Total body glucose metabolism in the conscious, unrestrained piglet and its relation to body- and organ weight

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1. Neonatal hypoglycaemia is a relatively common clinical problem in children but ethical constraints limit the investigations that may be made in the newborn.

2. As a preliminary step to assess the suitability of the piglet as a model for glucose metabolism in man, whole-body glucose turnover and glucose pool size were measured using [2-^{3}H]glucose in forty piglets from ten litters.

3. Glucose pool size was linearly related to brain weight. However, multiple regression showed that the most useful predictors of pool size were body-weight and resting plasma glucose concentration.

4. Glucose turnover was related to both brain weight and body-weight alone, but multiple regression showed that better predictors of turnover were liver weight, spleen weight and pancreas weight.

5. Similarities between our own results in piglets and those obtained in human neonates by Bier *et al.* (1977) extend not only to glucose turnover, but also to its relationship with body- and brain weight. These findings suggest that the piglet may be a useful model for the study of glucose metabolism in babies.

Although neonatal hypoglycaemia is a relatively common clinical condition in children, ethical considerations limit the investigations that may be carried out directly on human neonates, so that animal models are necessary if detailed studies are to be made of this problem. Several animal species have been examined including the puppy (Kornhauser *et al.* 1970), the rhesus monkey (Sherwood *et al.* 1976) and the lamb (Cowett *et al.* 1976). The piglet has certain morphological and physiological similarities to man, and is also a species that naturally develops neonatal hypoglycaemia (Goodwin, 1957), but relatively little work has been carried out to investigate its glucose kinetics during the perinatal period.

As a preliminary step to assess the suitability of the piglet as a model for glucose metabolism in babies, we have measured total body glucose turnover, using radio-labelled glucose as a tracer, in piglets whose ages ranged from 4 h to 142 d (sexual maturity in the pig occurs at approximately 180 d). Similar studies in man (Bier *et al.* 1977) have related glucose turnover to body-weight and to estimated brain weight. It may be, however, that more information is to be gained by studying glucose turnover in relation to the weights of other organs which is clearly impractical in man.

MATERIALS AND METHODS

Experimental animals

Pregnant Large White × Welsh sows were obtained from a commercial supplier and maintained in conventional conditions until parturition. The piglets produced were housed with their dams until required for experiment. In all, forty piglets from ten litters were used. Their ages at the time of experiment ranged from 4 h to 142 d.

Experimental procedures

Under light general anaesthesia, catheters were inserted into the external jugular vein and carotid artery (Flecknell, 1979) to facilitate stress-free sampling and injection of tracers.

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The catheters were kept patent by flushing with heparin saline (9 g sodium chloride/l; heparin 100 unit/ml).

Piglets aged 0–21 d were housed in a thermoneutral environment (Mount, 1959) and given water *ad lib.* but no food for 16 h before experiment. Piglets aged 4 h received no food following delivery. Animals were weighed at the time of experiment and their rectal temperatures monitored to ensure that the environmental temperature was high enough to prevent chilling. D-[2-^{3}H]glucose (100–500 μCi; The Radiochemical Centre, Amersham, Bucks) was injected rapidly into the jugular catheter, and flushed with a volume of normal saline equivalent to ten times the dead space of the catheter. Blood samples were obtained from the carotid catheter over the following three h at 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150 and 180 min, and collected in fluoride-oxalate tubes. At the end of this period the animals were killed and the brain and other major organs removed and weighed.

Chemical methods

Plasma glucose was measured by the glucose oxidase (EC 1.1.3.4) method. Radioactive glucose was measured in 0·2 ml plasma samples deproteinized by the method of Somogyi (1945). The deproteinized plasma was evaporated to dryness at 42° and resuspended in 1 ml distilled water. Unisolve 1 (10 ml; Koch-Light Labs) was used as the scintillant and the ^{3}H activity of each sample measured in an LKB 81000B liquid scintillation counter. Sufficient counts were collected to ensure that the counting uncertainty amounted to a relative standard deviation of 2% or less after correcting for background radioactivity. Quench corrections were not required.

Since this method of sample preparation assumes that ^{3}H is present in the serum only as [^{3}H]glucose or as ^3H_2O following its metabolism (Katz *et al.* 1974), a subsidiary experiment was performed to ensure that no significant transfer of ^{3}H to other metabolic products of glucose metabolism had occurred in vivo. For this purpose, duplicate plasma samples from nine experiments were deproteinized and then processed as described previously, either immediately or after passing them down an ion-exchange column (Dowex–1–acetate).

Kinetic analysis

Total body glucose turnover rate and total-body glucose pool size were calculated from the plasma specific activity values by conventional compartmental methods using a computer program (Fortran IV). Plasma specific activity curves were fitted by exponential curves of the form:

$$P = \Sigma A_i \exp(-B_i t),$$

where P is plasma specific activity, t is time and A_i and B_i are the fitted coefficients of the ith exponential term.

Single, dual, triple etc. exponential curves were fitted successively to the plasma specific activity values using the non-linear optimisation procedure of Nelder & Mead (1965). The curve-fitting process was terminated when addition of an extra exponential term did not significantly improve the fit at $P < 0{\cdot}05$ using an F test.

Glucose turnover rate (T) was calculated as:

$$T = \frac{D}{\Sigma A_i/B_i},$$

where D is the dose injected; and pool size (G) as:

$$G = \frac{D}{\Sigma A_i}.$$

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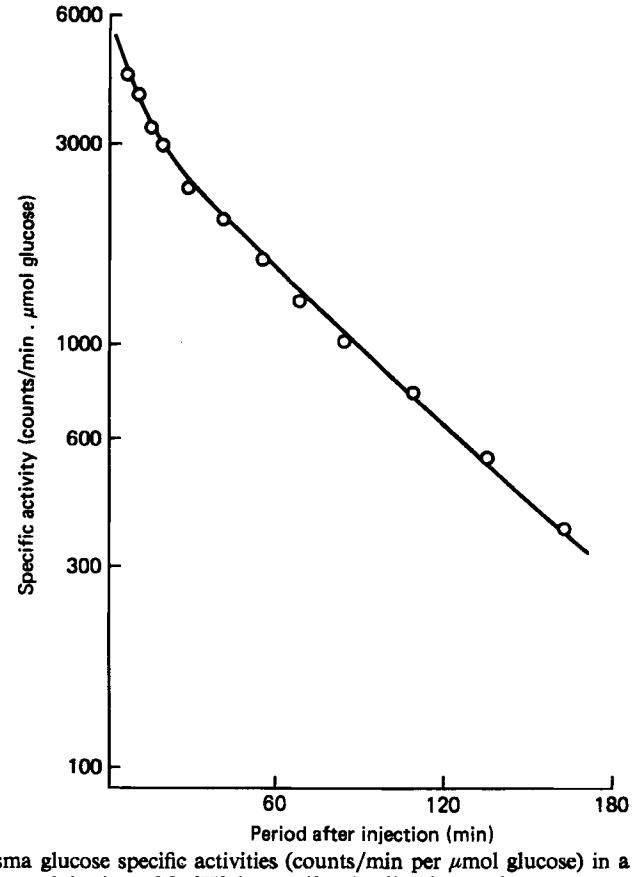

Fig. 1. Plasma glucose specific activities (counts/min per μmol glucose) in a piglet following the injection of [2-^{3}H]glucose (for details of procedures, see p. 193).

Statistical methods

Linear, curvilinear and multiple regression were carried out using the computer program GLIM3 (Royal Statistical Society, 1978). However, there was a tendency for the variance of the kinetic results to change with the magnitude of the result, the correlation coefficient between the estimated variance in glucose turnover and body-weight being 0·81 (n 39, $P < 0{\cdot}001$) for instance. Regression lines were therefore fitted by the weighted least squares method, using weights which were inversely proportional to the estimated variance of the values (Draper & Smith 1966). The variances were estimated by regressing the SD's on the means and using the fitted straight line as the estimator. Differences between multiple regression models (e.g. between a linear and a quadratic, or between a univariate and bivariate model) were tested by examining the change in the variance explained with the new model using an F test with the appropriate number of degrees of freedom. This is equivalent to calculating the significance of the change in multiple regression coefficient R^2, by use of the new model.

RESULTS

Recycling of label

Differences between the results calculated for the nine sets of samples processed by the two methods described previously were tested with a paired t test, after a logarithmic transforma-

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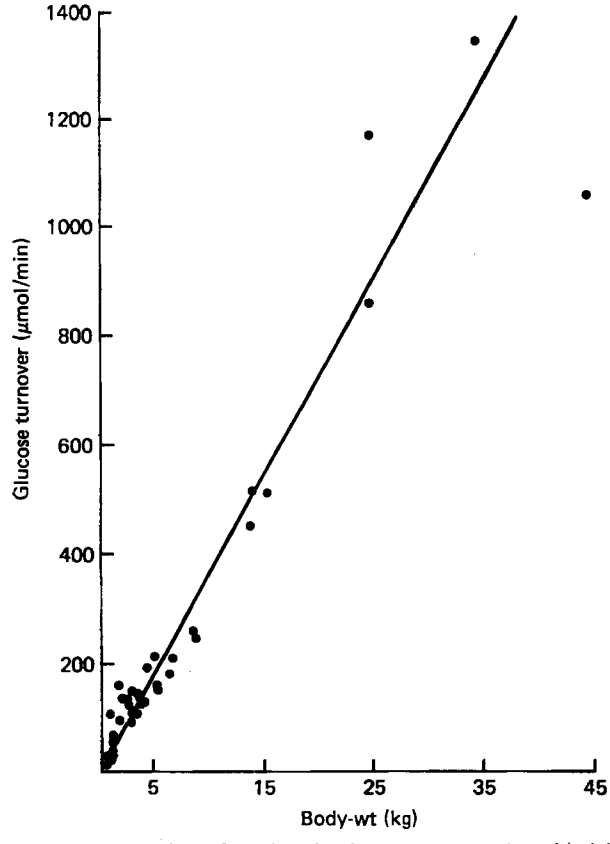

Fig. 2. Weighted linear regression of total-body glucose turnover (μmol/min) *v.* body-weight (kg) in piglets after injection of [2-^{3}H]glucose (for details of procedures, see p. 193).

tion to normalize the distributions. There was no significant difference in turnover rate (t 0·36, $P > 0·1$) or pool size (t 0·51, $P > 0·1$) and we therefore concluded that no significant transfer of ^{3}H to metabolic products of glucose metabolism, other than water, had occurred in vivo.

Glucose kinetics

In all of the experiments the specific activity data obtained were fitted adequately by one or two exponentials (Fig. 1). Plasma glucose concentration remained relatively constant throughout the experimental period in all piglets.

Glucose turnover and body- and organ weights

A regression of glucose turnover measured in forty normal piglets was carried out *v.* body-weight and brain weight. There was a linear relation between turnover and body-weight (Fig. 2) but it was found that a quadratic regression of turnover on brain weight produced a significant ($P < 0·05$) improvement in fitting the data (Fig. 3). A multiple regression of turnover on body- and brain weights showed that given either variable, a significant improvement ($P < 0·05$) in predicting power was obtained by adding the other; and that glucose turnover was better predicted by body-weight alone ($F_{1,37} = 110$) than by brain weight alone ($F_{2,36} = 39·7$).

A step-down multiple regression (Davies & Goldsmith, 1977) of glucose turnover on

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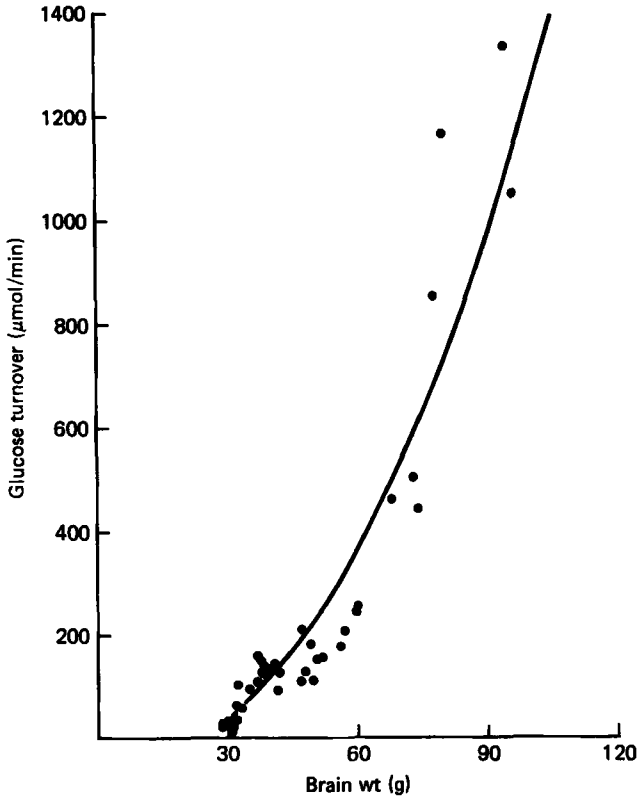

Fig. 3. Weighted quadratic regression of total body glucose turnover (μmol/min) *v.* brain weight (g) in piglets after injection of [2-^{3}H]glucose (for details of procedures, see p. 193).

Table 1. *Factors assessed in step-down multiple regression of glucose turnover v. body- and organ weights of piglets*

(Probabilities (using *F* test) that apparent association between the variables listed and glucose turnover and pool size are due to chance only)

Variable	Glucose turnover	Glucose pool size
Body-wt	NS	$P < 0{\cdot}001$
Brain wt	NS	NS
Heart wt	NS	NS
Kidney wt	NS	NS
Liver wt	$P < 0{\cdot}001$	NS
Lung wt	NS	NS
Pancreas wt	$0{\cdot}01 < P < 0{\cdot}025$	NS
Resting plasma glucose concentration	NS	$0{\cdot}01 < P < 0{\cdot}025$
Spleen wt	$P < 0{\cdot}001$	NS

NS, not significant ($P > 0{\cdot}05$).

body- and organ weights allowed the relative contribution of each of these factors to be assessed (Table 1). Only three of the nine factors were significant at the 5% level as a predictor of glucose turnover, and of these, liver weight was by far superior. The results were checked by computing regressions containing all possible subsets of the factors. Table 2

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Table 2. *Prediction of glucose turnover from organ weights of piglets*

(The general equation is: Predicted glucose turnover (μmol/min) = $C_0 + C_1 \times$ liver weight (g) $+ C_2 \times$ spleen weight (g) $+ C_3 \times$ pancreas weight (g). In the absence of pancreas weight, coefficients of equation B, are used while if liver weight alone is available, coefficients of equation C are used)

Equation	C_0	C_1	C_2	C_3	Confidence limits*
A	20·16	0·53	2·18	5·88	±42
B	12·56	0·82	2·86	—	±46
C	12·41	1·06	—	—	±58

* 95% confidence limits for a predicted glucose turnover of 156 μmol/min.

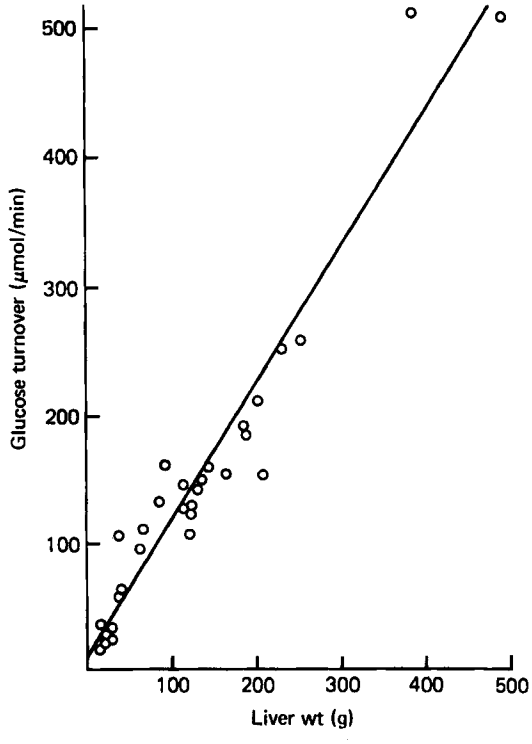

Fig. 4. Weighted linear regression of total-body glucose turnover (μmol/min) *v.* liver weight (g) in piglets after injection of [2-^{3}H]glucose (for details of procedures, see p. 193).

gives the regression equations for predicting glucose turnover from liver, spleen, and pancreas weight. Fig. 4 shows the regression of glucose turnover on liver weight alone.

Pool size and body- and organ weight

Glucose pool size was analysed in a similar manner to glucose turnover. A quadratic regression of pool size *v.* body-weight (Fig. 5) was found to be significantly ($P < 0{\cdot}05$) better than a linear regression which was, however, adequate to describe the relation between pool size and brain weight (Fig. 6). Multiple regression showed that only two factors were significantly associated with glucose pool size, i.e. body-weight and resting plasma glucose concentration (Table 1). Of these, body-weight was by far superior as a predictor.

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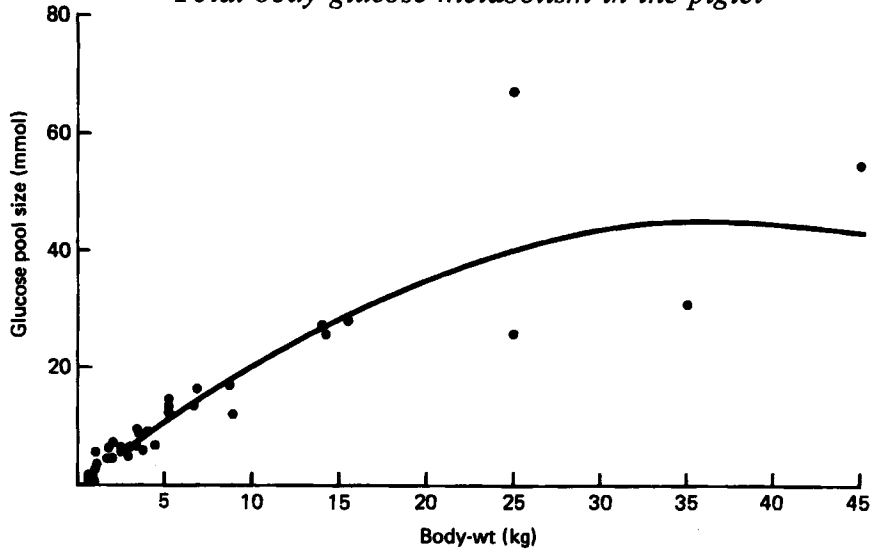

Fig. 5. Weighted quadratic regression of glucose pool size (mmol) *v.* body-weight (kg) in piglets after injection of [2-^{3}H]glucose (for details of procedures, see p. 193).

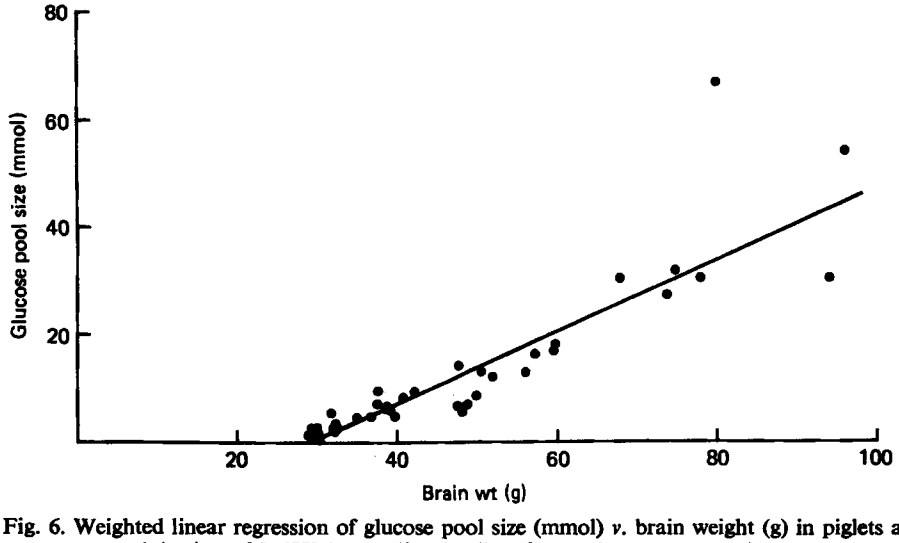

Fig. 6. Weighted linear regression of glucose pool size (mmol) *v.* brain weight (g) in piglets after injection of [2-^{3}H]glucose (for details of procedures, see p. 193).

The results were confirmed by computing regressions containing all possible subsets of the factors. Table 3 gives the regression equations for predicting glucose pool size from body-weight and resting plasma glucose concentration.

DISCUSSION

A fundamental assumption inherent in the use of compartmental analyses of tracer kinetic data is that steady-state conditions prevail during the period of measurement. To ensure that the piglets were not stressed during blood sampling, and consequently to avoid possible perturbations in resting plasma glucose concentrations and in glucose turnover rate, we used the chronic catheterization technique described by Flecknell (1979) which enables

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Table 3. *Prediction of total body glucose pool size from body-weight and plasma glucose concentration in piglets*

(The general equation is: predicted glucose pool size (mmol) = $C_0 + C_1 \times$ body-weight (kg) $+ C_2 \times$ glucose concentration (mmol/l))

Equation	C_0	C_1	C_2	Confidence limits*
A	−2·98	1·66	0·85	±3·6
B	1·37	1·75	—	±3·9

* 95% confidence limits for a predicted glucose pool size of 9·0 mmol.

arterial and venous blood samples to be obtained from the conscious, unrestrained piglet. This is particularly advantageous in view of the fact that previous workers have reported marked alterations in glucose concentrations following anaesthesia in adult swine (Meyer *et al.* 1962).

A further assumption inherent in the use of compartmental analyses is that no recycling of the tracer occurs and in attempts to circumvent this difficulty a variety of radio-labelled glucose preparations have been employed (Segal *et al.* 1961; Dunn *et al.* 1967; Kornhauser *et al.* 1970; Hetenyi *et al.* 1972; Judson & Leng, 1972; Katz *et al.* 1974). It is generally agreed, however, that ^{14}C-labelled glucose undergoes significant recycling (Katz *et al.* 1974) of the order of 10–40%. Glucose labelled with tritium in the various possible positions is thought to undergo little recycling as such, but may be subject to so-called futile cycling in the liver, the result being an over-estimate of turnover (Dunn *et al.* 1976). Although variations in calculated turnover result from the use of different ^{3}H labels, the differences are relatively small (Judson & Leng, 1972; Dunn *et al.* 1976). Since there is some measure of agreement that labelling at C_2 offers advantages with regard to lack of recycling (Katz & Dunn, 1967; Issekutz *et al.* 1972), we chose it as our tracer for the present study.

Although it has been reported that no recycling occurs when tritium-labelled glucose is used as a tracer (Katz *et al.* 1974), we wanted an indication of whether any significant recycling was occurring in piglets. For this reason we passed deproteinized samples from nine experiments down ion-exchange columns. The use of one column, (Dowex–1–acetate), provides an incomplete estimate of the recycling; two columns in tandem (Dowex–50–H^+ and Dowex–1–acetate) being necessary to estimate total recycling (Katz *et al.* 1974). However, as we required only an indication of the degree of recycling a single column was used. Our results showed that there was no significant difference between those portions which had been passed down the columns and those which had been processed by evaporation to dryness. We concluded that in the piglet, as in other species, significant recycling of the tritium label does not occur.

Studies in man have shown that glucose turnover is proportional to body-weight and to brain weight (Bier *et al.* 1977), and the present work demonstrates a similar relationship in the piglet. Not only is the increase in turnover rate with body-weight (i.e. the slope of the regression lines) remarkably similar in the two studies, but the turnover rates at a given body-weight are almost identical (Fig. 7). The animals on which these conclusions are based were all of normal birth weight as we have specifically excluded results from any undersized animals that had experienced intra-uterine growth retardation.

Using the values from Bier *et al.* (1977; Fig. 5), we have estimated the mean (±SD) glucose turnover rate of the eight full-term human neonates in their study as 33·9 ± 6·9 μmol/kg per min. During the same postnatal period, our own results obtained in twenty-two piglets are 44·6 ± 20·7 μmol/kg per min, which is not significantly different ($P > 0·05$) using Student's *t* test. Bier *et al.* (1977) also fitted a separate regression line to their values

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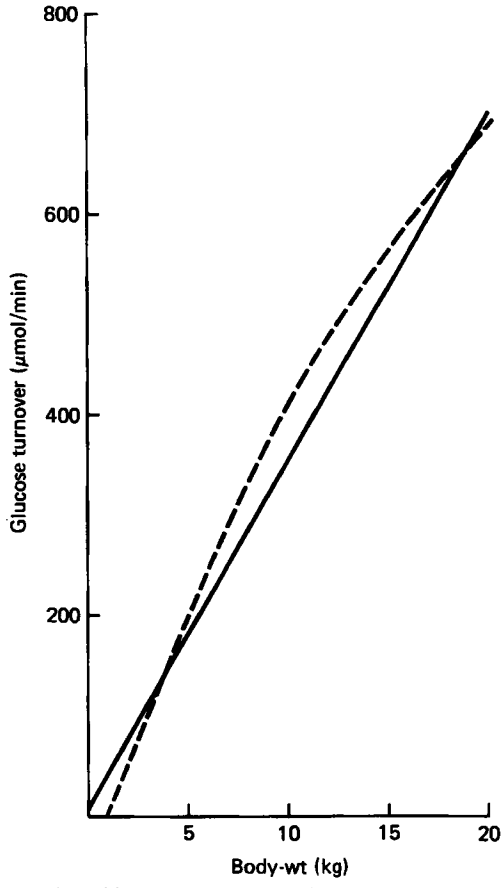

Fig. 7. Glucose turnover (μmol/min) in relation to body-weight: results from the present study (—) compared with those reported by Bier *et al.* (1977) in children (----).

of the first 14 d, in comparison with which (Fig. 8) our values in piglets over the same period are very similar. It has been proposed that the linear relationship between turnover and brain weight in man is the result of cerebral requirements comprising the major proportion of total body glucose utilization. It seems likely that this is also the situation in piglets, but as yet no information about neonatal piglet cerebral metabolism is available.

The conventional approach to the analyses of glucose kinetics in man and in animals has been to normalize the results to body-weight, to brain weight, or to liver weight alone. Inspection of our results shows that glucose turnover is highly significantly ($P < 0{\cdot}001$) related not only to body- and brain weight, but also to the weight of the other organs studied. Since the weights of the organs are intimately related to the weight of the body, the question therefore arises whether, given body-weight, any further improvement in predictive power can be obtained from knowledge of the weights of one or more organs. To answer this question, we have used multiple regression techniques. Although the present study considers only a limited number of variables, nine, it is clear that the majority seem to be irrelevant in the present context. Although both body-weight and brain weight (for example) alone are significantly associated with glucose turnover, the best single predictor is liver weight (Table 1). Given liver weight, the next best predictor is spleen weight, followed by pancreas weight. Using these three variables, no further predictive power can be obtained by the use of body-weight, brain weight, heart weight, kidney weight, lung weight or resting

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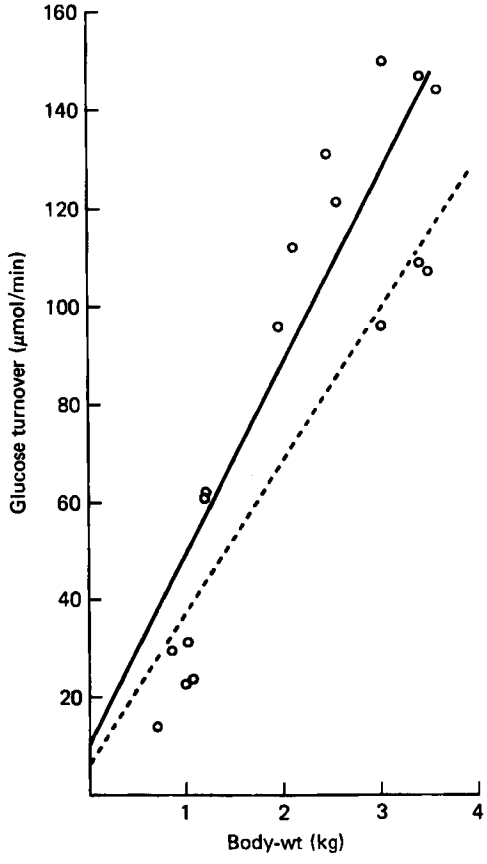

Fig. 8. Glucose turnover (μmol/min) and body-weight (kg) in piglets aged 0–14 d (o, —). For comparison the regression line of Bier *et al*, (1977) for human infants aged 0–14 d (----) is given.

plasma glucose concentration. The reasons for this must remain speculative at present, since other, unmeasured variables may also influence turnover to some extent, and their relative contribution remains to be assessed. However, in our sample, it is clear that liver, spleen and pancreas weight are the most important factors. The importance of liver weight as a predictor of glucose turnover is not unexpected in view of the central role of this organ in carbohydrate metabolism. The association of turnover with spleen and pancreas weights may be due to the weights of these organs reflecting more closely the mass of tissue which accounts for the greatest utilization of glucose than does total body-weight, which, of course, includes tissues which use relatively small quantities of glucose, for example fat and bone. The relative contribution of exocrine and endocrine tissue to total pancreas weight has not been measured, but it may be that the latter is the important factor in the association of turnover with pancreas weight. In contrast to glucose turnover, glucose pool size is most closely associated with body-weight and resting plasma glucose concentration, a result which is unsurprising in the light of current views of the distribution of glucose in the body.

Glucose turnover in the neonates of other species has been investigated, including the puppy (Kornhauser *et al.* 1976), the rhesus monkey (Sherwood *et al.* 1976) and the lamb (Cowett *et al.* 1976). Although turnover at birth in these species has been reported to be of a similar order to that of the piglet and human, the possible relationships to body-weight, organ weights or other variables in the growing animal have not been studied. We were

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unable to find any previously published measurements of turnover in neonatal pigs, but some comparison can be made with the results obtained by Freeman *et al.* (1970) who examined piglets aged 6–12 weeks. These workers normalized the turnover by expressing it in terms of body-weight$^{0.75}$. Expressing our results in this way, turnover was 65.5 ± 4.1 (SD) μmol/kg body-weight$^{0.75}$ per min, in comparison to 55.5 ± 8.0 μmol/kg body-weight$^{0.75}$ per min. This difference (of marginal significance), is consistent with the occurrence of approximately 20% recycling of the ^{14}C label used by Freeman *et al.* (1970) as has been reported in other species (Katz *et al.* 1974).

Several animal models have been proposed for the study of neonatal glucose metabolism and all have various advantages and disadvantages. In this study, the close similarity of the results obtained in the piglet with those reported in man encourages its use as a suitable animal model. Neonatal hypoglycaemia occurs most frequently in light-for-dates infants, and is recognized as an important clinical problem (Oh, 1977). Many litters of piglets contain one or more relatively undersized animals which are more susceptible to hypoglycaemia than are their littermates, hence the neonatal piglet may provide a suitable model for the study of glucose metabolism in both the normal and light-for-dates infant. A further advantage of this animal is that the state of its development and its size at birth make possible a wide variety of investigations which would be unethical in babies and enables detailed metabolic studies to be made in the neonatal period.

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