The distribution and metabolism of glycerol in the rabbit

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1. The effective volume of distribution of labelled glycerol was studied in conscious young adult rabbits provided with in-dwelling cannulae in the femoral blood vessels. This could be estimated after sampling arterial blood throughout an intravenous infusion of [2-³H]glycerol. The volume was calculated by using an algebraic method of graphical area analysis over 100 min of equilibration, and is symbolized $^{100}V_e$ or $^{100}V_e\%$. It occupied $34.1 \pm 2.2\%$ (mean $\pm$ S.E.M.; $n = 13$) of the body weight. The pool of endogenous glycerol occupying this space is distinguished in the present paper by calling it the transit pool, symbolized $^{100}M_e$. 2. The median time of transit of glycerol through this pool was approx. 6 min in these conscious rabbits with normal (<0.2 mM) blood glycerol concentrations. 3. The metabolism of glycerol was also studied in rabbits while anaesthetized with urethane or while conscious. On average, half of the change in glycerol concentration that occurred on overnight starvation could be attributed to a decrease in clearance, whereas half was due to an increase in lipolysis. 4. The correlation between the reciprocal of glycerol concentration and clearance showed that in these animals about a quarter of the variation in concentration was due to an association with clearance. The remainder of the variation was attributed to variations in the rate of glycerol formation (lipolysis). 5. The regression of glycerol turnover rate on concentration implied that turnover was positive at zero glycerol concentration. This confirms previous findings from studies on other species. The explanation offered for this phenomenon is that the well-known physiological changes induced by feeding (decreased lipolysis, increased splanchnic blood flow) may independently decrease the glycerol concentration by both decreasing its release into the blood and simultaneously increasing its clearance.

Glycerol is a compound of low molecular weight and high water solubility that is usually assumed to be able to permeate the tissues of the body with ease. Arising from this preconception, it is natural to suppose that any change in the rate of glycerol release or uptake should be buffered by a large, diffusely distributed pool, resulting in relatively slow changes in concentration.

Experience in many laboratories has not, however, supported such a prediction; changes in the lipolytic rate result in changes in both glycerol and plasma non-esterified fatty acid concentration that are almost equally abrupt (e.g. Carlson & Oro, 1963; Bergman, 1968). One of the purposes of the experiments described in the present paper was to establish why the concentration of glycerol is so labile, and in what space the glycerol pool appears to be distributed when considered in relation to a sample of the pool taken in blood.

There have been three studies published that give

estimates of the distribution volume of glycerol in the body (Holst, 1944; Larsen, 1963; Shafir & Gorin, 1963). In each of these, massive loads of glycerol were administered, disrupting normal gradients of glycerol concentration in the tissues, and saturating the metabolic pathways for the disposal of glycerol.

Despite its low molecular weight (92.1), the three hydroxy groups on the glycerol molecule attract molecules of water and effectively increase its molecular size, which prevents it from diffusing quickly across cell membranes by simple diffusion. In many species (e.g. rat, mouse, man, rabbit and guinea pig), facilitated-diffusion mechanisms are known to exist in the membranes of erythrocytes, but in others (e.g. pig, cat, dog, sheep and ox) no such mechanism is found (Jacobs, 1950). Nonetheless, massive glycerol concentration gradients will allow glycerol to enter most cells even in these species if sufficient time is allowed for the equilibration to take place, and in Larsen's experiments

(Larsen, 1963) the volume of distribution of glycerol in the cat averaged 60% of the body weight, 1–2 h after administering the load. In the experiments reported in the present paper in which the volume of distribution was estimated, the disruption of normal glycerol concentration gradients was avoided. The experiments were conducted in conscious rabbits with cannulated blood vessels that were given a continuous trace infusion of labelled glycerol by the intravenous route. The volume of distribution was estimated by following the rising curve of specific activity throughout the infusion, and, using a fitted curve, calculating the mass of labelled glycerol sequestered in the body when equilibration of the arterial labelled glycerol concentration was essentially (99%) complete.

The second principal objective of the experiments reported here was to establish whether or not variations in clearance seriously affect the reliability of glycerol concentration as an index of lipolytic rate *in vivo*.

Regressions of glycerol turnover rate on glycerol concentration have been published by several authors (Bergman, 1968; Winkler *et al.*, 1969; Bortz *et al.*, 1972). The conclusion reached in each of these studies was that there is a direct relationship between glycerol turnover rate and glycerol concentration, within the physiological range of the latter (up to 0.5 mM), and that glycerol concentration is a good index of lipolytic rate. However, in all these studies, the regression line intercepted the axis of turnover rate at a positive value, significantly differing from zero. Also, in each of these studies material was distinctly heterogeneous, giving weight to results from animals in which the lipolytic rate was unusually high.

Description of the clearance of glycerol has been omitted by previous investigators. Winkler *et al.* (1969) make the valid point that calculation of the related function, fractional turnover rate, is of somewhat limited value. This measure of the rate of uptake of glycerol depends on an unreliable estimate of pool size (all previous authors studying glycerol in this way have assumed a pool occupying 65% of the body weight). Also, and more fundamentally, fractional turnover rate declines as the absolute size of the animals studied increases. This is because the spaces in which metabolites are distributed are related directly to the body weight (in animals of similar body composition), whereas turnover rates are related to the overall metabolic rate, which only increases with body weight to the power 0.75 (Schmidt-Neilson, 1970). Estimates of clearance do not suffer from these disadvantages, however, and it is sensible to scale it in terms of (body weight)^{0.75}.

It will be shown in the present experiments that variation in clearance should not be underestimated as a potential source of variation in glycerol

concentration, particularly if the latter is low. Description of clearance values may also be found to be conceptually useful.

Experimental

Animals

All experiments were performed on fed or 24-h-starved New Zealand White rabbits of either sex, ranging in weight from 1.64 to 4.94 kg.

Anaesthesia

In the 13 experiments conducted on conscious animals, cannulae were implanted under halothane anaesthesia. Topical application of lignocaine hydrochloride (2% solution) to the wound during and at half-hourly intervals after the brief period of surgery further ensured analgesia, decreasing the animal's interest in, and interference with, the wound site.

A second series of 22 experiments was conducted under urethane anaesthesia. Urethane (carbamic acid ethyl ester; Hopkins and Williams) was given by intravenous injection as a 20% solution in saline at a dose of approx. 1 g/kg body wt.

Surgery

In all the experiments on conscious animals, polythene tubing (Portex Plastics PP 30) was tied into the femoral vein, and a nylon intravenous cannula (Portex Plastics, Pink Luer, 3FG 200/300/030) was tied into the femoral artery of the same leg. A loose cannula, for the instillation of local anaesthetic, was tied into the wound during closure.

A similar cannulation technique was used in the series of experiments under urethane anaesthesia, although in five of these experiments, vessels in the neck were cannulated.

Solutions administered

Tritiated glycerol solutions for infusion were prepared by dissolving approx. 0.4 mCi of [2-³H]glycerol (The Radiochemical Centre, Amersham, Bucks., U.K.) in 20 ml of sterile saline. The exact radioactive content of the solutions was determined in the scintillation counter.

Bulk glycerol infusions were prepared in saline, with glycerol (Analar grade; Hopkins and Williams) dissolved at a concentration of 150 mM. The rate of infusion varied between 13.4 μ mol/min and 60.3 μ mol/min; this represented a range of 0.5–7.8 times the endogenous glycerol turnover rate, which was subsequently determined.

Late in this series of experiments, three experiments were conducted in which equilibration was assisted by giving a priming dose of glycerol before infusion.

Sample processing

Small arterial blood samples of either 0.1 or 0.2 ml

(approximately) were added to pre-weighed stoppered tubes containing either 0.5 or 1 ml of ice-cold 0.085 M-zinc sulphate solution. The exact sample size was determined by reweighing the tubes, after which the addition of 0.5 or 1 ml of 0.083 M-Ba(OH)₂ solution completed the deproteinization procedure (Somogyi, 1945). The larger sample size was needed in experiments on conscious animals.

The supernatant was separated from the precipitate by centrifugation, and transferred to a clean tube. Glycerol assay and the extraction of tritiated glycerol as crystalline glycerol tribenzoate (conscious-animal experiments only) was either begun immediately, or after storing the supernatant at 4°C overnight or (occasionally) at -16°C for longer periods. Glycerol concentration in the supernatant was measured by the method of Laurell & Tibbling (1966).

Glycerol tribenzoate was prepared by the method of Winkler *et al.* (1967). As this procedure was originally applied to the extraction of ¹⁴C-labelled glycerol, its suitability for use with tritiated glycerol was thoroughly tested. ³H associated with glycerol was recovered almost completely ($97.3 \pm 0.5\%$, mean $\pm$ S.E.M.) and was not contaminated by ³H present in water, or in glucose, in the original mock supernatant (Wade, 1976). Radioactivity was measured in a model 3315 Packard Liquid Scintillation Spectrometer, using solvent NE250 (Nuclear Enterprises). Efficiency of counting was determined by either the channels ratio method, or by use of the automatic external standard.

Experimental protocol

(a) *Experiments on conscious animals.* Surgery (for the implantation of cannulae into blood vessels) was performed early on the day of the experiment, the whole period of anaesthesia (including induction) lasting approx. 40 min. The animals were allowed 3–4 h in which to recover from the effect of the anaesthetic. During this period, and throughout the experiment, they sat unfettered but under constant supervision in a small stall. Rectal temperature was monitored continuously to ensure that experiments were not conducted during pyrexial reactions. Control experiments had been performed to show that glycerol concentration was almost constant within 3–4 h of recovery from anaesthesia. Administration of the infusion, and the taking of samples, did not disturb the animals. At the end of the experiment, the animal was killed with an overdose of pentobarbitone sodium. Particular care was taken to ensure that these experiments were conducted in a quiet atmosphere, with minimal stress to the animal.

(b) *Experiments conducted under urethane anaesthesia.* Surgery was usually completed within 20–30 min of the induction of anaesthesia. After a

settling period of 15 min in lateral recumbency, four control samples of arterial blood (0.1 ml each) were collected at 5-min intervals. An intravenous infusion was begun, and a period of 15–30 min was allowed before collecting more samples; again a group of four samples was collected at 5-min intervals. In experiments with a single rate of infusion, the infusion was then switched off, and a period allowed (15–20 min) for glycerol concentration to decline; in experiments with two rates of infusion, the rate was increased (four experiments) or decreased (one experiment), and after a further equilibration period, four more samples were collected. The infusion was then switched off, and after allowing time for the glycerol concentration to decline, four more control samples were collected. At the end of the experiment, the animal was killed with an overdose of pentobarbitone sodium, given intravenously.

Analytical methods

(a) *Experiments on conscious animals.* When followed from the onset of continuous intravenous infusion, it was found that the arterial content of tritiated glycerol, expressed as nCi/g of blood, followed a reproducible pattern.

Inspection of the data before curve-fitting suggested the possibility that, empirical though such a fit might be, the data might be described by the equation of a rectangular hyperbola. The equation proposed to describe the data is given below as eqn. (1):

$$d = \frac{D_{\max} \cdot t}{K_t + t} \quad (1)$$

where d = radioactive glycerol concentration in blood (nCi/g), t = time (min), d tends to $D_{\max}$ as t tends to infinity, and $K_t = t$ when $d = D_{\max}/2$.

It is unwise to fit this curve by a simple least-squares regression using the reciprocals of d and t in a Lineweaver–Burk transformation, since the errors in d at low values of t become disproportionately great, and furthermore values in this region carry great weight in the fitting process. Accordingly, the computer-assisted method of curve fitting published by Cleland (1967) was used. This program also provided a statistical analysis of the fit obtained, giving standard errors for K_t and $D_{\max}$.

A practical advantage of this mathematical technique over the use of a multiple exponential analysis is that this technique is very robust, and will deal with any data, even though some points may deviate from the fitted curve. When using a multiple exponential analysis, on the other hand, it may be difficult or impossible to obtain a fit, due to the presence of singular regions (a consequence of the presence of transcendental functions in the equation), if the data do not conform quite closely to the ideal curve described by the equation.

In equilibrium conditions, the clearance of glycerol from the blood, C , in these experiments is given by:

$$C = \frac{IR}{D_{\max.}} \quad (2)$$

where IR is the rate of infusion of radioactive glycerol (nCi/min) and thus units of C are g of blood/min.

The calculation of the effective volume of distribution of glycerol, V_e , from the analysis of a curve of specific activity rising to a plateau during an infinite period of infusion has inescapable difficulties. In theory, unless the curve obeys certain mathematical conditions, the area below the curve (A') and the area above the curve, bounded by the plateau ordinate, (A''), will both become infinite at infinite time. To avoid the ridiculous conclusion that the space available to glycerol, and its minimal mass in the body, are both infinite, it is necessary to use a mathematical device that obtunds the area A'' . The most common solution to this problem is to fit the following multi-exponential expression to the data:

$$y = \sum B_i - B_1 e^{-\alpha_1 t} - B_2 e^{-\alpha_2 t} - B_3 e^{-\alpha_3 t} \dots B_i e^{-\alpha_i t} \quad (3)$$

Integration of this equation with respect to time for i terms leads to the expression:

$$A'' = \sum (B_i/\alpha_i) \quad (4)$$

It is found that with a finite infusion time and a limited number of data points, only two, three or, at the most, four terms can be fitted with a decrease in the variance as each final term is added. The experimenter therefore empirically obtunds the equation, at a level which is limited by the quality of his data, and obtains a single answer in his calculation.

The practical difficulties with the multiple exponential technique would be minimized if a single exponential term could be fitted. However, the initial rise in specific activity observed in the present experiments was so fast, and the final approach to the plateau so slow, that a single value of α is quite incapable of providing a good fit. It is obviously important, when subsequently making an analysis of an area bounded by the curve, that the data should be fitted well throughout the period of observations by the curve employed, and should not have a misfit forced on them.

In the analysis used in the present paper, an algebraic version of direct graphical analysis was employed, principally for practical reasons. The use of the equation of a rectangular hyperbola had two advantages: it provided a remarkably good fit, and it could always be fitted easily to the data. It also has a

disadvantage; again it is necessary to obtund the curve, but here there is no self-limiting process in the analysis to provide a single answer. The integral of the equation of a rectangular hyperbola is not finite, the area A'' being given by:

$$A'' = D_{\max.} K_t \ln \left(\frac{K_t + t}{K_t} \right) \quad (5)$$

It will be shown (see the Results section) that by 100 min, d_t approximates to $D_{\max.}$. The apparent volume of distribution by 100 min, symbolized $^{100}V_e$, was therefore investigated. The suffix e is included to denote the fact that this is an effective, not a real, volume of distribution.

To find $^{100}V_e$, the pool of tritiated glycerol that has not been consumed by metabolism by 100 min must be calculated. It is assumed that (a) during the observations, clearance is essentially constant and (b) the concentration of radioactive glycerol in blood is always representative of the concentration at sites of glycerol metabolism.

Then the residual pool, R_t , is given by:

$$R_t = A'' C \quad (6)$$

and

$$^{100}V_e = \frac{0.83 R_t}{d_t} \quad (7)$$

where 0.83 is a factor allowing for the water content of blood (by weight) and d_t is the final specific activity. (It is within experimental error to substitute $D_{\max.}$ for d_t at this point.)

$^{100}V_e$ may be conveniently expressed as a percentage of body weight, W :

$$\frac{^{100}V_e}{W} \times 100 = ^{100}V_e \% \quad (8)$$

In principle, the method of analysis applied here should lead on to an estimate of the minimal mass of glycerol, as calculated by the method of direct graphical analysis described by Katz *et al.* (1974). In their theoretical analysis of the interrelationships of different procedures for analysing curves of specific activity against time, Katz *et al.* (1974) reached the expression for minimal mass, $M_{\min.}$:

$$M_{\min.} = \frac{\text{Area} \times IR}{Sa_T^2} \quad (9)$$

of which the direct equivalent in the present terminology would be:

$$M_{\min.} = \frac{A'' \times IR}{D_{\max.}^2} \times G \quad (10)$$

where G is the glycerol concentration ($\mu\text{mol/g}$ of blood). (This can readily be shown by combining eqns. 2, 6 and 7.) However, Katz *et al.* (1974), who

equate this expression with the comparable analysis by multiexponential methods:

$$M_{\min.} = \frac{\sum (B_i/\alpha_i) \times IR}{(\sum B_i)^2} \quad (11)$$

did not make it clear that with the direct graphical analysis, obtaining the curve must either be due to a completely arbitrary decision or to a somewhat arbitrary decision based on judgement, whereas with the multiple exponential, obtaining the equation itself will be the empirical determining factor. Consequently, the numerical values of $M_{\min.}$ will only be identical if, fortuitously, the two blunting processes result in identical values of A'' . This is, of course, unlikely. It is therefore preferable not to use the term $M_{\min.}$ with data handled by these different techniques and the pool studied here will be called the transit pool, symbolized as $^{100}M_e$. This pool will have a turnover time or mean time of transit, analogous and similar to, but again probably not numerically identical with, the transit time, which can be determined for the minimal mass, $M_{\min.}$. This is symbolized by Katz *et al.* (1974) as $\bar{t}$. The equivalent transit time for $^{100}M_e$, symbolized as $^{100}\bar{t}_e$, is given by:

$$^{100}\bar{t}_e = \frac{A''}{d_t} \quad (12)$$

If d_t approximates $D_{\max.}$, as it does in the analysis used here by $t = 100$ min, this may be simplified still further:

$$^{100}\bar{t}_e = K_t \ln \left(\frac{K_t + t}{K_t} \right) \text{ min} \quad (13)$$

Thus, $^{100}\bar{t}_e$ depends solely on the value of K_t .

(b) *Experiments conducted under urethane anaesthesia.* The estimation of clearance depended on the estimation of the difference between the observed glycerol concentration during a glycerol infusion, and the concentration that would have been observed if the infusion had not been given. This I have called the 'endogenous glycerol concentration'. This was calculated either as the mean of pre- and post-infusion values, or (if post-infusion values were greater than pre-infusion values), only the pre-infusion values were used, on the assumption that in these cases, insufficient time had elapsed since discontinuing the infusion to allow the concentration to fall to its pre-infusion value. Clearance in these experiments was then calculated from the expression:

$$C = \frac{IR}{(G)} \text{ ml/kg}^{0.75} \text{ per min (whole blood)}$$

where C = clearance, IR = infusion rate ($\mu\text{mol/kg}^{0.75}$ per min), (G) = change in glycerol concentration due to the infusion (mm).

Results

Establishing the shape of the curve of radioactive glycerol concentration in blood against time

A series of seven experiments was performed in which the sampling times were standardized as far as possible. A maximum of 20 samples, each of 0.2 ml of blood, was taken from any one rabbit. The seven experiments were analysed collectively. Each measurement of the radioactive glycerol content of blood was adjusted to be that for a model rabbit weighing $1\text{ kg}^{0.75}$ and receiving an infusion of 270.3 nCi of $[2\text{-}^3\text{H}]\text{glycerol/min}$. The sample times used in further analysis were those for which four or more estimates of the radioactive glycerol content were available. Fig. 1 shows the mean and standard deviation of observations at each sampling time during infusion for 80 min, with the curve of a rectangular hyperbola fitted. The value of K_t was approx. 1, which implies that by 100 min, d_t would reach $0.99 D_{\max.}$.

Fig. 1. The curve of tritiated glycerol concentration in water of blood during the continuous infusion of $[2\text{-}^3\text{H}]\text{glycerol}$ from time zero to 80 min

The mean and standard deviation of observations at each sampling time are given, with the curve of a rectangular hyperbola fitted. The plateau value, $D_{\max.}$, was 2.606 ± 0.019 nCi/g of blood water (mean $\pm$ S.E.M.), and the time constant, K_t , was 0.983 ± 0.045 min (mean $\pm$ S.E.M.).

Estimates of $^{100}V_e$, $^{100}M_e$ and $^{100}t_e$

In 13 experiments, the volume of distribution of glycerol was calculated using the eqns. (5)–(8) (see under 'Analytical methods') after obtunding the area A'' between the curve of specific activity and the plateau ordinate.

The rate of change of A'' with time, if t is very much greater than K_t , will be approximately the rate of change of $\ln t$. If K_t is taken as 1, by $t = 1000$ min, $d_t = 0.999 D_{\max.}$, and $^{100}A'' = 1.5 ^{100}A''$. For practical purposes, such slow changes are likely to be of little interest; they would also depend on extrapolation far beyond the observations, and assume an unattainably perfect steady state.

Similar increases in A'' will occur with the multi-exponential method if many terms are fitted; thus, $M_{\min.}$ grows discontinuously if a two-term equation is substituted by a three-term equation, i.e. the equation for calculating A'' suddenly includes a much larger area above the 'tail'. It does not simply improve the fit during the period of observations.

The mean coefficient of variation of individual estimates of K_t was 15.3%, whereas that of individual estimates of $D_{\max.}$ was 2.1%. Values of $^{100}V_e\%$, $^{100}M_e$ and $^{100}t_e$ are given in Table 1. The mean value for $^{100}V_e\%$ was 34.1 ± 2.2 (mean $\pm$ S.E.M.). The median value for $^{100}t_e$ was 5.6 min. Median values of $^{100}M_e$ in fed and starved rabbits were 26 and $48 \mu\text{mol/kg body wt.}$ respectively.

Metabolism of glycerol

Values for the arterial concentration of glycerol, the clearance of glycerol from the blood, and the turnover rate of glycerol, on both conscious and anaesthetized animals, are given in Tables 2 and 3. It will be seen, from the results in Table 2, that there

Table 1. Individual results calculated for the effective volume of distribution ($^{100}V_e\%$), the mean time of transit through the transit pool ($^{100}t_e$) and the size of the transit pool ($^{100}M_e$), in 13 conscious rabbits

	Serial no.	$^{100}V_e$ (%)	$^{100}t_e$ (min)	$^{100}M_e$ ($\mu\text{mol/kg body wt.}$)
Fed	84	27.2	4.6	23.9
	86	33.7	5.2	26.0
	87	21.2	3.3	17.9
	89	44.9	5.6	32.5
	90	31.4	4.4	24.5
	91	30.9	7.6	—
	92	34.1	5.6	34.9
Starved	93	44.4	7.2	26.2
	95	42.4	10.4	102.7
	96	39.4	6.6	49.8
	97	31.3	4.6	38.5
	98	26.2	6.0	24.7
	100	35.7	5.1	44.3

Table 2. Analysis of data between groups of animals

In experiments using bulk glycerol infusion in anaesthetized animals, five experiments included two periods of infusion at different rates. These experiments are each represented by a single value, the mean of the two values, in group analysis. Abbreviations used: NS, not significant.

Experimental group	Number of animals	Glycerol concn.							Clearance of glycerol							Turnover rate						
		(μM)			Student's <i>t</i> test				[ml/(kg body wt.) ^{0.75} per min]			Student's <i>t</i> test				[$\mu\text{mol}/(\text{kg body wt.})^{0.75}$ per min]			Student's <i>t</i> test			
		$\bar{x}$	S.D.	S.E.M.	<i>t</i>	<i>f</i>	<i>P</i>	$\bar{x}$	S.D.	S.E.M.	<i>t</i>	<i>f</i>	<i>P</i>	$\bar{x}$	S.D.	S.E.M.	<i>t</i>	<i>f</i>	<i>P</i>			
Fed anaesthetized	6	61	24	10	0.593	12	NS	92.4	21.0	8.6	0.277	13	NS	5.5	2.0	0.8	1.197	12	NS			
Fed conscious*	7	66	11	4				94.5	13.0	4.6				6.5	1.0	0.4						
Starved anaesthetized	16	101	37	9	0.848	20	NS	76.8	14.8	3.7	0.967	21	NS	7.6	2.9	0.7	1.375	20	NS			
Starved conscious	5	118	48	21				84.3	16.4	7.3				9.6	2.8	1.3						
Fed (all results combined)†	13	64	17	5	3.572	33	0.005	93.6	16.2	4.3	2.810	34	0.01	6.0	1.6	0.4	2.278	33	0.05			
Starved (all results combined)	21	105	39	9				78.5	15.1	3.3				8.1	3.0	0.6						

* $n = 8$ for clearance data only.

† $n = 14$ for clearance data only.

was no significant difference between results obtained in anaesthetized- or conscious-animal experiments, but that glycerol concentration and turnover rate were both significantly higher in starved rabbits, whereas glycerol clearance in this subgroup was significantly lower. Table 3 gives individual results of glycerol clearance estimates made in urethane-anaesthetized rabbits studied with two rates of bulk glycerol infusion; these individual data are of value in establishing that this method did not impair the clearance of glycerol. A similar point was made by seeking a correlation between the infusion rate employed and the clearance estimates obtained in this series of experiments. The correlation coefficient was -0.174 (20 degrees of freedom), which did not differ from zero with any significant probability.

Regression of glycerol concentration on clearance of glycerol

The correlation coefficient of glycerol concentration on glycerol clearance, using both anaesthetized- and conscious-animal data, was -0.455 (significantly different from zero, degrees of freedom 32, $P < 0.01$). The data are plotted in this relationship in Fig. 2(a).

The relationship between clearance (expressed in relation to body size) and concentration is of the form:

$$xy = Z$$

where x is the clearance, y is the glycerol concentration and Z is the turnover rate. If turnover rate is variable, the data should lie on a family of curves,

Table 3. Individual results in five anaesthetized rabbits given bulk glycerol infusions at two rates
Starved rabbits had been deprived of food since the day preceding the experiment.

Experimental group	Serial no.	Infusion rate [$\mu\text{mol}/(\text{kg body wt.})^{0.75}$ per min]		Endogenous glycerol concn. (μM)	Clearance [$\text{ml}/(\text{kg body wt.})^{0.75}$ per min]	
		1	2		1	2
Fed	34	15.9	7.5	39	103.4	100.0
	35	8.92	18.3	43	92.0	100.2
	36	15.6	31.4	50	82.7	82.0
Starved	9	8.54	17.1	193	83.6	87.5
	11	8.99	18.0	123	77.5	79.9

Fig. 2. (a) The relationship between glycerol concentration (μM in whole blood) and the clearance of glycerol from whole blood, scaled for body size [$\text{ml}/(\text{kg body wt.})^{0.75}$ per min] and (b) the linearized relationship, reciprocal of glycerol concentration against clearance of glycerol, fitted by the line $y = a + bx$ where $a = 15.069$ and $b = 1.348$

For convenience, the reciprocal value was multiplied by 10^4 before the regression line was fitted (by the method of least squares). $\blacktriangle$ and $\triangle$, Rabbits anaesthetized with urethane and studied by bulk glycerol infusion; $\blacksquare$ and $\square$, conscious rabbits infused with tritiated glycerol in trace amounts. Results for fed animals are represented by filled symbols, and those for starved animals by open symbols.

Fig. 3. (a) Regression of glycerol turnover rate [$\mu\text{mol}/(\text{kg body wt.})^{0.75}$ per min] on glycerol concentration in whole blood (μM) in the rabbit and (b) the same regression (labelled Wade, present work) compared with similar regressions calculated from data published by other authors

In every case, the intercept ($x = 0$) value of y differs from zero. Symbols in (a) are explained in the legend to Fig. 2.

each of which has the form of a rectangular hyperbola.

If this equation is expressed as $1/Z \cdot x = 1/y$, the graphical form of x against $1/y$ becomes linear, with slope $1/Z$. The regression of the reciprocal of glycerol concentration on the clearance was calculated for the 34 points. The data are plotted in this form in Fig. 2(b). The correlation coefficient was 0.482. As these transformed data conform to a theoretically linear relationship, this estimate of the correlation coefficient is preferred to that obtained in direct regression analysis for estimating the proportion of variation in glycerol concentration that may be attributed to variation in clearance. This is given by the square of the estimated correlation coefficient (Snedecor & Cochran, 1967). The value of r^2 here is 0.232. The regression line between the reciprocal of concentration and clearance did not differ significantly from a line forced through both the origin and the mean values of the reciprocal glycerol concentration and glycerol clearance.

Regression of glycerol turnover rate on glycerol concentration

To allow direct comparison of the results in the present paper with those published by other authors, the regression of glycerol turnover rate on concentration is given in Fig. 3. Fig. 3(a) shows the

individual data points obtained in rabbits, with a line fitted by linear regression. The equation of the line drawn, $y = a + bx$, gave the constants $a = 1.886 \mu\text{mol}/\text{kg}^{0.75}$ per min, $b = 0.0605$ when the concentration, x , was expressed as μM . The intercept value was significantly different from zero ($P < 0.005$). The estimated correlation coefficient, r , was 0.855, so that $r^2 = 0.73$. This implies that 27% of the variation in concentration was not accounted for by variation in turnover rate. In Fig. 3(b), regression lines from three other studies are drawn, after rescaling data when necessary.

Discussion

The effective space occupied by the glycerol pool (in which the glycerol is assumed to be distributed at the same concentration, and at the same specific activity as that in the water of blood) is treated here as a single space; it is further assumed that sites of glycerol catabolism take up glycerol with a specific activity identical with that in blood. These very simple and crude modelling assumptions are those that lead to minimal estimates of glycerol mass in the body (Katz *et al.*, 1974). Given the assumptions employed, the mean value of the effective volume of distribution $^{100}V_c\%$ was 34.1 ± 2.2 (mean $\pm$ s.e.m.; $n = 13$) of the body weight. This value strongly

supports the view that in normal conditions the great majority of glycerol released into the venous blood is rapidly metabolized. It is easy to show that labelled glycerol does not distribute quickly and evenly into the total body water. If it did, $V_e\%$ would reach 70 only if the time of equilibration, t , was set at approx. 3 days, if the observed values of K_t and $D_{\max}$ are used in calculating A'' . Thus the conclusion must be that the space freely available to glycerol when normal concentration gradients are not disturbed is considerably less than the volume of the total body water. This is in marked contrast with the result which is obtained when a massive load of glycerol is injected, as was found in experiments published previously and cited in the Introduction.

Glycerol, produced and metabolized (at differing rates) widely throughout the body, undoubtedly normally exists in a three-dimensional matrix of concentration gradients, amongst which the concentration in blood must be relatively high. The specific radioactivity within the transit pool must also be at the highest value, as the label is added directly to the blood. The value in the cells of various tissues is unknown, but must depend on the balance between the rates of glycerol formation and metabolism within them, and the freedom of exchange with the fully labelled transit pool. It is a recognized limitation of a model with the assumptions made here (minimal mass assumptions) that this may result in underestimation of the total body glycerol turnover rate.

The second objective of the experiments reported here was to establish whether or not variations in clearance seriously affect the reliability of glycerol concentration as an index of lipolytic activity.

Before looking at effects of clearance on concentration, it is, of course, important to establish whether or not glycerol concentration directly affects clearance, through the saturation or partial saturation of glycerol uptake mechanisms.

There is good evidence that, provided the change in concentration is not extreme, glycerol clearance is not decreased by rising glycerol concentration. This comes both from the present series of results, and from previous publications. The results presented here included the following points. 1. Glycerol clearance estimates in experiments with bulk infusion of glycerol (at moderate rates) were not correlated with the rate of infusion employed during their determination. 2. When glycerol infusion rate was doubled or halved, it had no effect on the clearance estimate obtained.

Other points in support of this view are to be found in the literature. Tibbling (1969) has also shown (in man) that stepwise changes in the rate of bulk glycerol infusion did not alter the estimate of fractional turnover rate which he obtained, and Bergman (1968) provided data that may be recal-

culated to show that lipolysis is not decreased, and clearance is not decreased by giving bulk glycerol infusion at moderate rates in sheep. Furthermore, Winkler *et al.* (1969) deliberately set out to saturate uptake mechanisms in conscious dogs by using progressively higher rates of bulk glycerol infusion, and showed that the relationship between concentration and uptake was linear at least up to a concentration of $500\mu\text{M}$. Finally, in one important bed for glycerol uptake (the splanchnic, including the liver), Basso & Havel (1970) have given values for extraction efficiency which were unaffected by quite severe changes in endogenous glycerol metabolism in dogs, induced by the infusion of noradrenaline, or by diabetic acidosis.

Quantitative evidence that variation in clearance does significantly affect glycerol concentration, both in apparently homogeneous material, and in material with a distinct experimentally induced difference (starvation) was obtained in these experiments.

The correlations of the reciprocal of glycerol concentration on clearance, and of turnover rate on concentration, both suggest that about one-quarter of the variation in glycerol concentration was linked to variation in clearance under the experimental conditions in all the rabbits studied. This is an average value, and the wide range of clearance (from about 40 to $120\text{ml/kg}^{0.75}$ per min) represents a variation of $\pm 50\%$ of the mean. If it is assumed that turnover rate is linearly related to glycerol concentration, some serious errors of interpretation might arise.

In Fig. 3, the regression lines of glycerol turnover rate on concentration are plotted based on data derived from several previous papers and the data in the present paper. The slopes of these regressions vary, and so do the intercepts with the y axis. These variations reflect the extent to which high clearance values were associated with low concentrations, and vice versa, in the material studied. Such simultaneous changes in clearance and glycerol concentration result in the rotation of the slope of the regression of turnover rate on concentration.

The data on rabbits had the lowest slope, and so were the most affected by the conjugation of simultaneous clearance and turnover rate changes, probably because this series included fed and mildly starved animals in roughly equal proportions (21 starved, 13 fed), and the glycerol concentration was, at its highest, only $203\mu\text{M}$. Bergman (1968) used only five fed animals (among 40 experiments) and this and the other two series used relatively severe stimuli to lipolysis, weighting the regressions in favour of points at higher turnover rates. In these series, therefore, less of the variance in glycerol concentration would have been provided by variation in glycerol clearance, and more by the turnover rate of glycerol in the sampled pool.

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