

# DETERMINATION OF THE HEPATIC ELIMINATION CAPACITY ( $L_m$ ) OF GALACTOSE BY SINGLE INJECTION

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## Determination of hepatic elimination capacity ( $L_m$ )

Most physiological functions normally proceed with submaximal intensity, having a reserve which can be activated during extraordinary situations. An injured organ often appears to function normally because the reserve is more or less constantly in action, and the injury is only detected if the total functional capacity is tested. This principle has been successfully applied in tests of, e.g. renal, pulmonary, cardiac, muscular, and mental functions, but only sporadically in liver function tests. This is probably explained by the complicated metabolic and vascular regulations of most known liver-functions.

Attempts to measure the functional capacity of the liver have mostly been based on modifications of the technique used for determination of the tubular excretion or reabsorption capacity, and, on the analogy of the term  $T_m$ , for tubular mass,  $L_m$ , or liver mass, has been used (Mason, 1948). Mason's original method, based on the elimination of bromsulphalein, and the modifications suggested by Lewis (1950) have proved physiologically untenable, as only the capacity to excrete bromsulphalein into the bile, and not its elimination from the blood, can be measured by a rather complicated procedure (Wheeler, Meltzer & Bradley, 1960).

The functional capacity of an elimination process can be assumed to be saturated when

further increase in the concentration of the substance to be eliminated does not accelerate the rate of elimination, i.e. when the elimination rate is independent of the concentration. The maximal elimination rate may be taken as an expression of the liver mass.

## The hepatic galactose elimination

Several observations indicate that the hepatic elimination rate of galactose is independent of the concentration at levels which are easily obtained in clinical galactose tests. Stenstam (1946) demonstrated that continuous infusions of galactose, exceeding about 400 mg/min, resulted in rectilinearly rising plasma concentrations. Liver-vein catheterisation studies have shown that the arterio-hepatic venous concentration difference is constant in a wide concentration interval (Tygstrup & Winkler, 1954, Nakamura et al., 1961). Waldstein, Greenburg, Biggs & Corn (1960) demonstrated that the extrarenal elimination of galactose from the body would reach a maximum during galactose infusions, and Segal & Blair (1961) found that the conversion of  $^{14}\text{C}$ -galactose to  $^{14}\text{CO}_2$  was delayed by injection of 20 g of carrier galactose.

This is in agreement with the observed low  $K_m$  values for galactose of mammalian liver galactokinase (Ballard, 1966), provided that the phosphorylation of galactose is the limiting step in galactose breakdown. Enzymekinetic consi-

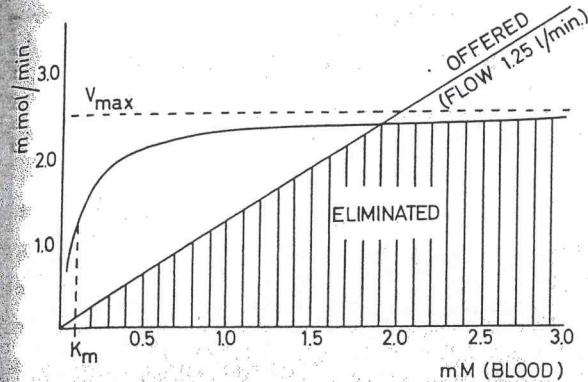


Fig. 1. Elimination rate in relation to concentration. The amount of galactose supplied to the liver is determined by the concentration in portal venous and hepatic arterial blood, which are assumed to be the same, and by the hepatic blood flow. At constant flow it is directly proportional to the concentration. The curved line shows the conversion rate in relation to concentration for an enzymatic reaction with Michaelis-Menten kinetics,  $K_m$  0.1 mM and  $V_{max}$  2.5 mmoles/min. At concentrations below 1.9 mM (330 mg/l) the amount supplied is less than the possible elimination rate with the indicated flow; at slightly higher concentrations the elimination rate is 95 per cent of  $V_{max}$ , and at still higher concentrations it increases very little. The transition from saturated to unsaturated elimination may be less abrupt than shown, since the elimination in centrilobular cells may become unsaturated at higher peripheral concentrations owing to the concentration gradient along the sinusoid. In cirrhosis the splay may be larger owing to intrahepatic shunts and to increased values of  $K_m$ .

derations indicate that this is the case unless galactose-1-phosphate accumulates in the cells (Cuatrecasas & Segal, 1965). Fig. 1 illustrates that a  $K_m$  value of 0.1 mM for the galactokinase reaction will ensure 95% saturation of the process when the amount of galactose supplied to the liver by portal and hepatic arterial blood equals or exceeds the elimination capacity ( $V_{max}$ ) of 2.5 mmoles/min (450 mg/min).

#### The elimination curve in blood

The hepatic elimination of galactose may be calculated fairly accurately in experiments with

continuous infusions of galactose (Waldstein et al., 1960, Tygstrup, 1963), but the procedure is too complicated for routine work.

For a clinical test the single injection method represents the maximum of complexity which can be accepted.

In theory, calculation of a constant elimination rate from an elimination curve is simple, since the curve can be described as

$$c_m = \frac{A}{V} - \frac{Q}{V} t \quad (1)$$

where  $c_m$  is the mean concentration in the volume of distribution  $V$ ,  $A$  the amount injected,  $Q$  the elimination rate and  $t$  the time. It follows from the equation that the slope of the curve  $dc_m/dt$ , is constant, i.e. the elimination curve is a straight line.

Conflicting statements have been made regarding the shape of galactose elimination curves in man. Several authors (Fischberg, 1930, Stenstrom, 1946, Colcher, Patek & Kendall, 1946, Vink, 1959, Tengström, 1966) have considered the curve to be a single exponential function, described by

$$c = c_0 e^{-kt} \quad (2)$$

because it seems to be rectilinear in a semilogarithmic system. This function presupposes an "unsaturated" elimination dependent upon concentration. Analysis of the shape of the elimination curve was performed in dogs by Dominguez & Pomerene (1944). They concluded that the extrarenal (i.e. mainly hepatic) elimination consisted of two components; one, dependent on, and the other, independent of, concentration.

The results of two attempts at curve analysis in man are conflicting. In one study (Tygstrup & Winkler, 1954), in which a part of the curve was examined separately, a significant systematic deviation of the experimental data from the

exponential function (eq. 2) was demonstrable, whereas the deviations from the straight line (eq. 1) were random.

The other group (Bernstein, Wheeler, Bond, Rohmsdahl & Dougherty, 1960) who studied the entire elimination curve obtained the opposite result. The discrepancies, however, between these results may be only apparent. Strictly speaking, the first study only shows that the intermediate section of the curve (from 20 minutes after the injection to concentrations of about 400 mg/l) is *not* exponential; and the second study shows that the entire curve (from 10 minutes to 200 mg/l) is *not* rectilinear. Positive evidence regarding the function of a curve is impossible, since deviations, caused by the analytical error, i.e. the random deviations, may cover a wide variety of functions; and physiological considerations must be decisive in determining which method should be preferred.

The delimitation of the curve to be analyzed is important. If the interval is too small, it will be very difficult to demonstrate significant deviations from any of the functions tested. On the other hand, if the interval becomes too wide, there will be a risk of different functions operating consecutively. If this is the case, an analysis of only one function becomes meaningless.

The delimitations made by Tygstrup & Winkler (1954) were based on the physiological assumption that such changes in function do occur, owing to distribution phenomena during the first part of the curve, and to unsaturation of the elimination mechanism during the last part. These phenomena, which are discussed below, will cause both ends of the elimination curve to take a curvilinear course; and when increasing parts of these sections are included in the curve analysis, the deviations from the straight line increase and may soon exceed the deviation from the exponential function. If a rectilinear, intermediate section is pronounced, how-

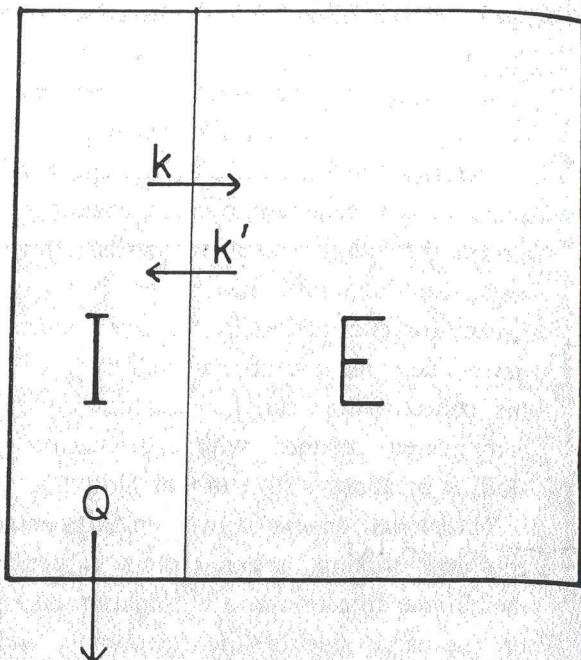


Fig. 2. Model.

ever, it may still be manifest as a downwards open angle on the elimination curve drawn in a simple logarithmic system. As this was actually found to be the case by Bernstein et al. (1961) in some curves, it is possible to surmise that only the interpretation and not the observations were different in the two materials.

#### The physiological meaning of the delimitation of the curve

Figure 1 shows that, at low concentrations, the amount of galactose removed by the liver is proportional to the concentration (if the hepatic blood flow is constant). This is in agreement with the observation that the hepatic venous concentration of galactose approximates zero, when the arterial concentration is below the arterio-hepatic venous difference observed at higher concentrations, i.e. about 350—450 mg/l (Tygstrup & Winkler, 1954, 1958, Nakamura et al., 1961). The elimination rate of galactose,

during this interval of a single injection curve (the "unsaturation period"), is mainly determined by the hepatic blood flow; and its kinetics should undoubtedly be analyzed separately.

During the first period of the curve a large amount of the galactose injected leaves the blood stream through the capillary walls. At a later stage this amount must re-enter the blood stream for elimination in the liver or the kidneys. These movements must greatly influence the concentration changes in the blood in a complicated manner which are most easily studied by analysis of a model (Fig. 2).

The model consists of two compartments with complete mixing, separated by a permeable membrane. Injection and elimination take place on the same side of the membrane, and the elimination rate ( $Q$ ) is assumed to be constant. If  $I$  and  $E$  are the quantities of galactose present in each compartment, then the change in these quantities are described by

$$\frac{dI}{dt} = -Q - kI + k'E \quad (3)$$

and

$$\frac{dE}{dt} = kI - k'E \quad (4)$$

where  $t$  is time and  $k$  and  $k'$  are the fractions of  $I$  and  $E$  respectively which pass through the membrane per time unit. It follows that the concentration course in compartment  $I$  is described by

$$\frac{dc_i}{dt} = -\frac{Q}{V_i} - k(c_i - c_e) \quad (5)$$

With the initial conditions,  $c_i = A/V_i$  and  $c_e = 0$  at  $t = 0$ , the solution of the differential equation is as follows (6), where  $e$  is the base of natural logarithms, and the other symbols have their previous significance:

$$c_i = \frac{A}{V} - \left( \frac{V_e}{V} \right)^2 \frac{Q}{V_i k} + \left[ \frac{A}{V} \frac{V_e}{V_i} + \left( \frac{V_e}{V} \right)^2 \frac{Q}{V_i k} \right] e^{-\frac{V}{V_e} kt} - \frac{Q}{V} t \quad (6)$$

It is evident that the variation in concentration with time depends on two components; one is exponential, decreasing with time, and the other is constant.

If the values of the constants were known, the time could be calculated when the exponential component becomes insignificant in relation to the constant component, i.e. the length of time that must elapse before the concentration curve can be considered as approximately linear. In eq. (6) most of the constants can be replaced by approximate values known from human physiology, except for the constant  $k$ .

Two approaches can be considered for determination of  $k$ . One is to analyze the elimination kinetics of the initial part of the curve after subtraction of the following section, which is assumed to be rectilinear. The disadvantages of this method are that it presumes rectilinearity of part of the curve after a single injection, and that, owing to osmotic forces, the injection will cause a temporary increase in the intravascular volume, producing disturbing changes in concentration.

The other possibility is to determine  $k$  from the preceding term of eq. (6), which is independent of time. The meaning of this term is evident if it is assumed that the exponential term has become insignificant, and the remaining part of eq. (6) is subtracted from eq. (1), which also applies to the model. The result is

$$c_m - c_i = \frac{1}{k} \frac{Q}{V_i} \left( \frac{V_e}{V} \right)^2 \quad (7)$$

i.e. the difference between the mean concentration and the intravascular concentration. It also

clearly shows that the mean concentration and the intravascular concentration run a parallel course under these conditions. Instead of the concentration difference it may be found preferable to study the time difference, which is the horizontal distance between the two curves ( $T$ ), where

$$T = \frac{1}{k} \frac{V_e^2}{V_i V} \quad (8)$$

The time displacement  $T$  may be estimated experimentally in man during continuous infusions of galactose (Tygstrup, 1963). Fig. 3

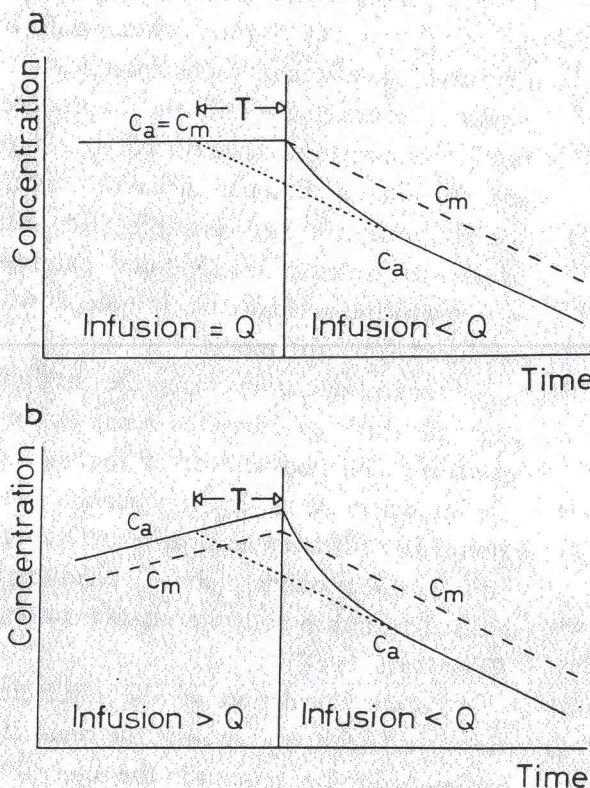


Fig. 3. Determination of the time displacement. Hypothetical experiments with infusions equal to, greater than, and smaller than the elimination rate  $Q$ . The intravascular concentrations ( $c_a$ ) are measured, the mean concentrations in the volume of distribution ( $c_m$ ) are drawn parallel with the rectilinear parts of the intravascular curves (see text). The time displacement  $T$  is measured as the time from the intercept between the first curve and the extrapolation of the second curve, to the start of the second period. Most of the actual experiments for determination of  $T$  have been performed as b

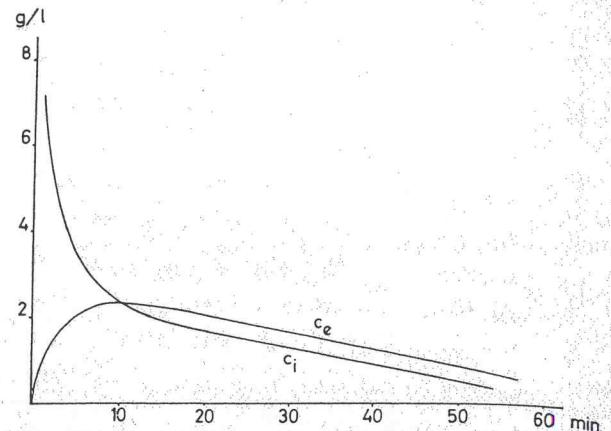


Fig. 4. Concentration course in the model after a single injection. Calculated intravascular ( $c_i$ ) and extravascular ( $c_e$ ) concentrations according to eq. (3) and (4) after instantaneous injection of 35 g (194 mmoles) of galactose at  $t = 0$ , if  $Q = 0.5$  g/min (2.78 mmoles/min),  $V_i = 4$  l,  $V_e = 9$  l, and  $T = 7$  min. The curves are described by ( $c = \text{mM}$ ):

$$c_i = 13.45 + 35.13 e^{-0.322 t} - 0.214 t$$

and

$$c_e = 15.91 - 15.91 e^{-0.322 t} - 0.214 t$$

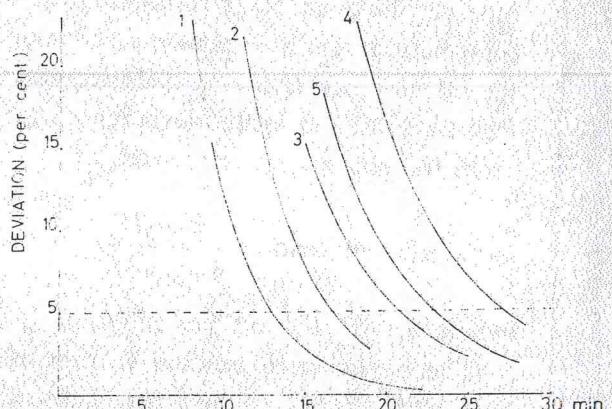


Fig. 5. Duration of equilibration period. The equilibration period is assumed to end when the exponential elimination is less than 5 per cent of the total elimination from the intravascular compartment, i.e. when the curve crosses the broken line. The curves are calculated from eq. (9).

Curve 1: "normal man", same conditions as in Fig. 4.

Curve 2: "fluid retention",  $V_e$  increased 50 per cent, otherwise as 1.

Curve 3: "inhibited diffusion",  $k$  reduced to 66 per cent, otherwise as 1.

Curve 4: combination of 2 and 3.

Curve 5: "cirrhosis with reduced elimination capacity",  $Q$  reduced to 40 per cent, otherwise as 4.

shows the principle on which this determination is based. The necessary conditions are that the elimination rate is constant during the whole experiment, and that a sufficiently long time can elapse to permit the exponential component to approach zero. A series of such determinations in patients who in this respect were regarded as normal, showed that the time displacement was about 7 minutes. This value for  $T$  may be used for the calculation of  $k$ , or  $T$  may be used directly in eq. (6), which simplifies it a little.

If  $T$  and the other constants are replaced by values assumed to be found in a normal man, the course of the intravascular (and the extravascular) concentrations may be constructed (Fig. 4). The curves seem to be approximately rectilinear from about 15 minutes after injection.

A more precise estimate of the distribution time may be obtained by computing the relative contribution of the exponential component to the fall in concentration at different times. This may be expressed as the deviation of the curve from the straight line calculated by

$$\text{deviation (per cent)} = \frac{nf + T}{f - t - T} e^{-\frac{k}{T}t} 100 \quad (9)$$

where  $n = V_e/V_i$  and  $f = A/Q$ .

The deviation in relation to time is shown in Fig. 5. If it is assumed that a deviation less than five per cent is undetectable, then, under the given conditions for the example in Fig. 4, the curve will be rectilinear 13 minutes after injection. Under pathological conditions, however, the situation may be different, as shown in curves 2—5; but even then the deviation from rectilinearity will rarely be significant 20 minutes after the injection.

Thus the theoretical considerations confirm that, under the given conditions, the delimitation of the test period as the interval starting

20 minutes after injection and ending when the concentration falls below 400 mg/l, is physiologically justified.

### Can the model be applied to man?

In the model the elimination rate ( $Q$ ) is easily calculated from the amount injected ( $A$ ) and the slope of the curve ( $Q/V$ ). It may be questioned, however, if the deductions from a simple model can be applied to the elimination curve in man after a single injection. If it could be proved that the curve is rectilinear during the test period—with the small deviations predicted from eq. (9)—the answer would be affirmative, since other, more complicated, conditions are extremely unlikely to produce this result. As previously mentioned, this proof is not feasible, and some deviations from the straight line are still possible, for instance, owing to distribution to some extravascular spaces which equilibrate much more slowly than predicted from the model.

A certain deviation from the straight line must be expected from the renal excretion of galactose. The mechanism of the excretion is not known in detail, but it probably consists of glomerular filtration and partial active reabsorption in the proximal tubules, resulting in increased clearance with the plasma concentration (Tygstrup, 1961).

Accurate calculation of the resulting function is difficult, and even if the renal clearance is assumed to be constant in the interval studied, the equation which describes the concentration curve is complicated:

$$c = \left( c' + \frac{Q}{Cl} \right) e^{-\frac{Cl}{V}(t-t')} - \frac{Q}{Cl} \quad (10)$$

where  $c'$  is the concentration at a given time  $t'$ , and  $Cl$  is clearance. This equation is not suitable for routine use.

The renal clearance of galactose in normal subjects amounts to about 50 ml/min, and the deviation from the straight line produced by this clearance value was within the analytical error. This does not mean that the renal loss of galactose can be omitted from the calculations, but that the urinary excretion rate can be regarded as constant during the test period.

This implies that in the equations  $Q$  represents the sum of the hepatic elimination rate and the mean renal excretion rate. It was found, however, that a simpler and sufficiently accurate correction for urinary loss of galactose can be obtained by subtracting the total amount excreted during the test ( $U$ ) from the amount injected ( $A$ ) (Tygstrup, 1961). Further simplification of the correction is possible if mainly patients with normal liver function and cirrhosis are examined, as in these cases the urinary excretion is  $0.1 \times$  the amount injected ( $\pm 0.05$ ).

### Conclusion

For calculation of the hepatic galactose elimination rate in man, eq. (1) may consequently be slightly modified to  $c_m = (A - U)/V - (Q/V)t$ . The slope of the curve,  $Q/V$ , is calculated from the arterial concentrations during the test period.

The elimination rate is the product of the slope and the volume of distribution, and the latter may be calculated from the modified eq. (1) for  $t = 0$  as  $V = (A - U)/c_{m_0}$ , where  $c_{m_0} = c_{i_0} + (Q/V)T$ ;  $c_{i_0}$  is the intercept of the test period of the arterial curve with the ordinate, and  $T$  is the time displacement of the intravascular curve in relation to the mean concentration curve (cf. Fig. 5).

Alternatively  $Q$  can be calculated by rearrangement of the modified eq. (1) as  $Q = (A - U)/t - (V/t)c_m$  for  $c_m = 0$ . The corre-

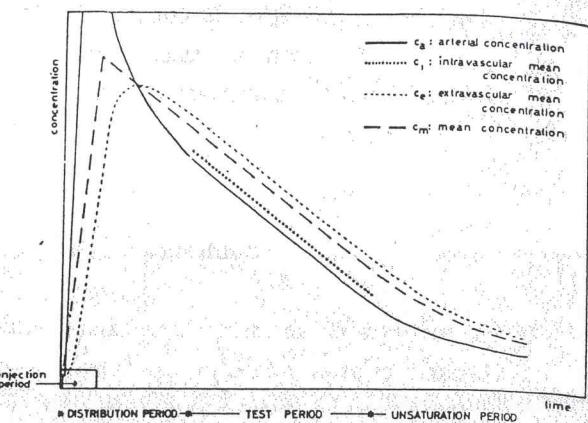


Fig. 6. Concentration course in man after a single injection. The full line shows the concentrations which may be found by analysis of arterial blood, whereas the broken lines show the assumed concentrations in other parts of the volume of distribution.

sponding value of  $t$  is determined as  $t_{c_m=0} = t_{c_i=0} + T$ ;  $t_{c_i=0}$  is the intercept of the test period of the arterial curve with the abscissa. For  $T$  the value 7 minutes may be generally used. This procedure is easy to apply in routine work: The concentrations measured are marked on linear paper, a straight line is drawn through the points for which  $t > 20$  minutes, and  $c > 400$  mg/l, the time of the (graphical) intercept of the line with the time axis is increased by 7, and the difference between the amount injected and the amount excreted in the urine is divided by the resulting value, thus

$$Q = \frac{A - U}{t_{c_i=0} + 7} \quad (11)$$

In order to obtain a sufficient number of determinations during the test period, the amount injected should be about 500 mg/kg body weight, injected intravenously in the course of about 5 minutes. Samples should be drawn of arterial (or capillary) blood every 5 minutes, from 20 minutes to 45 minutes after injection is begun. If the amount injected is calibrated carefully (within  $\pm 5$  per cent), and the corre-

tion for urinary loss by constant factor is considered sufficiently accurate, the numerator of eq. (11) can be calculated directly as  $450 \times \text{kg}$  body weight.

### Summary

A method is described for calculation of the hepatic elimination capacity ( $L_m$ ) of galactose after a single intravenous injection. Only the intermediate part of the elimination curve in blood is sufficiently free from disturbing factors (distribution phenomena, flow-dependent elimination) to permit calculation of the elimination capacity. Furthermore a correction must be made for uneven distribution of galactose between intra- and extravascular volumes of distribution. Approximate values for these corrections and for correction of the urinary loss of galactose are given. With these modifications, determinations of the  $L_m$  of galactose may be used as a clinical, routine liver-test.

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