Biochem. J. (1979) **182**, 565–575
Printed in Great Britain

Metabolism of Glucose in Hyper- and Hypo-thyroid Rats *in vivo*

GLUCOSE-TURNOVER VALUES AND FUTILE-CYCLE ACTIVITIES OBTAINED WITH ^{14}C- AND ^{3}H-LABELLED GLUCOSE

By Fumikazu OKAJIMA and Michio UI

Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

(*Received* 6 *February* 1979)

1. A trace amount of glucose labelled with ^{14}C uniformly and with ^{3}H at position 2, 3 or 6 was injected intravenously into starved rats to measure the turnover rate of blood glucose. 2. Reliable estimates were made based on the semilogarithmic plot of specific radioactivity of the glucose contained in whole blood samples taken from the tail vein. 3. Glucose turned over more rapidly in hyperthyroid and more slowly in hypothyroid than in euthyroid tats. The percentage contribution of glucose recycling (determined from the difference in replacement rates between [U-^{14}C]glucose and [6-^{3}H]glucose) to the glucose utilization increased on induction of hyperthyroidism. 4. Futile cycles between glucose and glucose 6-phosphate (determined from the difference between replacement rates of [2-^{3}H]glucose and [6-^{3}H]glucose) were activated and inactivated by induction of hyperthyroid and hypothyroid states respectively. 5. The hepatic content of glycogen was much lower in hyper- and hypo-thyroid than in euthyroid rats. The enhanced glucose production in hyperthyroid rats resulted from not only activation of hepatic gluconeogenesis but also diversion of the final product of gluconeogenesis from liver glycogen to blood glucose. In hypothyroidism, the inhibition of gluconeogenesis led to suppression of both glucose production and glycogenesis in the liver.

We have shown (Okajima & Ui, 1978) that hyperthyroid rats were characterized by an elevation of the starvation concentration of blood glucose associated with a slight hyperinsulinaemia, whereas hypothyroid rats showed a trend for hypoglycaemia despite a slight hypoinsulinaemia. The difference in the blood glucose concentration between normal and hyper- or hypo-thyroid animals, though rather small in magnitude, may not be accounted for solely by the hypoglycaemic action of plasma insulin, and hence suggests that other factors would be also involved as determinants of blood glucose metabolism in altered thyroid states.

The blood glucose concentration is dependent on both hepatic glucose production and peripheral glucose utilization; an increase in the rate of glucose production and a decrease in the rate of glucose utilization should cause hyperglycaemia, whereas changes in these rates in the reverse direction should be responsible for hypoglycaemia. Kusaka & Ui (1977*a*,*b*) have reported, however, that changes in the rate of hepatic glucose production were liable to be associated with changes in the same direction in peripheral glucose utilization in rats, thereby altering the glucose-turnover rate without appreciable changes in the blood glucose concentration. It is conceivable, therefore, that small changes in blood glucose concentration in the hyper- and hypothyroid states would be associated with marked changes in the turnover rate of blood glucose.

The purpose of the present paper is to estimate glucose turnover directly in hyper-and hypo-thyroid rats by means of a tracer technique. The turnover rate determined with [U-^{14}C]glucose was compared with the rate determined by the use of glucose labelled with ^{3}H at the 2, 3 or 6 position. As first described by Dunn *et al.* (1967) and Katz & Dunn (1967), ^{14}C will be, but ^{3}H will not be, incorporated into newly formed glucose owing to recycling, which occurs during metabolism of the labelled glucose injected. Thus the difference between replacement rates of ^{14}C-labelled and ^{3}H-labelled glucose was used for calculation of the rate of glucose recycling including futile cycles. Futile cycles will be possibly dependent on thermoregulatory endocrine factors such as the thyroid.

Experimental

Animal techniques and determinations

Male albino rats (body wt. 100–300g) of the Wistar-derived strain were used after 20h starvation. Rats were rendered hyperthyroid by daily injection of thyroxine (50μg/100g body wt.) for 8–12 days or

were rendered hypothyroid by giving a 0.3% solution of methylthiouracil (4-hydroxy-6-methyl-2-thiopyrimidine) instead of drinking water for 21–35 days, as described elsewhere in detail (Okajima & Ui, 1978). Where indicated, cortisol (suspended in water) was injected subcutaneously twice 24 and 3h before experiments, the dose being 2.5mg/100g at each time.

[U-^{14}C]Glucose (0.5μCi/100g) and [^{3}H]glucose (5μCi/100g) were injected into the femoral vein under pentobarbital anaesthesia. Blood samples (0.1ml) withdrawn from the tail vein at intervals were haemolysed in a small amount of water, deproteinized and assayed for [^{14}C]- or [^{3}H]-glucose by the method of Yajima & Ui (1974). Glucose was determined enzymically (Bergmeyer & Bernt, 1963). For analysis of ^{3}H content of glucose, the sample was evaporated to dryness to eliminate $^{3}H_2O$ before being counted for radioactivity.

The specific radioactivity of blood glucose calculated from these values was then used for estimation of kinetic parameters for glucose turnover as described below. This blood-sampling technique was used as a routine procedure in the present study, since turnover rates, rate coefficients and recyclings thus calculated for blood glucose were essentially the same as those respective values obtained with plasma taken from an indwelling catheter in the jugular vein. Thus blood samples taken from the tail vein without catheterization reflect the rapidly mixing pool of glucose in the same degree as blood samples directly taken from the central vessels. Heath (1973) reported that glucose in erythrocytes was much smaller in amount than, and was not in rapid equilibrium with, glucose in plasma.

To determine liver glycogen labelled with ^{14}C or ^{3}H, the abdominal cavity of the anaesthetized rat was opened at the end of the consecutive blood samplings and the liver was quickly excised and immediately frozen in a clamp cooled in liquid nitrogen. [^{14}C]- and [^{3}H]-Glycogen were determined as described previously (Yajima & Ui, 1974). An intravenous glucose-tolerance test was carried out by the method of Shikama & Ui (1975*a*); the rats were injected intravenously with [^{14}C]bicarbonate to follow the incorporation of ^{14}C into blood glucose and liver glycogen simultaneously. Radioactivity was measured in a Beckman liquid-scintillation spectrometer (model LS230). Toluene/Triton X-100 (2:1, v/v) containing 3.5g of PPO (2,5-diphenyloxazole) +200mg of POPOP [1,4-bis-(5-phenyloxazol-2-yl)-benzene]/litre was used as the scintillant.

Calculation of the glucose-turnover rate, the and coefficient and glucose recycling

All of the experiments in the present study were carried out in the starved state, in which the blood glucose concentration in each rat was maintained fairly constant throughout the course of experiments. Under such conditions, i.e. in the steady state, the rate of glucose release into the circulating blood (the inflow rate) should equal the rate of glucose removal from the circulation (the outflow rate), which will be referred to as the turnover rate of blood glucose. The glucose-turnover rate (R, mg/min per 100g body wt.) was estimated by means of a single injection of a tracer amount of labelled glucose followed by repeated samplings of blood to determine changes in specific radioactivity of glucose. Two calculation methods were used, as follows.

The linear plot of glucose specific radioactivity versus time (S–t curve) was first constructed. R was calculated according to eqn. (1) by using the graphical-analysis method of Katz *et al.* (1974*a*,*b*):

$$R = D/A \quad (1)$$

where D is the total radioactivity injected (c.p.m./100g) and A is the area under the S–t curve from zero to infinite time. Alternatively, a curve of the log of specific radioactivity versus time was constructed. As shown in Fig. 1, a straight line was obtained from 5 to 100min after the injection of the tracer, suggesting that glucose turned over in an instantaneously mixing pool (a single compartment) during this period. This straight line was represented by an exponential equation

$$S = S_0 e^{-kt} \quad (2)$$

where S is the specific radioactivity (c.p.m./mg) of glucose in a blood sample (S_0, the value at zero time) and k is the first-order rate constant or the fractional turnover rate (min^{-1}). k could be determined by the slope (b) of the straight line by the relationship $k = b \times \ln 10$. Then $R = Mk$, where M is the glucose-pool size (mg/100g) and could be determined by $M = D/S_0$. This calculation of R will be referred to as the monoexponential analysis (Hetenyi & Norwich, 1974). Since glucose-pool size is the product of the blood glucose concentration (C, mg/ml) and the space occupied by the glucose (the pool space, V, ml/100g), then $R = kCV = k'C$. k' (ml/min per 100g) can be obtained by dividing the turnover rate by the blood glucose concentration and is referred to as the rate coefficient of glucose turnover, which represents the turnover rate corrected for by changes in the blood glucose concentration.

The method for calculation of the glucose-turnover rate as described above is based on the assumption that glucose newly synthesized and released into the circulation is not labelled with radioisotopes. The validity of this assumption depends on a choice of radioisotope for labelling blood glucose; ^{14}C is recycled back into glucose released by liver, whereas ^{3}H is liable to be lost during glycolysis and gluconeogenesis, thereby minimizing its own recycling to the glucose pool. By comparing the turnover rates

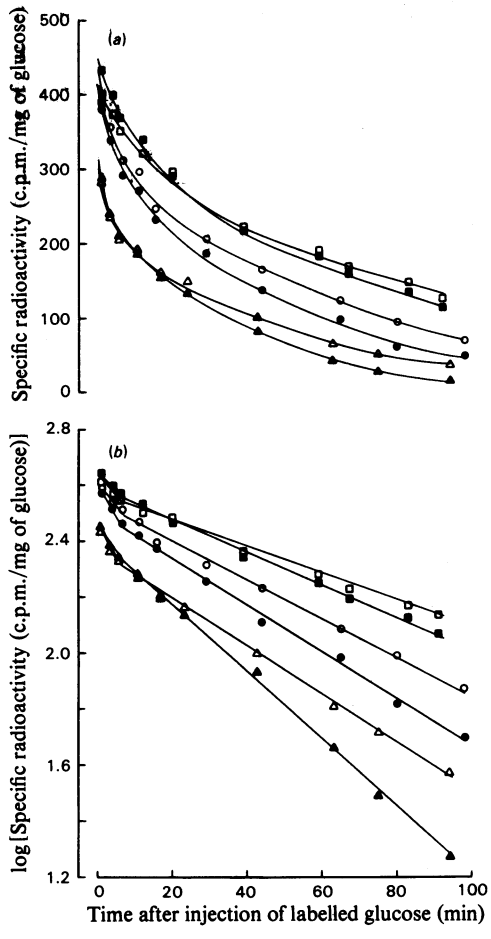

Fig. 1. *Linear and semilogarithmic plots of plasma glucose specific radioactivity in rats injected with [U-^{14}C,6-^{3}H]-glucose*

Euthyroid (○, ●), hyperthyroid (△, ▲) and hypothyroid (□, ■) rats were injected intravenously with [U-^{14}C,6-^{3}H]glucose at zero time. Linear (*a*) and semilogarithmic (*b*) plots of specific radioactivities of plasma [^{14}C]glucose (open symbols) and [^{3}H]glucose (solid symbols) are shown. The dose of radioactivity is normalized at 10000c.p.m./100g body wt.

obtained with [^{14}C]glucose with those obtained with [^{3}H]glucose, therefore, the rates of recycling of glucose carbon from extrahepatic tissues to liver (i.e. the Cori cycle plus the glucose–alanine cycle) and of the futile cycles were calculated as follows.

The real rate of the blood glucose turnover was obtained by the use of [6-^{3}H]glucose. Then the Cori-cycle (plus the glucose–alanine-cycle) activity is represented by $R_{6H}-R_c$, and its percentage contribution to the blood glucose turnover is $100\times(R_{6H}-R_c)/R_{6H}$, where R_{6H} and R_c are the turnover rates calculated with the use of [6-^{3}H]glucose and [U-^{14}C]-glucose respectively. Since futile cycles catalysed by hexokinase and glucose 6-phosphatase (followed by the rapid phosphoglucoisomerase reaction) will cause detritiation of [2-^{3}H]glucose and futile cycles catalysed by phosphofructokinase and fructose 1,6-bisphosphatase (followed by the rapid triose phosphate isomerase reaction) will cause detritiation of [3-^{3}H]glucose in the liver, the activity of the former futile cycles (glucose–glucose 6-phosphate cycle) is expressed as $R_{2H}-R_{6H}$ [its percentage contribution is $100\times(R_{2H}-R_{6H})/R_{6H}$] and the activity of the latter cycles (fructose 6-phosphate–fructose 1,6-bisphosphate cycle) is expressed as $R_{3H}-R_{6H}$ [its percentage contribution is $100\times(R_{3H}-R_{6H})/R_{6H}$], where R_{2H} and R_{3H} are the turnover rates of blood glucose calculated with the use of [2-^{3}H]glucose and [3-^{3}H]glucose respectively.

Doubly labelled glucose was used for determination of turnover rates, i.e. glucose specifically labelled with ^{3}H was injected into rats in combination with [U-^{14}C]glucose. Turnover rates and other kinetic parameters were then calculated for each rat and combined within a group of rats receiving the same treatment to give the mean ± S.E.M. for each parameter. The difference between the means was determined by Student's *t* test unless otherwise stated. The rate of the Cori cycle (plus the glucose–alanine cycle) was also calculated for each rat and subjected to the *t* test, but the rate for futile cycles, which has to depend for calculation on comparison of the ^{3}H data of one group of rats with those of another group, was presented without standard error and hence no statistical analysis was undertaken. In these cases, the difference between R_H and R_c (e.g. $R_{2H}-R_c$ or $R_{6H}-R_c$) was calculated for each rat and the mean value for the difference within a group of rats was used for calculation of the rate of futile cycles [e.g. $(R_{2H}-R_c)-(R_{6H}-R_c)$ for the glucose–glucose 6-phosphate cycle].

Chemicals

Sources of reagents were as follows: thyroxine and cortisol, Sigma Chemical Co., St Louis, MO, U.S.A.; [U-^{14}C]glucose and $NaH^{14}CO_3$, Daiichi Pure Chemicals, Tokyo, Japan; [2-^{3}H]-, [3-^{3}H]- and [6-^{3}H]-glucose, New England Nuclear Corp., Boston, MA, U.S.A. Other reagents were of analytical grade from commercial sources.

Results

Turnover rates calculated by monoexponential analysis with whole blood samples taken from the tail vein as a routine procedure

Handling of animals including operative procedures for catheterization and blood withdrawal should be

as minimal as possible during experiments for glucose turnover to be maintained in the steady state under physiological conditions. A routine method has developed on this principle, as described in the Experimental section.

Euthyroid, hyperthyroid and hypothyroid rats were injected with [U-^{14}C,6-^{3}H]glucose intravenously and the specific radioactivities of glucose were determined in whole blood taken from the tail vein at intervals for 4h until the radioactivity content of glucose became negligible. The concentration of blood glucose was maintained virtually constant during this period. By using the specific-radioactivity–time curves as shown in Fig. 1, turnover rates for [^{14}C]glucose and [^{3}H]glucose as well as glucose recycling were calculated by means of both graphical analysis and monoexponential analysis (Table 1). The graphical analysis tended to provide kinetic parameters slightly and insignificantly smaller than those obtained by the monoexponential analysis. The effect of changing thyroid function on glucose kinetics was found to be similar with the two methods used for the calculations; glucose turned over at a rate twice as high in hyperthyroid and half as high in hypothyroid as in euthyroid rats. The percentage glucose recycling was also increased in hyperthyroid rats, but was not affected by induction of the hypothyroid state.

On the basis of these findings, further analysis of glucose kinetics was conducted by the monoexponential method on whole blood samples taken from the tail vein.

Kinetic parameters of blood glucose in hyper- and hypo-thyroidism

Table 2 summarizes kinetic parameters of blood glucose as determined by using [U-^{14}C,6-^{3}H]glucose in various thyroid states. The concentration of blood glucose was significantly higher in hyperthyroid rats than in euthyroid rats, but no significant difference was detected between euthyroid and hypothyroid rats in this experiment. ^{14}C or ^{3}H decay was used for calculation of the pool size and the glucose space; either decay yielded essentially the same value. The pool size of glucose was expanded by induction of the hyperthyroid state; this expansion was explainable by hyperglycaemia only, since the glucose space was never affected by changing thyroid activity.

The fractional turnover rate and the rate coefficient of [6-^{3}H]glucose were higher than those of [U-^{14}C]-

Table 1. *Glucose-turnover rates and recycling calculated by two analytical methods*
Starved rats were injected with [U-^{14}C,6-^{3}H]glucose, and blood samples were taken at intervals from the tail vein. Mean values ± S.E.M. from four animals calculated as described in the Experimental section are given. Significance of difference from euthyroid rats: $^{*}P<0.05$, $^{**}P<0.01$.

	(*a*) Graphical analysis			(*b*) Monoexponential analysis		
	Turnover rate (mg/min per 100g body wt.)		Glucose recycling (%)	Turnover rate (mg/min per 100g body wt.)		Glucose recycling (%)
Thyroid state	R_{6H}	R_c		R_{6H}	R_c	
Euthyroid	0.64 ± 0.07	0.58 ± 0.07	10 ± 1.4	0.68 ± 0.07	0.58 ± 0.06	15 ± 3.1
Hyperthyroid	1.23 ± 0.07**	0.88 ± 0.04**	28 ± 5.3**	1.31 ± 0.07**	0.94 ± 0.04**	28 ± 2.4**
Hypothyroid	0.32 ± 0.03**	0.31 ± 0.03*	11 ± 4.0	0.34 ± 0.02**	0.30 ± 0.02**	13 ± 2.6

Table 2. *Kinetic parameters for glucose turnover determined with [U-^{14}C,6-^{3}H]glucose in various thyroid states*
Experiments were carried out as in Fig. 1, and kinetic parameters were calculated by the monoexponential method. Blood glucose concentration for each rat was determined as an average of five samples taken at 30, 45, 60, 75 and 90min after the injection of label. Mean values ± S.E.M. from four rats are given. Significance of difference from the corresponding value in the euthyroid group: $^{*}P<0.05$, $^{**}P<0.01$.

Thyroid state	Blood glucose (mg/100ml)	Label used	Pool size (*M*) (mg/100g body wt.)	Space (*V*) (ml/100g body wt.)	Fractional turnover rate (*k*) (%/min)	Rate coefficient (*k'*) (ml/min per 100g body wt.)
Euthyroid	76 ± 7	6-^{3}H	33.0 ± 3.1	43.5 ± 2.8	2.06 ± 0.09	0.90 ± 0.10
		U-^{14}C	33.7 ± 2.6	44.5 ± 1.8	1.71 ± 0.08	0.77 ± 0.05
Hyperthyroid	100 ± 4*	6-^{3}H	47.4 ± 3.3*	47.1 ± 1.5	2.77 ± 0.09**	1.31 ± 0.05**
		U-^{14}C	47.0 ± 1.2*	47.0 ± 1.1	1.95 ± 0.01*	0.92 ± 0.02**
Hypothyroid	74 ± 2	6-^{3}H	31.6 ± 2.9	42.6 ± 3.7	1.10 ± 0.05**	0.46 ± 0.02**
		U-^{14}C	30.5 ± 2.7	41.2 ± 3.6	0.99 ± 0.05**	0.40 ± 0.03**

glucose in any thyroid state. These rates and rate coefficients were significantly higher in hyperthyroid and lower in hypothyroid than in euthyroid rats, in accord with the results in Table 1, indicating that thyroid activity is one of the determinants of the glucose-turnover rate.

Prolonged treatment of rats with either thyroxine (to induce the hyperthyroid state) or methylthiouracil (to induce the hypothyroid state) minimized their weight gain. Since a marked weight loss was occasionally observed during the methylthiouracil regime with younger rats, the hypothyroid state was induced in rats bigger than those used for induction of the hyperthyroid state. Accordingly, euthyroid rats of a wide range of body weight were used as their controls. The effect of body weight on the glucose-turnover rate is shown in Fig. 2, in which the rates determined with [2-^{3}H]-, [3-^{3}H]- or [6-^{3}H]-glucose or [U-^{14}C] glucose are plotted against body weights of rats. In euthyroid rats, all of these rates per 100g body wt. showed a trend to decline as body weight increased, with correlation coefficients of -0.338 (for 2-^{3}H, $P<0.025$), -0.707 (for 3-^{3}H, $P<0.025$), -0.441 (for 6-^{3}H, $P<0.01$) and -0.462 (for U-^{14}C, $P<0.001$). In

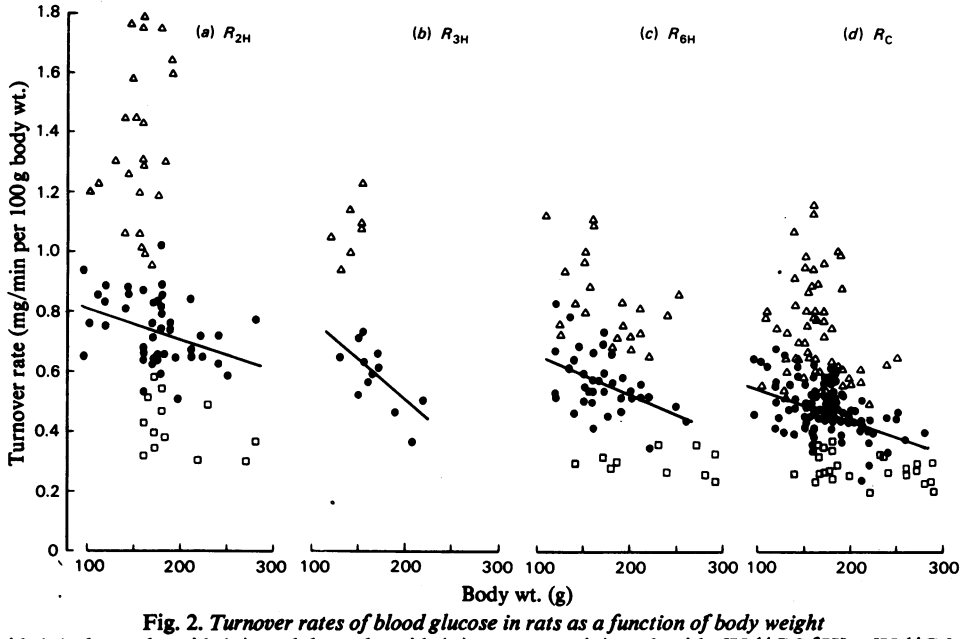

Fig. 2. *Turnover rates of blood glucose in rats as a function of body weight*

Euthyroid (●), hyperthyroid (△) and hypothyroid (□) rats were injected with [U-^{14}C,2-^{3}H]-, [U-^{14}C,3-^{3}H]- or [U-^{14}C,6-^{3}H]-glucose. Turnover rates (*a*, R_{2H}; *b*, R_{3H}; *c*, R_{6H}; *d*, R_c) calculated by the monoexponential analysis are plotted against body weights of rats. Straight lines represent regression lines drawn by least-squares fitting for the data with euthyroid rats. No significant regression was observed for hyper- or hypo-thyroidism in panels (*a*)–(*d*).

Table 3. *Activities of futile cycles in various thyroid states*

Values in this Table were calculated from the data in Fig. 2. Euthyroid rats with body weights smaller than 200g were used as the control for hyperthyroid rats, and those bigger than 200g served as the control for hypothyroid rats. Results (mg/min per 100g body wt.) are means ± S.E.M. for the numbers of rats shown in parentheses. Significance of difference from the corresponding value in column (*a*): *$P<0.01$.

Thyroid state	(*a*) $R_{6H}-R_c$ (glucose recycling)	(*b*) $R_{3H}-R_c$	(*c*) $R_{2H}-R_c$	Futile cycle (*c*)−(*a*)†	Futile cycle (*b*)−(*a*)‡
Euthyroid	0.11 ± 0.01 (29)	0.16 ± 0.02 (10)*	0.23 ± 0.02 (35)*	0.12	0.05
Hyperthyroid	0.23 ± 0.01 (22)	0.33 ± 0.02 (7)*	0.54 ± 0.03 (26)*	0.31	0.10
Euthyroid	0.07 ± 0.01 (8)		0.28 ± 0.03 (9)*	0.21	
Hypothyroid	0.04 ± 0.01 (10)		0.13 ± 0.02 (15)*	0.09	

† Futile cycles between glucose and glucose 6-phosphate.

‡ Futile cycles between fructose 6-phosphate and fructose 1,6-bisphosphate.

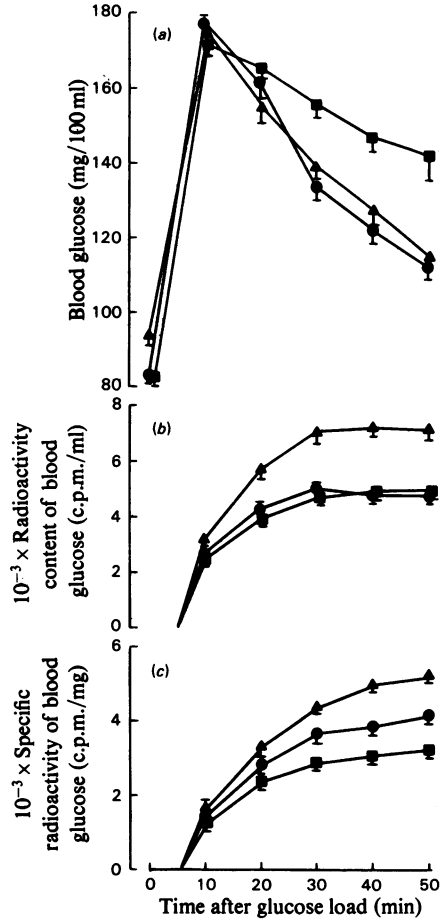

Fig. 3. *Incorporation of [^{14}C]bicarbonate into blood glucose in euthyroid, hyperthyroid and hypothyroid rats after intravenous glucose load*

Euthyroid (●), hyperthyroid (▲) and hypothyroid (■) rats were injected intravenously with a 50% (w/v) solution of glucose (0.15 ml/100 g body wt.) at zero time. [^{14}C]Bicarbonate was injected intravenously 5 min after the glucose load. Mean values ± S.E.M. from four animals are plotted against time. (*a*) Blood glucose concentrations, (*b*) ^{14}C content of blood glucose, (*c*) specific radioactivity of blood glucose.

hyperthyroid or hypothyroid rats, however, there was no significant correlation between body weight and the glucose-turnover rate per 100 g body wt. Co-variance analysis of the data in Fig. 2 taking the regression into consideration (Snedecor & Cochran, 1967) revealed that the glucose-turnover rate per 100 g body wt. was higher in hyperthyroid rats and lower in hypothyroid rats than in euthyroid rats regardless of body weight and the position of label in the glucose molecule ($P < 0.005$ for any comparison).

Cori-cycle and futile-cycle activities in hyper- and hypo-thyroid rats

From the data in Fig. 2, the differences between the glucose-turnover rate obtained from [U-^{14}C]-glucose (R_c) and the rates obtained from [^{3}H]glucose (R_H) were calculated as a measure of glucose recycling. Euthyroid rats weighing below 200 g and above 200 g (Table 3) were used as controls for hyperthyroid and hypothyroid rats respectively. The rates of the Cori cycle and of the futile cycles were then calculated based on these differences of turnover rates. Both the Cori-cycle (plus the glucose–alanine-cycle) activity and also the activity of futile cycles at the site between glucose and glucose 6-phosphate were doubled by induction of the hyperthyroid state, and were decreased in the hypothyroid state. Futile cycles at the site between fructose 6-phosphate and fructose 1,6-bisphosphate operated at only a low rate, though there was also a trend for increase in hyperthyroid rats. Owing to its low activity, this cycle was not determined in hypothyroid rats. Thus the thyroid-dependent acceleration of glucose turnover appeared to be associated with activated gluconeogenesis and futile cycles.

Glucose production and glycogenesis in the liver in hyper- and hypo-thyroid rats

The increase in hyperthyroid rats and the decrease in hypothyroid rats of glucose turnover as observed above are considered to indicate that the rates of hepatic production and peripheral utilization of glucose changed depending on thyroid activity. As a more direct measure of glucose production via gluconeogenesis, the incorporation of [^{14}C]bicarbonate into blood glucose was then studied. A large amount of glucose was injected simultaneously with [^{14}C]bicarbonate for the purpose of expanding the glucose pool, thereby decreasing the removal by peripheral tissues of newly synthesized [^{14}C]glucose. The incorporation of ^{14}C from bicarbonate into blood glucose and liver glycogen under these conditions reflects hepatic gluconeogenesis, as previously reported by Shikama & Ui (1975*a*,*b*).

Hyperglycaemia induced by the glucose load declined as rapidly in hyperthyroid rats as in euthyroid rats (Fig. 3*a*), in accord with our previous results (Okajima & Ui, 1978). Despite the same glycaemic response, much more ^{14}C was incorporated into blood glucose in hyperthyroid than in euthyroid rats (Fig. 3*b*). As a result, the specific radioactivity of blood glucose was significantly higher in the former than in the latter (Fig. 3*c*). On the other hand, hypothyroid rats showed an impaired glucose tolerance, in confirmation of our previous results (Okajima & Ui, 1978), without an increase in ^{14}C

Table 4. *Tissue concentrations and radioactivity contents of liver glycogen in euthyroid, hyperthyroid and hypothyroid rats*
In Expt. 1, liver was excised at 90min after injection of [U-^{14}C,2-^{3}H]glucose. In Expt. 2, glucose was injected as in Fig. 3, and liver was excised 60min later. Significance of difference from euthyroid: $*P<0.05$, $**P<0.01$.

(*a*) Expt. 1 (labelled with [U-^{14}C,2-^{3}H]glucose; without a glucose load)

Thyroid state	Glycogen (mg/g)	Radioactivity in glycogen (c.p.m./10mg of tissue) ^{14}C	^{3}H	Specific radioactivity of glycogen (c.p.m./10μg of glycogen) ^{14}C	^{3}H
Euthyroid	3.7±0.47	29±5.3	34±6.2	9±1.5	11±2.1
Hyperthyroid	0.4±0.04**	1±0.2**	2±0.2**	3±0.2**	4±0.4**
Hypothyroid	1.4±0.12**	16±3.7**	20±5.2*	8±2.2	10±3.0

(*b*) Expt. 2 (labelled with [^{14}C]bicarbonate; with a glucose load)

Thyroid state	Glycogen (mg/g)	^{14}C in glycogen (c.p.m./10mg of tissue)	Specific radioactivity of glycogen (c.p.m./10μg of glycogen)
Euthyroid	5.4±0.35	364±56	105±10
Hyperthyroid	3.4±0.53*	296±59*	86±8
Hypothyroid	2.8±0.84*	257±69*	90±10

content of blood glucose. Consequently, the specific radioactivity of blood glucose was lower in hypothyroid than in euthyroid rats (Fig. 3*c*). Thus hepatic production of glucose owing to gluconeogenesis was stimulated and suppressed in the hyper- and hypothyroid states respectively. Impaired glucose tolerance associated with suppressed glucose production by the liver is a strong indication of the inhibition of peripheral glucose utilization, in hypothyroid rats. Likewise, the lack of effect on glucose tolerance despite stimulated glucose production in hyperthyroid rats suggests that peripheral glucose utilization is also accelerated in the hyperthyroid state. The changes in hepatic glucose supply to the glucose pool, associated with changes in the same direction in the removal of blood glucose, are compatible with changes in glucose turnover as observed in Fig. 2 for hyper- and hypo-thyroid states.

Table 4 shows the hepatic content of glycogen as well as its labelling with [U-^{14}C,2-^{3}H]glucose in the starved state (Expt. 1) and with [^{14}C]bicarbonate after a glucose load (Expt. 2). The induction of either the hyper- or hypo-thyroid state decreased the starvation content of liver glycogen; the decrease was more striking in the hyperthyroid rats than in hypothyroid rats, in agreement with earlier reports (Takahashi & Suzuki, 1971; Battarbee, 1974; Porterfield *et al.*, 1975). The incorporation of [^{14}C]- or [^{3}H]-glucose into liver glycogen was inhibited by 95% on induction of hyperthyroidism. Thus the inhibition of glycogenesis was evident in hyperthyroid rats, since the decrease in specific radioactivity of precursor glucose induced by hyperthyroidism was only 40–60%. The excess glycogen content in Expt. 2 over Expt. 1 in Table 4 may reflect the net glycogenesis induced by glucose loading. It was not smaller in hyperthyroid than in euthyroid rats. Probably the marked hyperglycaemia induced by glucose loading resulted in partial restoration of glycogenesis in hyperthyroid rats, although glycogenic activity was still lower than in euthyroid rats, as evidenced by lower ^{14}C content despite higher specific radioactivity of blood glucose under these conditions (see Fig. 3*c*).

Both the tissue content of glycogen and also the incorporation of ^{14}C and ^{3}H from glucose into glycogen were lowered, despite the increased specific radioactivity of precursor glucose in blood (Fig. 1), by induction of the hypothyroid state. The net gain of liver glycogen induced by glucose load was also smaller in hypothyroid rats than in euthyroid rats. Thus hepatic glycogenic activity was decreased as thyroid activity was suppressed, although the decrease in glycogenesis appeared to be smaller than that observed on induction of the hyperthyroid state. It is likely that increased gluconeogenesis in association with decreased glycogenesis in the liver leads to an enhanced hepatic production of glucose in hyperthyroid rats, whereas inhibition of gluconeogenesis appears to be primarily responsible for diminution of both glucose production and liver glycogen in hypothyroid rats.

Progressive decrease in glucose turnover in methylthiouracil-treated rats and its reversal by additional treatments with thyroxine

Suppression of thyroid activity was not immediate, but only gradual, during treatment of rats with methylthiouracil. The 3-week treatment was required for the thyroid to be fully suppressed, as judged by impaired insulin-secretory response of hypothyroid rats to isoproterenol (Okajima & Ui, 1978). Table 5 shows progressive changes in rate coefficients and turnover rates of [U-^{14}C,2-^{3}H]glucose in rats chronically treated with methylthiouracil. The 1-

week regimen decreased the glucose pool significantly, but was without effect on its turnover. A significant inhibition of glucose turnover developed only after the 2-week treatment; it became more marked as the treatment was prolonged up to 4 weeks. The blood concentration of glucose, however, tended to be restored during this period of treatment, and was completely restored to the euthyroid value after 36 days (Table 6). When thyroxine was injected additionally in methylthiouracil-treated rats for 3 or 6 days, blood-glucose turnover was as rapid as, or more rapid than, in euthyroid rats (Table 6), indicating that methylthiouracil-induced suppression of glucose turnover was actually due to hypothyroidism.

The rats treated with methylthiouracil for 28 days, together with their control euthyroid rats, were injected with cortisol twice. As shown at the bottom of Table 6, cortisol was effective in causing hyperglycaemia, in decreasing the rate coefficient slightly and in increasing the glucose-turnover rate significantly. The expansion of the glucose pool was largely responsible for the increased turnover of glucose. These effects of cortisol were observed similarly in either euthyroid or hypothyroid rats. In other words, hypothyroid-induced suppression of glucose turnover was observed in cortisol-treated rats. In contrast, the suppressive effect of hypothyroidism on the starvation content of liver glycogen was no longer observable after cortisol (63 ± 1.4 and 58 ± 2.1 mg/g in hypo- and eu-thyroid rats respectively). Thus impaired secretion of adrenocortical hormones characteristic of hypothyroidism (Peterson, 1958) might play an important role in lowering the hepatic content of glycogen, but does not appear to be responsible for the decreased turnover of glucose, in hypothyroid rats.

Discussion

Validity of the present procedure as a routine method for estimating blood glucose turnover in rats in the steady state

Rapid labelling of blood glucose with ^{14}C or ^{3}H followed by analysis of subsequent changes in its specific radioactivity provides means for estimating

Table 5. *Progressive changes in glucose-turnover rates in methylthiouracil-treated rats*

Turnover rates and rate coefficients were determined with [U-^{14}C,2-^{3}H]glucose. Blood glucose concentrations were determined as in Table 2. Mean values ± S.E.M. are given, with the numbers of animals in parentheses. Significance of difference from day 0: $*P<0.05$, $**P<0.01$.

Time after start of methylthiouracil regimen (days)	Blood glucose (mg/100ml)	Turnover rate (mg/min per 100g body wt.)		Rate coefficient (ml/min per 100g body wt.)	
		R_{2H}	R_c	k'_{2H}	k'_c
0 (6)	79 ± 2.4	0.84 ± 0.06	0.55 ± 0.04	1.11 ± 0.06	0.72 ± 0.05
7 (3)	67 ± 2.8*	0.78 ± 0.06	0.57 ± 0.01	1.17 ± 0.13	0.84 ± 0.05
13 (3)	73 ± 2.2*	0.66 ± 0.03*	0.41 ± 0.02*	0.90 ± 0.05*	0.58 ± 0.01*
28 (3)	71 ± 4.2	0.54 ± 0.1**	0.33 ± 0.01**	0.73 ± 0.08**	0.45 ± 0.05**

Table 6. *Reversal by thyroxine of methylthiouracil-induced changes in blood glucose turnover*

Turnover rates, rate coefficients and blood glucose concentrations were determined with [U-^{14}C,2-^{3}H]glucose as in Table 5. Key: MTU, treated with methylthiouracil for 36 days; MTU plus thyroxine, treated with methylthiouracil for 36 days, together with thyroxine additionally for the final 6 days; Thyroxine after MTU, treated with methythiouracil for 36 days, then thyroxine for 3 days; None after MTU, treated with methylthiouracil for 36 days, then water for 3 days. In Expt. 2, cortisol was injected as described in the Experimental section into the control ('None') and 'MTU' groups. Mean values ± S.E.M. are given with the numbers of observations in parentheses. Significance of difference from 'None' in each Expt.: $*P<0.05$, $**P<0.01$.

Treatment	Blood glucose (mg/100ml)	Turnover rate (mg/min per 100g body wt.)		Rate coefficient (ml/min per 100g body wt.)	
		R_{2H}	R_c	k'_{2H}	k'_c
(*a*) Expt. 1					
None (8)	84 ± 3.8	0.67 ± 0.03	0.41 ± 0.02	0.80 ± 0.02	0.49 ± 0.02
MTU (4)	85 ± 4.1	0.33 ± 0.02**	0.24 ± 0.01**	0.39 ± 0.03**	0.29 ± 0.03**
MTU plus thyroxine (4)	89 ± 4.4	0.69 ± 0.05	0.45 ± 0.05	0.77 ± 0.05	0.50 ± 0.06
None after MTU (4)	82 ± 3.0	0.44 ± 0.03**	0.30 ± 0.03**	0.54 ± 0.05**	0.36 ± 0.05*
Thyroxine after MTU (4)	80 ± 1.7	0.81 ± 0.05*	0.40 ± 0.04	1.00 ± 0.07*	0.50 ± 0.06
(*b*) Expt. 2 (treated with cortisol)					
None (4)	133 ± 8.1	1.25 ± 0.09	0.76 ± 0.05	0.94 ± 0.07	0.57 ± 0.04
MTU (4)	111 ± 5.8	0.64 ± 0.04**	0.43 ± 0.03**	0.58 ± 0.03**	0.38 ± 0.03**

the blood glucose turnover. Several methods have been proposed for calculation of the turnover rate and related kinetic parameters, though the graphical method appears to afford the most reliable turnover rate, as recommended by Katz *et al.* (1974*a*,*b*). The monoexponential analysis, rather than graphical analysis, was adopted in the present study for the following reasons.

Firstly, blood samplings for obtaining the slope of the semilogarithmic plot of glucose specific radioactivity can be at longer intervals and for a shorter period, and hence the total volume of blood required can be much smaller, than the samplings and sample volumes required for the graphical analysis. Thus metabolic and vascular disturbances that would be otherwise incurred by repeated samplings or catheterization could be minimal in small animals such as rats, making it possible to withdraw blood samples from the tail vein.

Secondly, it would be expected that the glucose space is subject to only a much smaller variation than other kinetic parameters unless vascular effects are introduced. Thus the glucose pool size (the blood glucose concentration multiplied by the glucose pool space), and hence the glucose-turnover rate derived therefrom in the monoexponential analysis, should be proportional, though not equal, to the real value, since both the blood concentration and fractional turnover rate of glucose are accurately measurable.

Thirdly, the values for glucose-turnover rates determined by the monoexponential analysis essentially equalled their values obtained with the aid of the graphical analysis (Table 1), despite their large variations by induction of hyper- and hypo-thyroid states. Thus the simple analytical method used in the present study appears to be useful for practical purposes.

Estimation of glucose recycling and futile cycles by the use of glucose doubly labelled with ^{14}C and ^{3}H

The recycling of glucose had been measured *in vivo* with the use of [2-^{3}H,U-^{14}C]glucose (Katz & Dunn, 1967; Hetenyi & Mak, 1970; Issekutz *et al.*, 1972; Katz *et al.*, 1974*b*; Forichon *et al.*, 1976; Katada & Ui, 1976), [U-^{14}C,5-^{3}H]glucose (Heath *et al.*, 1977*a*,*b*) and [U-^{14}C,6-^{3}H]glucose (Dunn *et al.*, 1967, 1969; Brockman *et al.*, 1975). Differences in the metabolism *in vivo* of the various ^{3}H tracers have been also studied, showing that the rate of ^{3}H loss from the different carbon atoms of blood glucose decreased in the following order: $2 > 5 > 3 = 6$ in mice (Hue & Hers, 1974), $2 = 5 > 3 = 6$ in rabbit (Dunn *et al.*, 1976), $2 > 3$ (Altszuler *et al.*, 1975) and $2 > 6$ (Belo *et al.*, 1976) in dogs. Moreover, Dunn *et al.* (1976) described their unpublished results showing that ^{3}H was lost from the different carbon atoms in the order $2 > 5 > 3 > 6$ in fed or starved rats.

In the present study, [U-^{14}C,6-^{3}H]glucose was used to estimate net hepatic glucose ouptut and the extent of inter-organ carbon recycling (i.e. the Cori cycle and the glucose–alanine cycle). The activity of futile cycles between glucose and glucose 6-phosphate and between fructose 6-phosphate and fructose 1,6-bisphosphate in the liver was determined by estimating the ^{3}H loss from [2-^{3}H]glucose and from [3-^{3}H]-glucose respectively in excess of the loss from [6-^{3}H]-glucose. We used [3-^{3}H]glucose, rather than [5-^{3}H]-glucose, to estimate hepatic futile cycles between fructose 6-phosphate and fructose 1,6-bisphosphate, since the transaldolase reaction also causes ^{3}H loss from [5-^{3}H]glucose (Hue & Hers, 1974). The ^{3}H loss owing to the futile cycles between fructose 6-phosphate and fructose 1,6-bisphosphate depends on the activities of aldolase and triose phosphate isomerase. The rather low activity of this futile cycle observed in the present study might be partly explained in terms of an insufficient activity of triose phosphate isomerase, because the activity of this enzyme in the isolated hepatocyte has been reported (Rognstad *et al.*, 1975) to be much lower than the maximal capacity of the enzyme assayed under optimal conditions in a liver homogenate.

As regards futile cycles between glucose and glucose 6-phosphate, the phosphoglucoisomerase reaction directly involved in detritiation was in operation in the liver at a sufficiently high rate, as evidenced by the much lower $^{3}H/^{14}C$ ratio in glycogen (1.3 as shown in Table 4) than the ratio in blood glucose (5.8) at the time of death of the rats injected with [U-^{14}C,2-^{3}H]glucose. Thus the greater ^{3}H loss from [2-^{3}H]glucose than from [6-^{3}H]glucose appears to reflect the activity of futile cycles between glucose and glucose 6-phosphate, though the possibility for an involvement of the pentose phosphate cycle could not be excluded.

Effect of changing thyroid activity on metabolic rates and futile-cycle activities

Blood glucose turned over at a significantly higher rate in hyperthyroid rats and at a significantly lower rate in hypothyroid rats than in euthyroid rats. The rate coefficient (k') for the turnover also exhibited similar changes on induction of the hyper- or hypo-thyroid states, indicating that these changes in the turnover rate were not solely due to accompanying changes in the blood glucose concentration. Marecek & Feldman (1973) also reported that there was a 2-fold increase in glucose turnover after the development of hyperthyroidism in rabbits.

Hepatic gluconeogenesis appears to be the main source of the glucose that was released in an increased amount in hyperthyroidism. As shown in Table 4, glycogen formation from gluconeogenesis was suppressed in hyperthyroid rats. In addition to the

activation of the gluconeogenic pathway, the diversion of gluconeogenic products from liver glycogen to blood glucose may be favourable for increased glucose liberation, just as has been observed in adrenaline-induced hyperglycaemia (Shikama & Ui, 1975*a*, 1978). Moreover, an increased supply of glycerol, resulting from increased turnover (Tibbling, 1969), may also contribute to glucose production via gluconeogenesis. On the other hand, the inhibition of hepatic gluconeogenesis in hypothyroidism is likely to be responsible for a slower rate of glucose liberation into the circulation as well as for suppressed glycogenesis in the liver. In accord with the results of the present and other (Freedland & Krebs, 1967) studies *in vivo*, the liver isolated from hyperthyroid rats showed higher gluconeogenic activities than the liver from euthyroid rats during their perfusion *in vitro* (Menahan & Wieland, 1969; Singh & Snyder, 1978). Moreover, Sestoft *et al.* (1977) have reported that, during perfusion of rat liver with lactate, pyruvate and glycerol, the rate of glucose release in hyperthyroid rats was twice that in euthyroid rats, which in turn released glucose at twice the rate observed in hypothyroid rats.

The percentage contribution of glucose recycling to the glucose-turnover rate increased on induction of hyperthyroidism. Hepatic gluconeogenesis was supplied with more lactate and alanine that arose from glycolysis in extrahepatic tissues. As a result, a relatively smaller portion of the glucose that was taken up by the tissues seemed to be consumed as respiratory fuels in hyperthyroidism. A similar metabolic effect was produced by the injection of adrenaline or isoproterenol into euthyroid rats (Kusaka & Ui, 1977*b*).

Futile cycles operating between glucose and glucose 6-phosphate in the liver were stimulated and inhibited by inductions of hyper- and hypo-thyroidism respectively. Increases in glucokinase and glucose 6-phosphatase activities observed in hyperthyroidism (Winnick, 1970; Battarbee, 1974) may be partly responsible for the activation of the futile cycle. Heat production owing to ATP dissipation should take place during operation of futile cycles and of the Cori cycle (or glucose–alanine cycle). Thus the metabolic alterations observed in the present study may play some roles in occurrence of hyper- or hypothermia in the animal with abnormal thyroid functions.

The turnover rate of blood glucose per 100g body wt. declined significantly as rats grew, provided that their thyroid functions were normal (Fig. 2). No such correlation of the turnover rate with body weight was observed in hyper- or hypo-thyroid rats. Lack of such correlation might be explained in terms of the narrower body-weight range of rats used for hyper- or hypo-thyroidism than for euthyroidism. As for R_{6H}, however, the body-weight range of rats used for the estimation was essentially the same among the three groups: 120–260g for euthyroid, 110–250g for hyperthyroid and 140–290g for hypothyroid rats. Nevertheless, the correlation coefficient was -0.441 ($P<0.01$), -0.141 ($P>0.05$) and -0.130 ($P>0.05$) for these groups respectively. Conceivably, the body-weight-dependent suppression of the blood glucose turnover could be due to the age-dependent decrease in thyroid activities as observed by Brunelle & Bohuon (1972).

Possible roles of other endocrine functions in metabolic alterations caused by changing thyroid activity

The injection of glucocorticoids into rats or dogs caused marked increases in the turnover rate of glucose (Issekutz & Borkow, 1972; Issekutz & Allen, 1972; Altszuler *et al.*, 1974), whereas adrenalectomy was effective in inhibiting gluconeogenesis from amino acids (Dunn *et al.*, 1969). Although adrenocortical hormones are known to be secreted in smaller amounts in hypothyroid rats than in euthyroid rats (Peterson, 1958), the hypothyroid-induced suppression of glucose turnover appeared not to be due to insufficiency of glucocorticoids, since it was still observed even after treatment with cortisol (Table 6).

As discussed above, metabolic alterations observed in hyperthyroidism appear to resemble those induced by the injection of catecholamines in some respects. Insulin, the secretion of which is affected by thyroid functions (Okajima & Ui, 1978), is known to stimulate the removal of blood glucose from the circulation. The relation of these hormone actions to hyperthyroid-induced changes in the glucose-turnover rate is the subject of the subsequent papers (Okajima & Ui, 1979*a*,*b*).

References

Altszuler, N., Morrison, A., Gottlieb, B., Bjerknes, C., Rathgeb, I. & Steele, R. (1974) *Metab. Clin. Exp.* **23**, 369–374

Altszuler, N., Barkai, A., Bjerknes, C., Gottlieb, B. & Steele, R. (1975) *Am. J. Physiol.* **229**, 1662–1667

Battarbee, H. D. (1974) *Proc. Soc. Exp. Biol. Med.* **147**, 337–343

Belo, P. S., Romsos, D. R. & Leveille, G. A. (1976) *Proc. Soc. Exp. Biol. Med.* **152**, 475–479

Bergmeyer, H.-U. & Bernt, E. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.-U., ed.), pp. 123–130, Academic Press, New York

Brockman, R. P., Bergman, E. N., Pollak, W. L. & Brondum, J. (1975) *Can. J. Physiol. Pharmacol.* **53**, 1186–1189

Brunelle, P. & Bohuon, C. (1972) *Clin. Chim. Acta* **42**, 201–203

Dunn, A., Chenoweth, M. & Schaeffer, L. D. (1967) *Biochemistry* **6**, 6–11

Dunn, A., Chenoweth, M. & Schaeffer, L. D. (1969) *Biochim. Biophys. Acta* **177**, 11–16

Dunn, A., Katz, J., Golden, S. & Chenoweth, M. (1976) *Am. J. Physiol.* **230**, 1159–1162
Forichon, J., Jomain, M. J., Dallevet, G. & Minarire, Y. (1976) *Metab. Clin. Exp.* **25**, 897–902
Freedland, R. A. & Krebs, H. A. (1967) *Biochem. J.* **104**, 45P
Heath, D. F. (1973) *Br. J. Exp. Pathol.* **54**, 359–367
Heath, D. F., Frayn, K. N. & Rose, J. G. (1977*a*) *Biochem. J.* **162**, 643–651
Heath, D. F., Frayn, K. N. & Rose, J. G. (1977*b*) *Biochem. J.* **162**, 653–657
Hetenyi, G., & Mak, D. (1970) *Can. J. Physiol. Pharmacol.* **48**, 732–734
Hetenyi, G. & Norwich, K. H. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1841–1848
Hue, L. & Hers, H.-G. (1974) *Biochem. Biophys. Res. Commun.* **58**, 532–539
Issekutz, B. & Allen, M. (1972) *Metab. Clin. Exp.* **21**, 48–59
Issekutz, B. & Borkow, I. (1972) *Can. J. Physiol. Pharmacol.* **50**, 999–1006
Issekutz, B., Allen, M. & Borkow, I. (1972) *Am. J. Physiol.* **222**, 710–712
Katada, T. & Ui, M. (1976) *Biochim. Biophys. Acta* **421**, 57–69
Katz, J. & Dunn, A. (1967) *Biochemistry* **6**, 1–5
Katz, J., Rostami, H. & Dunn, A. (1974*a*) *Biochem. J.* **142**, 161–170
Katz, J., Dunn, A., Chenoweth, M. & Golden, S. (1974*b*) *Biochem. J.* **142**, 171–183
Kusaka, M. & Ui, M. (1977*a*) *Am. J. Physiol.* **232**, E136–E144
Kusaka, M. & Ui, M. (1977*b*) *Am. J. Physiol.* **232**, E145–E155
Marecek, R. L. & Feldman, J. M. (1973) *Res. Commun. Chem. Pathol. Pharmacol.* **5**, 493–504
Menahan, L. A. & Wieland, O. (1969) *Eur. J. Biochem.* **10**, 188–194
Okajima, F. & Ui, M. (1978) *Am. J. Physiol.* **234**, 196–201
Okajima, F. & Ui, M. (1979*a*) *Biochem. J.* **182**, 577–584
Okajima, F. & Ui, M. (1979*b*) *Biochem. J.* **182**, 585–592
Peterson, R. E. (1958) *J. Clin. Invest.* **37**, 736–743
Porterfield, S. P., Whittle, E. & Hendrich, C. E. (1975) *Proc. Soc. Exp. Biol. Med.* **149**, 748–753
Rognstad, R., Wals, P. & Katz, J. (1975) *J. Biol. Chem.* **250**, 8642–8646
Sestoft, L., Bartels, P. D., Fleron, P., Folke, M., Gammeltoft, S. & Kristensen, L. (1977) *Biochim. Biophys. Acta* **499**, 119–130
Shikama, H. & Ui, M. (1975*a*) *Am. J. Physiol.* **229**, 955–961
Shikama, H. & Ui, M. (1975*b*) *Am. J. Physiol.* **229**, 962–966
Shikama, H. & Ui, M. (1978) *Am. J. Physiol.* **235**, E354–E360
Singh, S. P. & Snyder, A. K. (1978) *Endocrinology* **102**, 182–187
Snedecor, G. W. & Cochran, W. G. (1967) *Statistical Methods*, Iowa State University Press, Ames
Takahashi, T. & Suzuki, M. (1971) *FEBS Lett.* **12**, 221–224
Tibbling, G. (1969) *Clin. Chim. Acta* **24**, 121–130
Winnick, S. (1970) *Endocrinology* **87**, 124–128
Yajima, M. & Ui, M. (1974) *Am. J. Physiol.* **227**, 1–7