PROCEEDINGS OF THE NUTRITION SOCIETY

The Four Hundred and Fifty-sixth Scientific Meeting (One Hundred and Seventy-eighth Scottish Meeting) was held at Heriot-Watt University, Edinburgh on 15/16 September 1988

SYMPOSIUM ON 'NUTRITION AND METABOLISM IN THE NEWBORN'

Animal models of metabolism in newborn infants

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Neonatal care has evolved to such a degree that intensive care is now provided for infants with birth weights of 500–750 g. One aspect of the care of these vulnerable infants is the optimum choice of the route and the composition of nutritional support. It is often difficult to provide enough protein and energy to meet growth requirements without some degree of toxicity (Finberg, 1967). One example of such toxicity is the frequent occurrence of hyperglycaemia during parenteral feeding (Dweck, 1976). The quantification of metabolic flux is central to the rational management of these infants.

Any discussion on animal models of metabolism could include the range of species studied, and the range of metabolic models used. These are two distinct areas. The first approach is the province of the comparative physiologist, and aims to study similarities between the metabolism of animals and the human infant. It is, however, difficult to match fetal metabolism, placental function, length of gestation, brain weight relative to body-weight, weight:surface area ratio, litter size and the rate of post-natal maturation. I do not propose to discuss this area and the reader is referred to more relevant work (Adlard *et al.* 1973; Flecknell *et al.* 1981).

The second aspect of animal models concerns the nature of the modelling process itself. This asks whether it is possible to study metabolism in the human infant directly, using animal studies to help model the assumptions contained within the models themselves. It is a more fundamental problem than the first, and would still have to be dealt with even if the perfect species had been found. The central problems in modelling are the nature and validity of the assumptions which form the basis for the mathematical models of all metabolic processes. This applies equally well to the study of glucose, protein, fatty acid or ketone body metabolism. This area will be discussed using glucose metabolism to illustrate the methodology and its limitations.

Mathematical models, a clear need?

Mathematical models form the cornerstone of the analytical techniques available to the investigator. This is because all quantitative work requires a series of assumptions which together form a model of the metabolic pathway being studied. This pathway might be studied by classical enzymology, or with tracers to measure flux in the intact animal. Progress has been made in the analysis of control systems of cellular pathways (Crabtree & Newsholme, 1987; Kacser & Porteus, 1987), but there is a lively controversy about the most appropriate models (correspondence from Welch & Keleti, 1987). Thus,

if it is at present not possible to predict whole body kinetics from an understanding of intracellular control theory, then the analysis of 'open-pool systems' (models of the kinetics of whole body metabolism) has to be the most practical approach. This subject has been extensively reviewed (DiStefano, 1982; Hetenyi *et al.* 1983), and only the principles behind the analysis of the steady state and the non-steady state will be discussed here. Particular reference will be made to the types of clinical questions which may be answered using these models. Before any of these models may be used, however, the human infant must itself be accurately classified.

Classification of the newborn animal

The neonatal animal is often classified by birth weight in relation to the expected weight for a given gestational age. An assessment of the nutritional status of the infant is difficult, and if sufficiently accurate methods are not used then the results of metabolic investigations will have a poor discriminatory value. This is illustrated by the infant with an above average fetal weight who suffers growth retardation such that the birth weight is now merely average; then the infant would still be within the 'normal' weight range. The ponderal index ($100 \times$ weight/length^3) has been suggested as a useful discriminator in this situation (Walther & Ramaekers, 1982). The method used by Brenner *et al.* (1976) overcomes additional variables by providing a correction nomogram for factors such as parity and socioeconomic status. An alternative method is currently being evaluated at the University of Kansas, which combines the fetal growth values with a clinical nutrition score (J. Metcoff, personal communication).

The aims of the modelling process

When the subgroup to which the infant belongs has been accurately defined, the investigator is confronted with the mathematical representation of the biochemical pathway being studied. This is as true for simple steady state kinetics (Lassen & Perl, 1979) as it is for the more complex non-steady state (Shipley & Clark, 1972). In the steady state, a metabolite enters and leaves the well mixed pool (a space containing the total mass of metabolite within the body) at equal rates (often referred to as the turnover rate and expressed as μmol/kg per min). The nature and validity of the assumptions in these models are the key to the successful prediction of these rates of entry (R_a, or appearance rate into a pool) and disappearance (R_d, or disappearance rate from the pool). The optimum set of assumptions differs with each metabolite and whether non-steady state conditions are present, i.e. whether R_a and R_d are themselves changing (μmol/kg per min per min). Thus in nitrogen metabolism it is assumed that a free amino acid pool exists, and that R_a comes from dietary intake or from protein breakdown. R_d is assumed to be dependent on oxidation rate and the reutilization of the amino acid for protein synthesis (Picou & Taylor-Roberts, 1969; Jackson *et al.* 1981). In glucose metabolism, R_a depends on the rate of entry of glucose from the gut and on the rate of *de novo* synthesis in the liver and kidney. R_d is determined by the rate of oxidation (to CO_2 and water) and on the rate of reutilization of the carbon skeleton. The aim of all modelling processes is to determine these rates using tracers. The models are easier to analyse in the steady state, but this is often difficult to maintain in the newborn.

Analysis of turnover in the steady state

There are two methods for the analysis of the steady state. The first calculates the turnover rate alone using the tracer dilution principle, and requires an infusion of tracer until the specific activity (counts/mol tracee) reaches a plateau. This may take a long time if the metabolite is dispersed in a large volume (e.g. urea or bicarbonate), and it may be

necessary to fill the pool with a bolus of tracer (the priming dose) before infusion and then to wait for a plateau to occur. This approach is undoubtedly the 'gold standard' against which all other methods have to be validated. This is because it requires the fewest assumptions. Its disadvantage is that it provides the least information about the distribution of metabolite within the body (for further discussion see Lassen & Perl, 1979; Norwich, 1977). In addition this method cannot be used to analyse acute changes in flux following any stimulus to the organism.

The second method also calculates turnover but provides additional information. It uses compartmental analysis (a compartment is a pool which is defined by the investigator). The tracer is allowed to mix in the pool of metabolite following a rapid injection (the spike dose), and the decline in its specific activity is plotted against time. The shape of this curve allows the determination of the distribution of metabolite in the body. The area under the curve is inversely proportional to the turnover rate. The principal advantage of this knowledge is that the equation of this fitted curve may then be used to analyse the non-steady state (Shipley & Clark, 1972; Norwich, 1977). The implicit assumption is that the pattern of decline of tracer will mirror the path taken by new tracee entering the pool. The principles governing all models will be discussed with reference to glucose metabolism.

Models of neonatal glucose metabolism

Factors influencing the choice of tracer. Many species have been used to study glucose metabolism in the neonatal animal (Girard *et al.* 1973; Grajwer *et al.* 1977; Sherwood *et al.* 1977; Radziuk *et al.* 1978; Flecknell *et al.* 1981). The problem which is common to all these studies is the selection of the type and site of labelling on the glucose ring of the tracer.

Types of tracer molecule. The tracer molecule is likely to contain a radioisotope in animal studies and a safe, naturally occurring, non-radioactive, stable isotope in studies on the human infant. Tracers containing stable isotopes have the disadvantage of needing complex mass spectrometers to measure the ratio, labelled tracer molecules:unlabelled tracee (enrichment). The term atoms percent excess is used to quantify the enrichment and is equivalent to the term specific activity for radiolabelled tracers. Stable labels (such as ^{2}H,^{13}C,^{15}N,^{18}O) have to be administered in quantities which may represent as much as 5% of the total pool size of the metabolite being studied to facilitate detection. In the case of some isotopes, such as ^{13}C, there is the additional problem of a high background enrichment of certain foods (the natural abundance).

Site of labelling on the tracer molecule. The problem of substrate cycling has also to be considered, and the site of labelling is critical in this respect. When glucose is metabolized in peripheral tissues, a proportion of the C skeleton returns to the plasma via gluconeogenesis from the liver and kidney. This has the effect of violating the dilution principle, which assumes that all endogenously produced glucose is free of tracer.

The degree of recycling of labelled atoms on the glucose ring has been reviewed (Judsen & Leng, 1972; Katz *et al.* 1974). These studies have shown that the hydrogens on C-6 of the glucose ring have very little chance of recycling. C labels, however, recycle to variable degrees. This difference may be exploited for the study of the distribution of different parts of the glucose molecule amongst intermediary metabolites by labelling the glucose molecule at more than one site. Thus the selection of a tracer will depend on the type of clinical question being asked (for studies including oral feeds, for example, see method of Issekutz *et al.* 1974). The analysis of the steady state would not be complete without a comment on the determination of the rates of oxidation (a component of R_d). The major problem here is the accurate measurement of the rate of CO_2 production and

https://doi.org/10.1079/PNS19890037 Published online by Cambridge University Press

the enrichment of $^{13}CO_2$ in the expired air of infants (Clugston & Garlick, 1983; Van Aerde *et al.* 1985; Robert *et al.* 1987).

Failure of the steady state in neonates. If any metabolic model is to be useful it must be powerful enough to deal with the clinical circumstances surrounding the sick newborn infant. Such an infant is often out of the steady state because of the multiple illnesses that are present, the immaturity of many enzyme systems and the process of perinatal adaptation to extra-uterine life (Girard *et al.* 1973; Grajwer *et al.* 1977). When R_a and R_d for any metabolite are not matched, non-steady state conditions prevail.

In glucose metabolism, it is assumed that the true locations of R_a and R_d in the body are in rapid equilibrium with the plasma (Steele, 1959). This assumption is reasonable in the steady state, but when the rate of appearance of hepatic glucose begins to change inhomogeneities begin to occur in the total glucose pool and a more complex analysis is needed (Issekutz *et al.* 1974; Radziuk *et al.* 1978; Wootton, 1985).

Modelling assumptions and the non-steady state

It is often the case that the most physiologically accurate models contain too many unknowns to be solved in the clinical setting (Norwich, 1977). One 'solution' has been to introduce more simplifying assumptions in order to be able to calculate R_a and R_d (Cobelli *et al.* 1983; Hetenyi *et al.* 1983). The validity of the assumptions then becomes a problem in its own right (DiStefano, 1982) and animal models may then help to select the assumptions for use in the newborn. The principles behind the selection process are well illustrated by non-steady state glucose kinetics. The reader is referred to specialist reviews for the modelling of other aspects of intermediary metabolism (Chiasson *et al.* 1977; Keller *et al.* 1981 (ketone bodies); Hetenyi *et al.* 1983).

Glucose kinetics in the non-steady state: which model?

The majority of the studies have used Steele's (1959) single compartment model to approximate the non-steady state. A compartment may be defined as the ideal equivalent of the real pool of metabolite. There are two assumptions implicit in this definition. The first is the variable which governs the exit of metabolite from the compartment. It is a mathematically convenient expression which approximates reality. The commonest assumption is that a constant proportion of the mass of metabolite contained within the compartment is removed in unit time. This constant proportion is called the rate constant of exit from the compartment. The second assumption is that there are no concentration gradients within the compartment, and that a molecule entering is instantaneously mixed. This means that more than one compartment may be present within the total pool.

Steele (1959) studied non-steady state glucose kinetics in the dog. He showed that appearance rates could be approximated by the use of only one compartment, but this assumption produced values for R_a which were too high, during validation studies. He found that it was possible to correct the results empirically by a factor called the 'pool fraction' which could take a value between 0 and 1. This is based on the assumption that only a part of the total glucose pool changes its mass in the non-steady state, and is in a sense a hybrid between the one compartment and two compartment models of non-steady state glucose metabolism. The problem in the newborn is to estimate this fraction in different groups of infants.

One answer is to stress each infant with a known change in the rate of glucose administration (Mehta, unpublished work). Steele's (1959) model is then interrogated at a series of different pool fractions until the 'correct' one is found. This is the fraction

which correctly predicts both the magnitude and time of the change in the rate of glucose administration (induced by the experimenter and blind to the model).

This is a labour intensive approach and quite impractical for most studies. A two-compartment model, which has been validated in the dog (Radziuk *et al.* 1978), overcomes many of the drawbacks of Steele's (1959) method. Its principal advantage over Steele's (1959) one-compartment model is that it is able to predict the true change in flux without resort to an arbitrary pool fraction. This approach may in the future yield important information on the dose–response relationships of R_a and R_d following hormonal stimuli which have hitherto been subjected to a semiquantitative analysis only (Mehta *et al.* 1987, 1988). Compartmental models are often criticized because they cannot distinguish compartments with similar rate constants. This is true, but it is equally true that the purpose behind the effort is to calculate changes in R_a and R_d in response to a stimulus, such as a rise in the concentration of a hormone. Such changes are very common in the perinatal period, and this makes the method particularly relevant in this situation.

N Metabolism

The principles outlined previously are equally applicable to N metabolism. The inter-organ exchange of intermediary N carriers involves the differential uptake of alanine, glutamine, ammonia, etc, between tissues such as gut, liver and muscle (see Jackson & Golden, 1981). The models used to try and measure protein turnover may be divided into two different groups, the end product model and the precursor pool enrichment model (for the theoretical aspects, see Hetenyi *et al.* 1983 and for the application to neonates, see Millward *et al.* 1986). The problems of the selection of the most appropriate tracer (glycine, leucine, etc.) and the assumptions of the model recur in N metabolism and will be discussed by others in this symposium.

Conclusion

Models of metabolism have problems which are common to all metabolites. Validation of all assumptions must be one of the first tasks of the investigator when these models are applied to the newborn infant.

REFERENCES

Adlard, B. P. F., Dobbing, J. & Smart, J. L. (1973). *Biology of the Neonate* **23**, 95–108.

Brenner, W. E., Edelman, D. A. & Hendricks, C. H. (1976). *American Journal of Obstetrics and Gynecology* **126**, 555–564.

Chiasson, J. L., Liljenquist, J. E., Lacy, W. W., Jennings, A. S. & Cherrington, A. D. (1977). *Federation Proceedings* **36**, 229–235.

Clugston, G. A. & Garlick, P. J. (1983). *Clinical Science* **64**, 231–233.

Cobelli, C., Ruggeri, A., Toffolo, G., Avagaro, A. & Nosadini, R. (1983). *American Journal of Physiology* **245**, R624–R632.

Crabtree, B. & Newsholme, E. A. (1987). *Trends in Biochemical Sciences* **12**, 4, 6, 8, 10, 12.

DiStefano, J. J. (1982). *American Journal of Physiology* **243**, R1–R6.

Dweck, H. S. (1976). *Postgraduate Medicine* **60**, 118–124.

Finberg, L. (1967). *Pediatrics* **40**, 1031–1034.

Flecknell, P. A., Wootton, R. & John, M. (1981). *Clinical Science* **60**, 335–338.

Girard, J. R., Cuendet, G. S., Marliss, E. B., Kervran, A., Rieutort, M. & Assan, R. (1973). *Journal of Clinical Investigation* **52**, 3190–3200.

Grajwer, L. A., Sperling, M. A., Sack, J. & Fisher, D. A. (1977). *Pediatric Research* **11**, 833–866.

Hetenyi, G., Perez, G. & Vranic, M. (1983). *Physiological Reviews* **63**, 606–667.

Issekutz, T. B., Issekutz, B. & Elahi, D. (1974). *Canadian Journal of Physiology and Pharmacology* **52,** 215–224.

Jackson, A. A. & Golden, M. H. N. (1981). In *Nitrogen Metabolism in Man,* pp. 203–213 [J. C. Waterlow and J. H. L. Stephen, editors]. London: Applied Science Publishers.

Jackson, A. A., Shaw, J. C. L., Barber, A. & Golden, M. H. N. (1981). *Pediatric Research* **15,** 1454–1461.

Judsen, G. J. & Leng, R. A. (1972). *Australian Journal of Biological Sciences* **25,** 1313–1332.

Kacser, H. & Porteus, J. W. (1987). *Trends in Biochemical Sciences* **12,** 5, 7, 9, 11, 13, 14.

Katz, J., Rostami, H. & Dunn, A. (1974). *Biochemical Journal* **142,** 161–170.

Keller, U., Sonnenberg, G. E. & Stauffacher, W. (1981). *American Journal of Physiology* **240,** E253–E262.

Lassen, N. A. & Perl, W. (1979). *Tracer Kinetic Methods in Medical Physiology.* New York: Raven Press.

Mehta, A., Wootton, R., Cheng, K. N., Penfold, P., Halliday, D. & Stacey, T. E. (1987). *Archives of Disease in Childhood* **62,** 924–930.

Mehta, A., Wootton, R. Cheng, K. N., Penfold, P. & Halliday, D. (1988). *Clinical Science* **74,** Suppl. 18, Abstr. 186, p. 50.

Millward, D. J., De Benoist, B. & Halliday, D. (1986). In *Clinical Nutrition and Metabolic Research. Proceedings of the 7th ESPEN Congress, Munich 1985*, pp. 178–191 [G. Dietze, A. Grünert, G. Kleinberger and G. Wolfram, editors]. Basel: Karger.

Norwich, K. H. (1977). *Molecular Dynamics in Biosystems.* Oxford: Pergamon Press.

Picou, D. & Taylor-Roberts, T. (1969). *Clinical Science* **36,** 283–289.

Radziuk, J., Norwich, K. H. & Vranic, M. (1978). *American Journal of Physiology* **234,** E84–E93.

Robert, J. J., Koziet, J., Chauvet, D., Darmaun, D., Desjeux, J. F. & Young, V. R. (1987). *Journal of Applied Physiology* **63,** 1725–1732.

Sherwood, W. G., Hill, D. E. & Chance, G. W. (1977). *Pediatric Research* **11,** 874–877.

Shipley, R. A. & Clark, R. E. (1972). *Tracer Methods for in vivo Kinetics.* New York: Academic Press.

Steele, R. (1959). *Annals of the New York Academy of Sciences* **82,** 420–430.

Van Aerde, J. E. E., Sauer, P. J. J., Pencharz, P. B., Canagarayar, U., Beesley, J., Smith, J. M. & Swyer, P. R. (1985). *Paediatric Research* **19,** 806–810.

Walther, F. J. & Ramaekers, L. H. J. (1982). *Acta Paediatrica Scandinavica* **71,** 437–440.

Welch, G. R. & Keleti, T. (1987). *Trends in the Biochemical Sciences* **12,** 216–217.

Wootton, R. (1985). In *Substrate and Energy Metabolism in Man,* pp. 16–26 [J. S. Garrow and D. Halliday, editors]. London: John Libbey.

Printed in Great Britain