From Medical Clinic B, Rigshospitalet, Copenhagen, Denmark.

Kinetics of Galactose Elimination.

By

NIELS TYGSTRUP and KJELD WINKLER.

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Galactose is known to be a substance well suited for the estimation of the liver function (BAUER 1906, DRAUDT 1913, SHAY, SCHLOSS and BELL 1931, BOLLMAN, MANN and POWER 1935). To get the best quantitative measure of its metabolism, it is of primary importance to know the rate at which it is removed from the blood.

The galactose blood-time curve has by FISHBERG (1930), DOMINGUEZ and POMERENE (1944) and STENSTAM (1946) been described as a decreasing exponential function which means that the amount of galactose removed from the blood is dependent on the concentration. The equation of the simple decreasing exponential function is

$$c = c_o \cdot e^{-g \cdot t} \qquad (1)$$

(c is the concentration, t time, e the base of natural logarithms and c_o and g are constants).

Preliminary experiments with intravenous administration of galactose have suggested to us that the amount of galactose eliminated per time unit is constant, independent of concentration within a certain range. Determination of the difference of galactose concentration in peripheral blood and blood obtained by catheterization of the hepatic veins has shown this difference to be constant above a certain level in the peripheral blood. When

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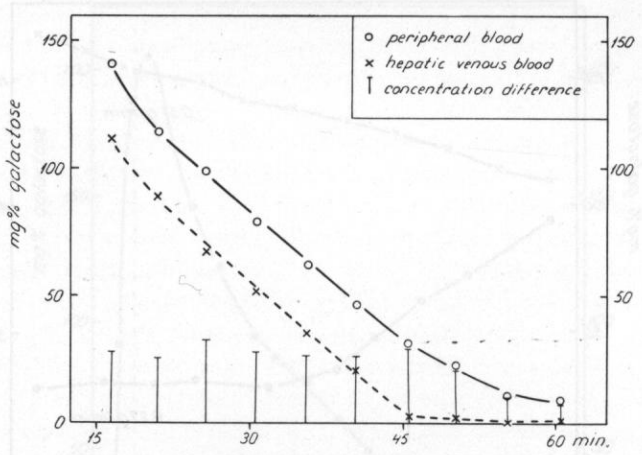

Fig. 1. *Catheterization experiment.* O. K. male, 17 years. 24 g of galactose intravenously in 5 minutes. Average concentration difference until about 45 minutes: 28 mg per cent.

this level is reached the galactose content of the hepatic veins approximates zero. A curve is shown in fig. 1. The catheterization experiments will be reported in detail in a separate communication (TYBJÆRG HANSEN, WINKLER and TYGSTRUP, to be published).

Furthermore, experiments with constant infusion of galactose (after a priming dose to shorten the time of equilibration and to raise the concentration to about 100 mg per cent) show that a constant plasma level only is found if the concentration falls below 50 to 30 mg per cent. Similar conclusions can be drawn from STENSTAM's results (1946). Fig. 2 shows an experiment in which 0.51 g of galactose was infused per minute after a priming dose of about 20 g, resulting in a continuous rise in concentration. The experiment was repeated a few days later in the same subject, only reducing the amount infused by ab. 50 per cent, which caused the concentration to fall until a level was obtained at ab. 30 mg per cent, as seen in the same figure.

The object of the present work is to decide whether the constant elimination, seen in these experiments, determines the shape of the elimination curve after a single intravenous injection which is the method generally employed in clinical intravenous galactose tolerance tests. If this be the case, expressions derived from equation (1), as for instance the "Galactose Removal Constant" of

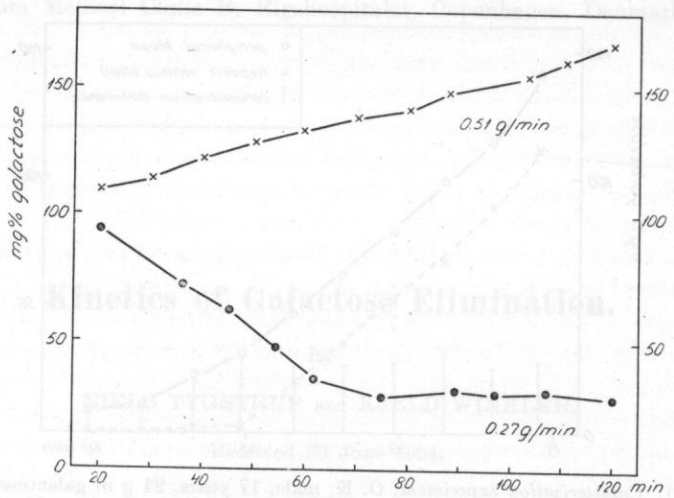

Fig. 2. *Infusion experiment.* K. S. male, 25 years. Priming dose 20 g of galactose, infusion of 0.51 g/min. (upper curve), and 0.27 g/min. (lower curve).

Colcher, Patek and Kendall (1946) cannot be expected to describe the galactose elimination.

Therefore we have investigated the elimination curve after a single injection using frequent blood sampling in order to determine its course more accurately.

Methods.

Sixteen galactose tolerance tests have been carried out in 12 male subjects of 15 to 57 years of age, with no history of liver disease, and with normal liver function tests. The test was done with the subject in the recumbent position, at least 3 hours after a meal. 0.5 g per kg body weight of galactose (Sandoz, puriss.) dissolved in 100 ml of sterile water was injected intravenously at a constant rate of 20 ml per minute. Before the injection and at intervals of 3 to 5 minutes during the first hour or so afterwards, venous blood was drawn through an indwelling needle (Gordh-needle) into centrifuge tubes, containing one drop of a 5 per cent heparin solution and 4 mg of potassium fluoride.

The galactose concentration of plasma was determined spectrophotometrically in protein-free filtrates after oxidation of glucose to nonreducing gluconic acid by a specific glucose oxidase Notatin[1]. The procedure is described in detail elsewhere (Tygstrup, Winkler, Lund and Engell 1954). Its standard deviation is approximately 2 mg per cent.

[1] Kindly supplied by Boot Pure Drugs Ltd. Nottingham, Engl.

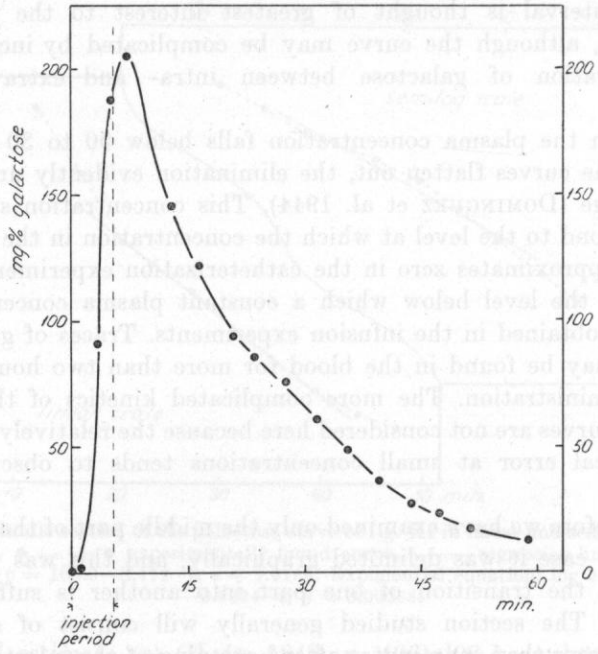

Fig. 3. *Single injection experiment.* W. B. male, 20 years. 32 g of galactose intravenously.

General Considerations.

A survey of the curves, obtained by plotting the plasma concentration against time as shown in fig. 3, reveals that each curve roughly can be divided into three sections, the first part consisting of a steep rise in concentration during the injection, with a maximum of 200 to 300 mg per cent about one minute after conclusion of the administration, followed by a rapid decrease in concentration for the next 10 to 15 minutes. During this period galactose removal from the blood is largely governed by diffusion to extravascular beds and by renal excretion (v. i.), therefore the curve is too complicated for study of the metabolism of galactose ("utilization"). This part of the curve has been examined in only 5 cases, while its transition into the second part has been ascertained in all cases.

During the second part the curves assume a more gradual decline for some time, averaging 22 minutes (12 to 29 minutes).

This interval is thought of greatest interest to the problem studied, although the curve may be complicated by incomplete equilibration of galactose between intra- and extravascular beds.

When the plasma concentration falls below 50 to 30 mg per cent, the curves flatten out, the elimination evidently undergoes a change (DOMINGUEZ et al. 1944). This concentration seems to correspond to the level at which the concentration in the hepatic veins approximates zero in the catheterization experiments, and also to the level below which a constant plasma concentration can be obtained in the infusion experiments. Traces of galactose often may be found in the blood for more than two hours after the administration. The more complicated kinetics of this part of the curves are not considered here because the relatively greater analytical error at small concentrations tends to obscure the details.

Therefore we have examined only the middle part of the curves. In each case it was delimited graphically, and this was possible because the transition of one part into another is sufficiently abrupt. The section studied generally will consist of samples drawn later than 20 minutes after beginning of the injection (the time required for distribution to extravascular beds?) and containing more than about 40 mg per cent (corresponding to the level of changed elimination).

Results.

If the amount of galactose removed from the blood after a single intravenous injection is independent of the concentration in the interval studied, the blood galactose curve here would be a straight line, the equation of which is

$$c = c_o - g \cdot t \qquad (2)$$

The fitting of the curves to equation (1) and (2) has been investigated in the following way. For each curve two regression equations were calculated by means of the method of least squares (*e. g.* FISHER 1950). In one calculation the concentrations and the other the logarithms of the concentrations were used as dependent variate, time being independent variate in both. The deviation of each experimentally found concentration from the value calculated from these two equations, in units of the standard

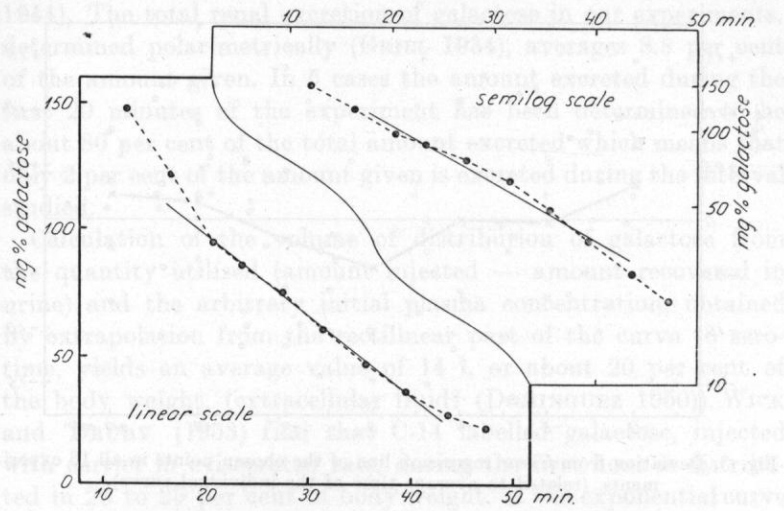

Fig. 4. Middle section of the galactose curve of fig. III in linear and semilogarithmic scale. ● - - - - ● experimentally found curve, ——— regression line. Linear equation: $c = 160.9 - 3.172 \cdot t$; $s = 1.0186$. Exponential equation: $\log c = 2.4616 - 0.0224 \cdot t$; $s = 0.00258$.

error of estimate (s) (HALD 1948, p. 406) has been determined ($\frac{c-C}{s}$ and $\frac{\log c - \log C}{s}$ respectively). A curve in linear and semilogarithmic scale with the corresponding regression lines is seen in fig. 4. Fig. 5 illustrates the deviations from the linear curve in all 16 experiments, and fig. 6 that from the exponential curve. In these figures the zero line represents the regression line used.

Discussion.

The criterion of fitting is random distribution of the deviations around the regression line. From figs. 5 and 6 it therefore appears that the curve-section studied is rectilinear while it deviates significantly from the exponential shape.

Furthermore the fitting of the galactose curves is the best obtainable with the analytical procedure employed, as the standard deviation of the analysis at the average concentration (80 mg per cent) is 1.8 mg per cent, corresponding to 0.0100 in the logarithmic system (HALD 1948, p. 193) and the average standard error of estimate of the linear curves is 2.0 mg per cent, while that of the exponential curves amounts to 0.0240.

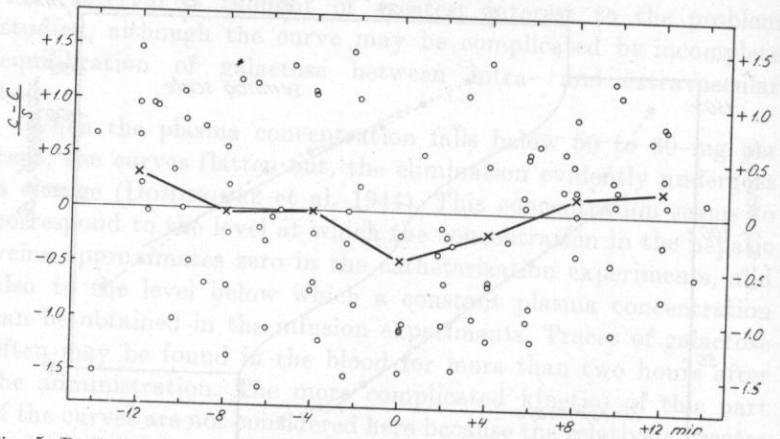

Fig. 5. Deviation from *linear* regression line of the chosen points in all 16 experiments (related to average time of the individual curve).

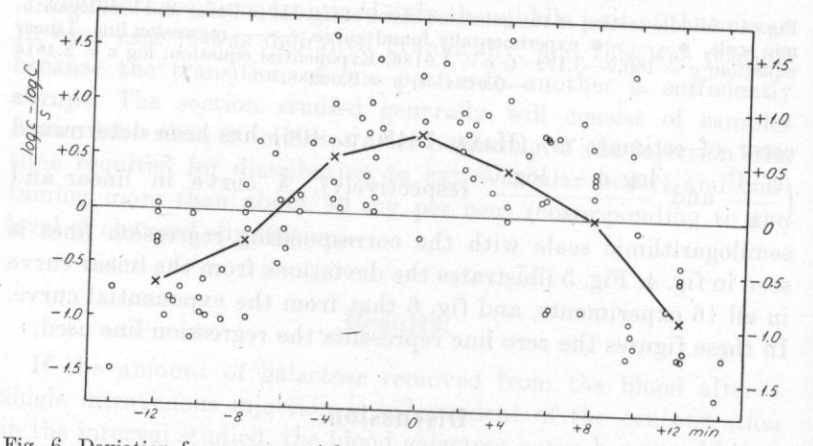

Fig. 6. Deviation from *exponential* regression line of the chosen points in all 16 experiments.

Whether there is some preponderance of negative values of the deviations in fig. 5 around the middle part of the line, balanced by positive ones at the extremities, indicating a slight exponential component, cannot be decided with certainty, because the deviation, if there be any, is of the same order of magnitude as the analytical error.

An exponential component might be attributed to renal excretion, which is known to be proportional to concentration (GAMMELTOFT and KJERULF-JENSEN 1943. DOMINGUEZ et al.

1944). The total renal excretion of galactose in our experiments, determined polarimetrically (GEILL 1934), averages 8.8 per cent of the amount given. In 5 cases the amount excreted during the first 20 minutes of the experiment has been determined to be about 80 per cent of the total amount excreted which means that only 2 per cent of the amount given is excreted during the interval studied.

Calculation of the volume of distribution of galactose from the quantity utilized (amount injected — amount recovered in urine) and the arbitrary initial plasma concentration, obtained by extrapolation from the rectilinear part of the curve to zero-time, yields an average value of 14 l, or about 20 per cent of the body weight, (extracellular fluid? (DOMINGUEZ 1950)). WICK and DRURY (1953) find that C-14 labelled galactose, injected with carrier in eviscerated rats, during the first hour is distributed in 20 to 25 per cent of body weight. If the exponential curve is used for extrapolation, the volume calculated averages 10 l. The value obtained by the first method seems independent of the slope of the rectilinear part of the curve, as it is the same in 6 patients with decreased galactose elimination as in the normals. We think this may support the concept of rectilinearity.

Conclusions.

These experiments indicate that a constant galactose elimination, such as is found in the catheterization and infusion experiments, results in a rectilinear part of the blood galactose curve after a single intravenous injection. It seems relevant to take this into consideration when estimating the liver function from intravenous galactose tolerance tests. Probably the slope of the curve is a measure of the galactose removal capacity of the liver (L_m of galactose (LEWIS 1952)).

Summary.

The galactose elimination curve in blood after a single intravenous injection is studied. Only a part of the curve is used for elimination analysis. This part is shown to be rectilinear which means that the amount of galactose removed is independent of concentration and therefore probably a measure of the galactose removal capacity of the liver.

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