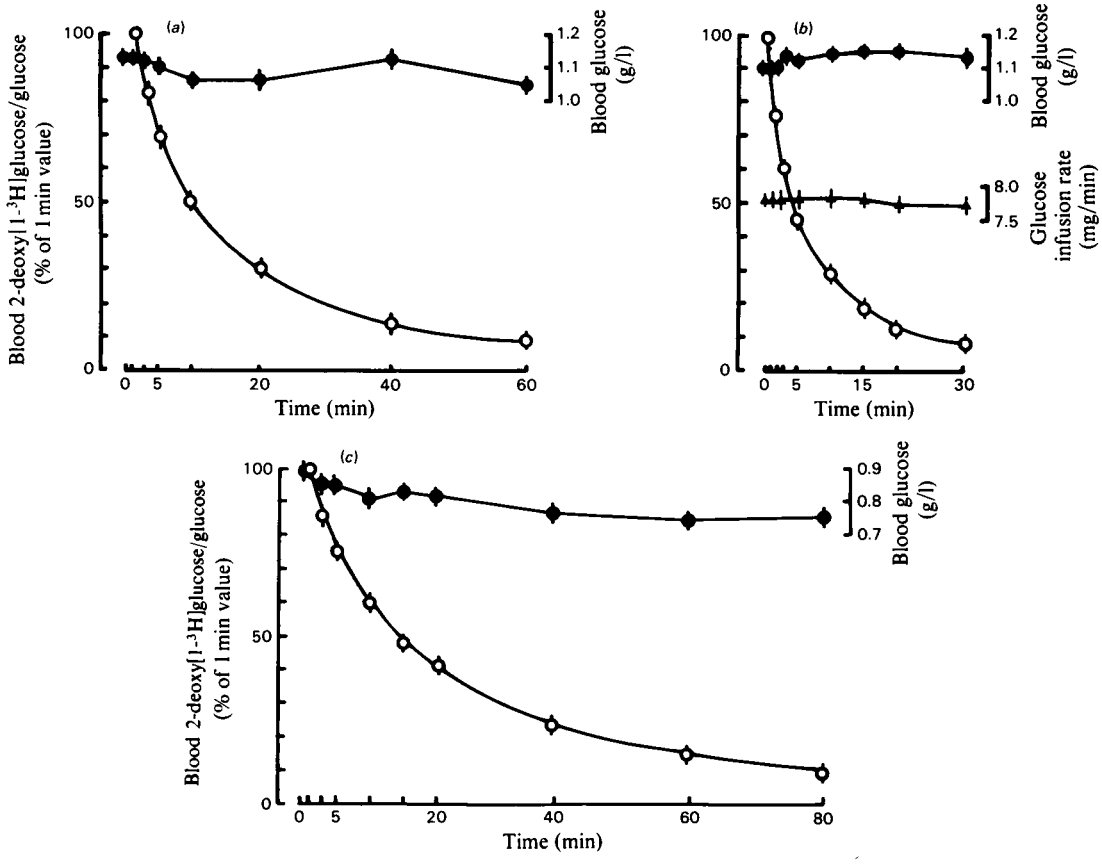

Fig. 1. *Blood glucose* (●) *and 2-deoxy[1-³H]glucose/glucose ratio* (○) *in post-absorptive controls* (*a*), *post-absorptive hyperinsulinaemic* (*b*) *and 48 h-starved rats* (*c*)

In the post-absorptive hyperinsulinaemic animals, the rate of exogenous glucose infusion is indicated (▲). Results are presented as means ± S.E.M. for five to seven determinations.

deoxyglucose and glucose. In muscles and adipose tissue, insulin increased glucose utilization 2–3-fold (Table 1). By contrast, lumped constants were not statistically different in muscles in the presence or absence of insulin. For the calculation of glucose-utilization rates *in vivo*, the values in the presence or absence of insulin were then pooled; their value are indicated in Table 1. In white adipose tissue, the lumped constant was decreased by only 17% in the presence of 1 munit of insulin/ml. Thus the mean value of the lumped constant was also chosen for the calculation of

glucose-utilization rates in white adipose tissue (see also the Discussion section).

Whole-body glucose-utilization rate and plasma insulin concentrations

Whole-body glucose-utilization rates, plasma insulin concentrations and glycaemia in the three groups of rats are reported in Table 2. During the hyperinsulinaemic–euglycaemic clamp the glucose-turnover rate increases 3.5-fold. Starvation decreases whole-body glucose utilization by 40%, together with a 32% fall in glycaemia.

2-Deoxyglucose and blood glucose concentrations

The pattern of blood 2-deoxyglucose/glucose ratio and blood glucose concentration during the experimental periods are shown in Fig. 1. Blood glucose is in a steady state, as required by the theoretical model (Sokoloff *et al.*, 1977).

Glucose-utilization rates in various tissues

Glucose-utilization rates given by eqn. (1), in post-absorptive control animals for two different lengths of experimental period, i.e. 30 and 60 min, were compared. For the 30 min experiment, values for glucose-utilization rates for the soleus, the extensor digitorum longus and the epitrochlearis muscles and the periovarian white adipose tissue were respectively 6.9 ± 1, 6.9 ± 0.5, 7.7 ± 1.9 and 2.7 ± 0.6 ng/min per mg, with five to seven determinations. No statistical difference in the rates could be detected when compared with the 60 min value (Fig. 2), thus validating the use of eqn. (1).

Glucose-utilization rates in various tissues in post-absorptive control, hyperinsulinaemic and 48 h-starved animals are reported in Fig. 2. In control animals brain utilizes 4–7-fold more glucose than skeletal muscles per unit wet weight, and 20-fold more than white adipose tissue. There is no

Table 2. *Blood glucose, whole-body glucose-turnover rate and plasma insulin in fed control, fed hyperinsulinaemic and 48 h-starved rats*

Results are presented as means ± S.E.M. for five to seven determinations.

	Blood glucose (mg/ml)	Glucose-turnover rate (mg/min per kg)	Plasma insulin (μunits/ml)
Post-absorptive control	0.97 ± 0.02	8.4 ± 0.2	104 ± 11
Post-absorptive hyperinsulinaemic	0.97 ± 0.03	28.0 ± 1.0	7800 ± 1000
48 h-starved	0.66 ± 0.02	5.2 ± 0.3	51 ± 11

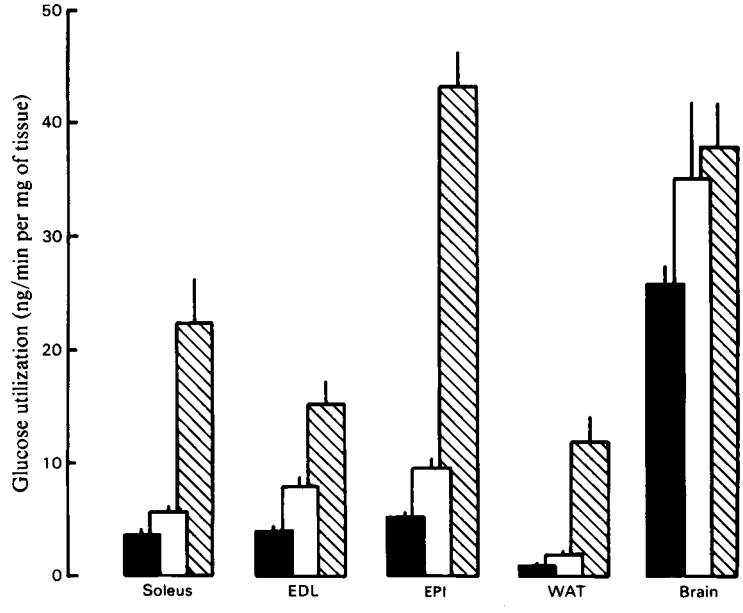

Fig. 2. *Glucose utilization in vivo in soleus, extensor digitorum longus (EDL), epitrochlearis (EPI), periovarian white adipose tissue (WAT) and brain, in post-absorptive controls, post-absorptive hyperinsulinaemic and 48 h-starved rats*

Glucose utilization was calculated according to eqn. (1). Results are presented as means ± S.E.M. for five to seven determinations. □, Control; ▨, hyperinsulinaemic; ■, starved.

marked difference in glucose-utilization rates between soleus and extensor digitorum longus, whereas glucose utilization is significantly higher in epitrochlearis. During the hyperinsulinaemic–euglycaemic clamp, brain glucose utilization is not significantly enhanced, whereas it is increased 5.7-fold in periovarian white adipose tissue and respectively 2.0-, 4.3- and 4.3-fold in the extensor digitorum longus, soleus and epitrochlearis muscles. Starvation leads to a 30–50% decrease in the glucose-utilization rate in skeletal muscles, white adipose tissue and brain.

Discussion

Determination of lumped constants

The constancy of the lumped constant has been challenged by Pardridge (1983), since it depends on the relative rates of transport and phosphorylation, which might vary with the metabolic situation. Obviously it might also vary between the conditions *in vivo* and *in vitro*. It was thus critical to us to examine whether the lumped constant was modified in a given tissue for a wide range of transport rates.

In vitro, glucose utilization in muscles increases 2–3-fold in presence of 1 munit of insulin/ml, but the values of the lumped constants are not significantly affected. In white adipose tissue, despite a 2.3-fold increase in glucose utilization, the lumped constant is decreased by only 17%. Thus, if one compares metabolic situations for which the rate of glucose utilization in adipose tissue is largely changed, the observed variability of the lumped constant is negligible. The validity of lumped constants determined *in vitro* is strengthened by the fact that, in our experimental conditions, the flux of glucose *in vitro* in the muscles and in white adipose tissue is in the same range as the values obtained *in vivo* (Fig. 1 and Table 2).

In conclusion, under the metabolic conditions encountered in the present study, the use of lumped constants determined *in vitro* seems valid for the calculation of rates of glucose utilization *in vivo*.

Use of arterial blood 2-deoxyglucose/glucose ratio

As stated by Sokoloff *et al.* (1977), the blood glucose and 2-deoxyglucose concentrations should represent mean capillary plasma glucose and 2-deoxyglucose concentrations. They can be approximated by the arterial blood concentrations when glucose and thus 2-deoxyglucose fractional extractions by the tissues are low (Sokoloff *et al.*, 1977). In muscles, in the presence of high plasma insulin, glucose extraction might be high. However, it is the ratio of blood 2-deoxyglucose/glucose concentrations rather than their absolute values which is important [Sokoloff *et al.* (1977) and eqn. (1)]. Since in skeletal muscle glucose and 2-deoxyglucose extractions are very similar (lumped constants close to 1), the capillary 2-deoxyglucose/glucose ratio should be nevertheless similar to the arterial 2-deoxyglucose/glucose ratio.

Utilization of 2-deoxyglucose in tracer amounts

The amount of 2-deoxyglucose injected in the rat corresponds to 1.5 nmol, and accumulation of 2-deoxyglucose 6-phosphate in tissues is in the nanomolar range. Thus 2-deoxyglucose can be considered as a true tracer in our experiments. 2-Deoxy[1-^{3}H]glucose cannot be used to determine whole-body glucose utilization, since significant amounts of labelled 2-deoxyglucose are found in urine (results not shown).

Glucose utilization

In the present study, results are obtained in anaesthetized animals. Anaesthesia decreases whole-body turnover rates in rats by 20% (Heath *et al.*, 1977). Brain glucose utilization is decreased by 30–40% in rats during anaesthesia (Sokoloff *et al.*, 1977), and it is likely that glucose utilization is also decreased in other tissues, especially in postural muscles. However, absolute values and half-maximal concentrations for insulin stimulation of glucose-utilization rates are similar in anaesthetized and unanaesthetized Wistar rats (Leturque *et al.*, 1984; Kraegen *et al.*, 1983).

In the brain, to correct for the 2-deoxyglucose analogue effect, we have used the lumped constant determined by Sokoloff *et al.* (1977) for anaesthetized rats (0.51) in the three metabolic conditions that we have studied, since the constant seems relatively insensitive to variations of glucose utilization (Sokoloff *et al.*, 1977). The value for brain glucose utilization thus obtained in post-absorptive anaesthetized control rats is close to the value found by Hawkins *et al.* (1974) for the whole brain in anaesthetized Wistar rats and to the range of values found by Sokoloff *et al.* (1977) for various brain regions in the anaesthetized Sprague–Dawley rat. As predicted from previous studies (see the review by Pardridge, 1983), brain glucose-utilization rate is not affected by insulin. By contrast, brain glucose utilization is decreased by 30% during starvation. Again, this was not unexpected, since rat brain can utilize ketone bodies as a carbon source in states of hyperketonaemia (Hawkins *et al.*, 1971). Assuming a brain weight of 2 g for a rat of 200–300 g, the contribution of brain glucose utilization to whole-body glucose turnover is then 5% in the post-absorptive state, 2% in hyperinsulinaemic animals and 4% in starved animals. In contrast with humans, brain glucose utilization in rats thus represents a marginal fraction of total

glucose utilization, although on a weight basis it utilizes much more glucose than resting muscles or white adipose tissue.

As predicted by previous data in the literature, glucose utilization in muscles is increased by insulin and decreased by starvation. The quantitative comparison of the values found in the present study for muscle glucose metabolism with values found *in vitro* is difficult, since *in vivo* glucose metabolism in muscle is affected by work load, blood flow, glycaemia, plasma insulin, counter-regulatory hormones and alternate-substrate concentrations. In the perfused hindlimb, which is the closest preparation to our conditions *in vivo*, for glucose concentration of 5–5.5mM in the medium, basal glucose utilization is in the range of 2–6ng/min per mg and under maximal insulin stimulation is in the range of 18–44ng/min per mg (Ruderman *et al.*, 1974; Berger *et al.*, 1975; Richter *et al.*, 1982; Goodman *et al.*, 1983). These values compare favourably with those shown in Fig. 2.

The various muscles studied are representative of the range of fibre-type composition, since the composition in terms of slow-twitch oxidative, fast-twitch oxidative glycolytic and fast-twitch glycolytic fibres is respectively 84%, 16%, 0% for the soleus, 3%, 59%, 38% for the extensor digitorum longus, and 15%, 15%, 70% for the epitrochlearis (Ariano *et al.*, 1973; Nesher *et al.*, 1980). When comparing the data from Fig. 2, it is clear that, in anaesthetized rats, epitrochlearis is the muscle that has the greatest glucose utilization in basal and insulin-stimulated conditions, as well as the greatest decrease during starvation. This fits with the fact that epitrochlearis is very much dependent on glucose as substrate and has the highest potential capacity for glycogen synthesis, as seen from the fibre composition. The pattern of glucose utilization is not different in soleus and extensor digitorum longus.

The exact quantitative fibre composition of the whole muscle mass is unknown. It is thus difficult to calculate from our data the contribution of muscles to whole-body glucose-turnover rate. We can nevertheless give a value by averaging the glucose utilization for the soleus and for the epitrochlearis and by assuming that the muscle mass represents 40% of the body weight. This leads to a 36% contribution in post-absorptive control animals, 50% during the hyperinsulinaemic–euglycaemic clamp and 38% during starvation, or, in other words, it indicates a major role of the muscle mass in glucose utilization, even in the anaesthetized state, and specially in insulin stimulation.

In white adipose tissue the expected changes in glucose metabolism were observed: an increase in hyperinsulinaemic animals and a decrease during starvation compared with post-absorptive control rats (Fig. 2). From a quantitative point of view, glucose utilization in white adipose tissue is much less than in resting muscle or brain. Assuming a mass of adipose tissue representing 12% of body weight, the contribution of white adipose tissue to overall glucose turnover rate is 3% in post-absorptive controls, 5% in insulin-stimulated rats and 2% in starved animals. This conclusion has to be tempered by the fact that adipose tissues from different sites might have different glucose-utilization rates, although among white adipose tissues, and from data for adipocytes, periovarian adipose tissue seems to be in the average range (Fried *et al.*, 1982). Thus, in non-obese animals, alterations in the uptake of glucose by white adipose tissue play a minor role in the overall glucose utilization by the animals.

Conclusion

We have developed a technique to study glucose metabolism in individual tissues of the rat. The results obtained seem to correspond qualitatively and quantitatively to expected values in tissues of known physiological characteristics. This technique can be extended to other tissues as long as they do not possess a high glucose-6-phosphatase activity and if it is possible to determine a correction factor for the discrimination against 2-deoxyglucose, *in vivo* (Sokoloff *et al.*, 1977) or *in vitro* (the present study).

However, a number of limitations exist. (1) Since measurements have to be made in steady-state conditions for glucose utilization, it precludes the analysis of rapid variations of glucose utilization. (2) Owing to possible small variations in the lumped constant between two metabolic conditions, it does not seem possible to detect safely variations of the glucose utilization of less than 10–15%. (3) As discussed above, in tissues (liver, kidney) where a substantial glucose-6-phosphatase activity exists, the use of this technique is not possible.

We are indebted to J. Kande and P. Maulard, for their skilful technical assistance, to D. Chamereau for taking care of animals and to I. Coquelet for the preparation of the manuscript. This work was supported by grants 82-L-101 from the Ministère de l'Industrie et de la Recherche and 6931-75 from the Centre National de la Recherche Scientifique.

References

Ariano, M. A., Armstrong, R. B. & Edgerton, V. R. (1973) *J. Histochem. Cytochem.* **21**, 51–55

Berger, M., Hagg, S. & Ruderman, N. B. (1975) *Biochem. J.* **146**, 231–238

Dole, V. P. & Meinertz, H. (1960) *J. Biol. Chem.* **235**, 2595–2599

Ferré, P., Pégorier, J.-P., Marliss, E. B. & Girard, J. R. (1978) *Am. J. Physiol.* **234**, E129–E136
Fried, S. K., Lavau, M. & Pi-Sunyer, F. X. (1982) *Metab. Clin. Exp.* **31**, 876–883
Goodman, M. N., Dluz, S. M., McElaney, M. A., Belur, E. & Ruderman, N. B. (1983) *Am. J. Physiol.* **244**, E93–E100
Hawkins, R. A., Williamson, D. H. & Krebs, H. A. (1971) *Biochem. J.* **122**, 13–18
Hawkins, R. A., Miller, A. L., Cremer, J. E. & Veech, R. L. (1974) *J. Neurochem.* **23**, 917–923
Heath, D. F., Frayn, K. N. & Rose, J. G. (1977) *Biochem. J.* **162**, 643–651
Hom, F., Goodner, C. J. & Berrie, M. A. (1984) *Diabetes* **33**, 141–152
Kipnis, D. M. & Cori, C. F. (1959) *J. Biol. Chem.* **234**, 171–177
Kraegen, E. W., James, D. E., Bennett, S. P. & Chisholm, D. J. (1983) *Am. J. Physiol.* **245**, E1–E7
Kraegen, E. W., James, D. E., Jenkins, A. B. & Chisholm, D. J. (1984) *Diabetologia* **27**, 298A
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
Lackner, R., Chaliss, J., West, D. & Newsholme, E. A. (1984) *Biochem. J.* **218**, 649–651
Leturque, A., Gilbert, M. & Girard, J. (1981*a*) *Biochem. J.* **196**, 633–636
Leturque, A., Satabin, P., Ferré, P. & Girard, J. R. (1981*b*) *Biochem. J.* **200**, 181–184
Leturque, A., Burnol, A.-F., Ferré, P. & Girard, J. (1984) *Am. J. Physiol.* **246**, E25–E31
Nesher, R., Karl, I. E., Kaiser, K. E. & Kipnis, D. M. (1980) *Am. J. Physiol.* **239**, E454–E460
Olefsky, J. M. (1975) *J. Clin. Invest.* **56**, 1499–1508
Olefsky, J. M. (1976) *J. Clin. Invest.* **58**, 1450–1460
Pardridge, W. M. (1983) *Physiol. Rev.* **63**, 1481–1535
Rennie, M. J., Idström, J.-P., Mann, G. E., Schersten, T. & Bylund-Fellenius, A.-C. (1983) *Biochem. J.* **214**, 737–743
Richter, E. A., Garetto, L. P., Goodman, M. N. & Ruderman, N. B. (1982) *J. Clin. Invest.* **69**, 785–793
Ruderman, N. B., Ross, P. S., Berger, M. & Goodman, M. N. (1974) *Biochem. J.* **138**, 1–10
Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O. & Shinohara, M. (1977) *J. Neurochem.* **28**, 897–916
Somogyi, M. (1945) *J. Biol. Chem.* **160**, 69
Weber, G., Banerjee, G. & Ashmore, J. (1960) *Biochem. Biophys. Res. Commun.* **3**, 182–190