Acta physiol. scand. 1961. 51. 263—274

From Medical Department B, Rigshospitalet, København, Denmark

The Urinary Excretion of Galactose and its Significance in Clinical Intravenous Galactose Tolerance Tests

By

NIELS TYGSTRUP

Received 12 November 1960

Abstract

TYGSTRUP, N. *The urinary excretion of galactose and its significance in clinical intravenous galactose tolerance tests.* Acta physiol. scand. 1961. *51.* 263—274. — The disappearance of galactose from the blood after intravenous administration may be used for measurement of the liver function, if correction for the urinary loss of galactose is made. The present study was performed in order to determine the significance of this loss. It was found that urinary excretion of galactose was unimportant in relation to hepatic elimination at plasma concentrations below about 500 mg/l (approximately 3 per cent of the total elimination), but at higher concentrations the ratio between urinary excretion and hepatic elimination increased, as the former rises with the concentration, and the latter remains constant. After a single injection of about 500 mg/kg body weight on an average 10 per cent of this amount was excreted in normal subjects as well as in patients with cirrhosis. This percentage could be used for a practical calculation of the contribution of the urinary loss to the total elimination rate of galactose during the interval in which the latter remains essentially constant. The experiments indicate a low renal threshold for galactose, but no evidence for a reabsorption T_m was found within the range of plasma concentrations studied (up to 3,000 mg/l). The renal clearance of galactose was significantly decreased in patients with cirrhosis. The implications of these findings for the calculation of the maximal hepatic galactose elimination rate from the plasma concentration curve following a single injection are examined in an appendix.

The fact that the plasma galactose concentration-time curve following a single intravenous injection of galactose is essentially rectilinear over a considerable range of plasma concentrations (TYGSTRUP and WINKLER 1954), indicates that in this range the elimination of galactose mainly depends on processes which are saturated at such concentrations. As galactose is predominantly eliminated by the liver, the maximal galactose elimination rate is determined largely by the metabolic status of the liver.

It is well known, however, that some extrahepatic elimination of galactose does occur (BOLLMAN, MANN and POWER 1935), chiefly by way of renal excretion. If urinary loss of galactose is significant under the conditions used in clinical intravenous galactose tolerance tests, determination of the hepatic galactose elimination rate from elimination curves in plasma requires correction for this extrahepatic elimination.

The renal excretion of galactose in patients with normal and with reduced liver function therefore has been investigated in order to determine the magnitude of this correction. The renal excretion was studied partly under conditions of continuous intravenous galactose infusions (with constant or constantly rising plasma galactose concentrations) and partly under conditions of falling plasma concentrations (following a single intravenous injection).

Methods

The patients were studied in the morning while lying in their bed, more than 12 hours after their last meal. They were allowed to drink moderate amounts of water, but forced drinking to ensure high diuresis was avoided, as this apparently influences the excretion of galactose.[1]

Galactose was administered as analyzed solutions by calibrated, motor-driven syringes. Infusions of galactose were maintained for one to three hours, and the urine samples examined were collected either in periods with constant plasma concentrations, or in periods with constantly rising concentrations, depending on whether the galactose infusion rate was smaller or greater than the maximal galactose elimination rate of the patient.

In experiments with single injection of galactose about 500 mg/kg body weight was injected at a constant rate in the course of 6 minutes.

Determination of galactose in plasma was performed as previously described (TYGSTRUP *et al.* 1954). As galactose is mainly distributed in extracellular fluid (except in the liver), concentrations are expressed as mg/l plasma water.

Urine was collected through an indwelling bladder catheter. The bladder was emptied by suction, washed twice with 20 ml of sterile saline, and urine and washing fluid were mixed.

When the excretion was studied at falling or rising plasma concentrations, the galactose of the urine samples was related to the mid-concentration in plasma 8 minutes

[1] In 2 patients a single injection of the same amount of galactose was given during high and low diuresis. After deprivation of water for 24 hours the urine flow of these patients during the test was 0.55 and 0.60 ml/min, and-after administration of one liter of water in the morning 8.0 and 6.6 ml/min. The excretion of galactose was increased by 29 and 36 per cent, respectively, during high diuresis.

prior to the mid-time of the urine collection interval. This correction for the average delay-time in the urinary tract was applied because in one experiment (see Fig. 2) in which the excretions were studied during rising as well as during falling plasma concentrations, application of this correction resulted in essentially identical renal clearances at comparable plasma concentrations.

For determination of galactose in urine the specimen was diluted to an estimated concentration of about 500 mg/l. The reducing power was determined by the same method as used for plasma, partly after treatment with a galactose fermenting strain of yeast,[1] partly without treatment. The yeast, cultured in a galactose containing broth, was washed three times in saline to remove reducing substances. About 5 g of washed yeast was suspended in 30 ml of 0.2 M phosphate buffer (pH = 5.6). Two ml of diluted urine and 2 ml of yeast suspension were mixed in a flask which was stoppered, placed in a water bath at + 30 C, and automatically shaken for 90 min.

The difference between reduction values of the yeast-treated and the untreated sample was taken as concentration of galactose. Treatment with yeast for determination of the unfermentable (non-galactose, non-glucose) rest reduction was found necessary, especially when the concentration of galactose was low, as the rest reduction varied widely from sample to sample, and in most cases amounted to 500—1,500 mg/l of urine, expressed as galactose. The standard deviation of this analytical procedure is about 3 per cent.

As glucose is fermented by this strain of yeast as well as galactose, glucosuria will give falsely high values. In earlier experiments, when glucosuria was suspected from previous urinalysis, galactose was determined as the difference between a yeast-treated and a Notatin[2] treated sample. In more recent experiments this procedure was used when the urine showed reaction with Notatin-impregnated paper (Clinistix®).

Results

A. *Infusion experiments with constant plasma concentration*

The rate of galactose infusion was lower than the maximal galactose elimination rate of the patients. In patients with normal liver function the infusion rate was about 300 mg/min, and in patients with diseases of the liver about 200 mg/min. Consequently the plasma concentrations were lower than 400 to 500 mg/l, *i. e.* the level at which the eliminating mechanism becomes saturated (TYGSTRUP and WINKLER 1954). The material comprises 32 determinations in 29 subjects with normal hepatic and renal function and 10 determinations in patients with cirrhosis of the liver or acute epidemic hepatitis.

In the subjects with normal liver function the rate of galactose excretion averaged 9 mg/min (s. d. 5.6) or 3.1 per cent of the infusion rate, and in the patients with reduced elimination of galactose 4.1 mg/min (s. d. 3.1) or 1.8 per cent of the infusion rate. Fig. 1 shows the relationship between the amount of galactose excreted in the urine per minute and the concentration in plasma water. It is seen that the correlation between the two is poor, and the calculated renal clearance of galactose therefore varies widely. In patients with normal liver function it averages 35 ml/min (s. d. 18), and in the patients with hepatic diseases 17 ml/min.

[1] Kindly supplied by Carlsberg Laboratories, Copenhagen.

[2] Generously supplied by Leo Pharmaceutical Ltd., Copenhagen.

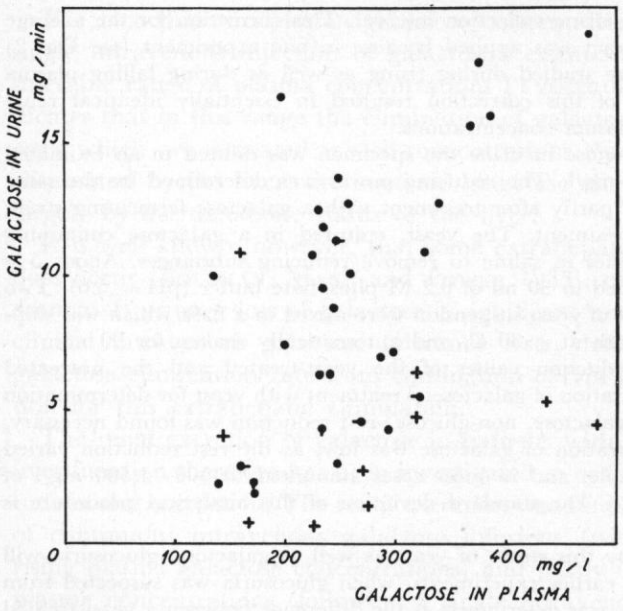


Fig. 1. Urinary excretion rate of galactose at low, constant plasma concentrations.

• = subjects with normal liver function,

+ = patients with diseases of the liver.

•–, ⁞, and –• are patients in whom two successive determinations were performed.

B. *Infusion experiments with rising plasma concentration*

The rate of galactose infusion was between 400 and 800 mg/min which was greater than the maximal galactose elimination rate of the patients. The material consists of 8 subjects with normal liver function and 3 patients with cirrhosis of the liver. Most data were obtained in intervals during which the concentrations were rising at rates varying from 0.2 to 12 mg/l/min. In one case the urinary excretion during a steeper rise (34 mg/l/min) was compared to that occurring during a falling curve (— 51 mg/l/min) found after interruption of the infusion. (This experiment was used for assessment of the average delay-time in the urinary tract.)

The relationship between the urinary excretion rate of galactose and the concentration in plasma water is shown in Fig. 2. In this concentration interval the correlation is more definite than at low concentrations, although the individual variation is still appreciable. Furthermore it is seen that the renal clearance tends to fall at low concentrations. In the intermediate concentration interval of 1,000 to 2,000 mg/l it averages 46 ml/min in the subjects with normal liver function, and at higher concentrations 57 ml/min.

C. *Single injection experiments*

The rate of galactose elimination may be calculated from the rectilinear plasma concentration-time curve by multiplying the absolute value of the slope of the curve by the volume of distribution of galactose, the latter being

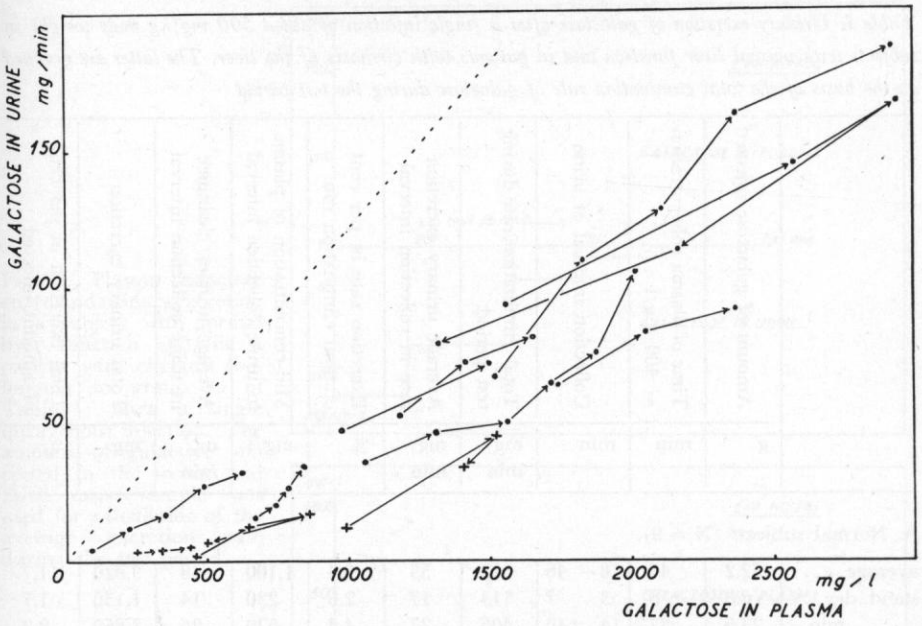


Fig. 2. Urinary excretion rate of galactose at higher, changing plasma concentrations. The arrows indicate whether the observation was made during rising or falling concentration. • = subjects with normal liver function, + = patients with cirrhosis of the liver. The dotted line indicates the amount of galactose filtered per minute at different plasma concentrations, assuming the glomerular filtration rate to be 120 ml/min.

obtained by dividing the amount injected by the extrapolated concentration at zero time. The rectilinear part of the curve, the *test period*, generally starts about 20 minutes after beginning of the injection and ends when the concentration falls below about 400 mg/l.

Quantitative collection of the urine excreted during the entire test period is not practicable, as the exact duration of this period is not known beforehand in the individual patient. In about one fourth of the experiments the test period was found to be inadequately covered by the urine collection intervals employed. Excluding these, 9 experiments remained in subjects with normal liver function, and 32 in patients with cirrhosis of the liver which were satisfactory for an evaluation of the relationship between the rate of urinary excretion and the total elimination rate during the test period. (Table I.) Fig. 3 illustrates the experimental findings in two typical examples. The patients with cirrhosis were arbitrarily divided into 3 groups on the basis of the total elimination rate.

It is seen from the table that the urinary excretion rate during the test period decreases with decreasing total elimination rate, and in all 4 groups

Table I. Urinary excretion of galactose after a single injection of about 500 mg/kg body weight in subjects with normal liver function and in patients with cirrhosis of the liver. The latter are grouped on the basis of the total elimination rate of galactose during the test period

	Amount of galactose injected	Time of plasma concentration = 400 mg/l	Collection interval of urine	Total elimination rate during test period	Average urinary excretion rate in collection interval	Excretion rate in per cent of total elimination rate	Mid-concentration of plasma in urine collection interval	Average urinary clearance in urine collection interval	Total urinary excretion	Total excretion in per cent of amount injected
	g	min	min	mg/min	mg/min	%	mg/l	ml/min	mg	%
A. Normal subjects (N = 9).										
average	32.2	47	18— 46	572	53	9.4	1,100	48	3,820	11.7
stand. dev. ..	6.8	8	3 7	115	17	2.6	230	14	1,130	1.7
range: min.	23.5	37	14 40	406	27	4.3	670	26	2,660	9.2
range: max.	43.0	56	22 55	732	79	14.4	1.530	74	5,330	15.2
B. Cirrhotics, total elimination rate > 300 mg/min (N = 11).										
average	29.4	67	20— 54	356	42	11.6	1.140	35	3,090	10.5
stand. dev. ..	5.2	9	6 15	45	20	5.2	210	12	940	3.9
range: min.	24.6	58	16 41	304	17	5.3	840	20	1,490	4.9
range: max.	40.7	82	23 73	456	74	20.3	1,500	52	4,300	14.8
C. Cirrhotics, total elimination rate < 300, > 210 mg/min (N = 10).										
average	32.0	102	20— 85	246	27	11.2	1,120	23	3,080	10.1
stand. dev. ..	6.1	15	3 12	29	13	6.3	130	10	1,570	6.2
range: min.	26.5	69	16 72	215	10	3.8	890	9	1,290	3.7
range: max.	46.8	128	24 115	298	52	24.1	1,309	44	6,660	25.1
D. Cirrhotics, total elimination rate < 210 mg/min (N = 11).										
average	29.5	120	20— 89	188	23	11.9	1,100	21	2,310	8.3
stand. dev. ..	3.8	26	2 30	19	11	5.2	150	11	930	3.6
range: min.	25.0	91	17 71	156	3	1.7	835	2	930	2.5
range: max.	37.6	179	24 178	209	43	20.7	1,389	41	3,910	15.2

averages about 10 per cent of the latter. As the mid-concentrations of the groups in the sampling interval are almost identical, the values of renal clearance of galactose also show a decrease roughly parallel to the fall in total elimination rate.

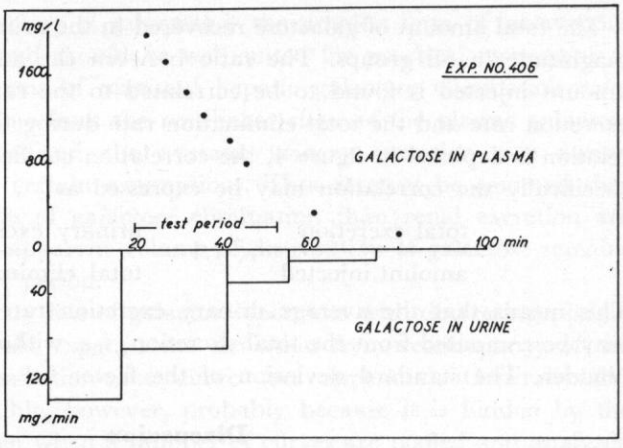
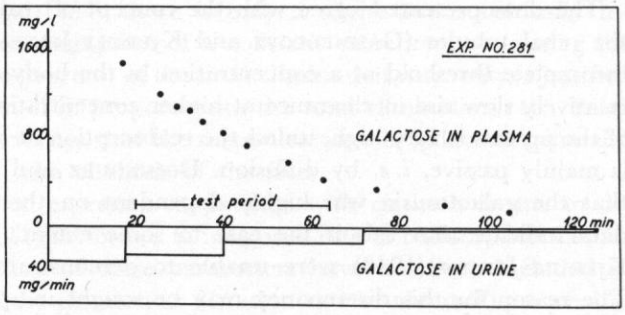


Fig. 3. Plasma galactose curve and urinary excretion in a subject with normal liver function and in a patient with cirrhosis (belonging to group C of Table I) after a single intravenous injection. The amount of galactose excreted in the second and third urine sample was used for calculation of the average excretion rate during the test period.



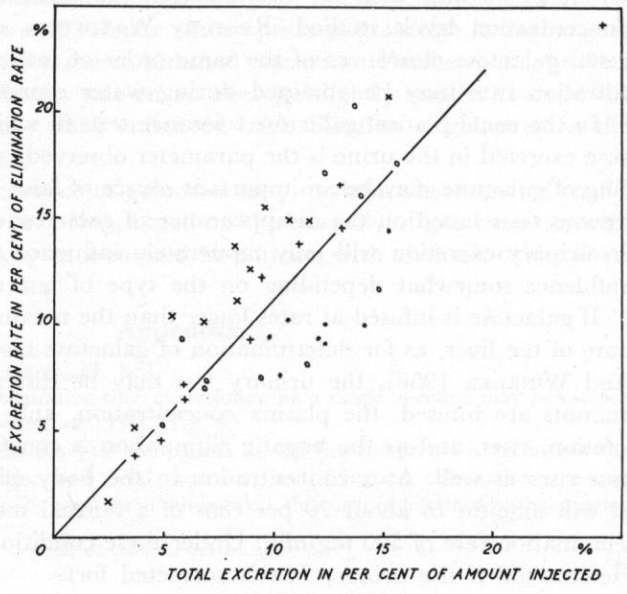


Fig. 4. Correlation between the average urinary excretion rate during the collection interval in per cent of the total elimination rate during the test period and the total urinary excretion in per cent of the amount injected.

• = subjects with normal liver function,

∘ = patients with cirrhosis and total elimination rate greater than 300 mg/min,

+ = patients with cirrhosis and total elimination rate between 300 and 210 mg/min,

× = patients with cirrhosis and total elimination rate smaller than 210 mg/min. The line shows the average relationship between the values in all the patients.

The total amount of galactose recovered in the urine is of the same order of magnitude in all groups. The ratio between the amount excreted and the amount injected is found to be correlated to the ratio between the urinary excretion rate and the total elimination rate during the test period. This correlation is depicted in figure 4, the correlation coefficient (r) is + 0.80. More specifically the correlation may be expressed as

$$\frac{\text{total excretion}}{\text{amount injected}} \times 1.1 = \frac{\text{urinary excretion rate}}{\text{total elimination rate.}}$$

This means that the average urinary excretion rate during the test period may be computed from the total excretion, *i. e.* without catheterisation of the bladder. The standard deviation of the factor 1.1 is 0.3.

Discussion

The data presented agree with the concept of reabsorption of galactose in the renal tubules (GAMMELTOFT and KJERULF-JENSEN 1943) with a low and incomplete threshold at a concentration in the body of 100 to 200 mg/l. The relatively slow rise in clearance at higher concentrations indicates that the T_m of the process is very high, unless the reabsorption at these high concentrations is mainly passive, *i. e.* by diffusion. DOMINGUEZ and POMERENE (1944) found that the galactosuria was highly dependent on the rate of urine flow; our data indicate that this is the case to some extent, while GAMMELTOFT and KJERULF-JENSEN (1943) were unable to demonstrate any such relationship. The reason for this discrepancy may be sought in species differences, in different procedures used for production of diuresis, and in different plasma concentration levels studied. Recently WALDSTEIN *et al.* (1960) found that renal galactose clearances of the same order of magnitude as the glomerular filtration rate may be obtained during water diuresis.

In the usual peroral galactose tolerance tests in which the amount of galactose excreted in the urine is the parameter observed, an abnormal renal handling of galactose may be an important source of false interpretations. In intravenous tests based on the disappearance of galactose from the blood, changes in urinary excretion will only moderately influence the result, the degree of influence somewhat depending on the type of test used.

If galactose is infused at rates lower than the maximal galactose elimination rate of the liver, as for determination of galactose blood clearance (TYGSTRUP and WINKLER 1958), the urinary loss may be disregarded. When larger amounts are infused, the plasma concentration, and thereby the urinary excretion, rises, and as the hepatic elimination is constant, the relative urinary loss rises as well. At a concentration in the body of for instance 2,000 mg/l it will amount to about 20 per cent of a normal maximal hepatic galactose elimination rate of 500 mg/min. Under these conditions the latter only can be determined if the urinary loss is corrected for.

Technically single injection of galactose is the simplest form of intravenous galactose tolerance test and should be well suited for practical assessment of the liver function in terms of maximal hepatic galactose elimination rate.

Calculation of this value from the rectilinear slope of the plasma galactose concentration-time curve and the average urinary excretion rate during the test period requires certain assumptions. Thus it must be assumed that other extrahepatic routes of galactose elimination than renal excretion are negligible, and that the apparent volume of distribution of galactose remains constant during the test period.

Since the rate of urinary loss of galactose decreases with decreasing plasma concentrations, one should expect some deviation from rectilinearity of the plasma galactose concentration-time curve (see appendix). Generally this deviation is not discernible, however, probably because it is hidden by the analytical error, and even when a number of curves are pooled and analyzed (TYGSTRUP and WINKLER 1954) there is only a slight and statistically insignificant deviation. The assumption of a constant excretion rate during the test period, used for the correction of the total elimination rate, only is justified if the excretion rate is small in proportion to the total elimination rate. From this point of view it is fortunate that the renal clearance of galactose generally is decreased in patients with cirrhosis of the liver.

Whether the diminished excretion in patients with cirrhosis is due to diminished filtration rate of galactose or to increased rate of reabsorption cannot be stated, as determination of these parameters have not been included in this study. Reduction of the glomerular filtration rate to about 50 per cent of normal has been demonstrated in patients with decompensated cirrhosis of the liver (ÖNEN 1960), and if tubular reabsorption of galactose is unchanged, this may partly account for the diminished excretion. Furthermore the rate of urine flow in the cirrhotics was on an average slightly smaller than in the normals, a fact which may have enhanced back-diffusion of galactose in the tubules.

The reduced renal clearance of galactose may explain why negative results of peroral galactose tolerance tests, based on the quantity of galactose excreted in the urine, are frequently encountered in patients with cirrhosis of the liver.

Appendix

(In collaboration with Chr. GRAM, M. Sc.)

The galactose plasma concentration-time curve following a single injection may be divided into 3 parts (TYGSTRUP and WINKLER 1954). (Fig. 5.)

If the disappearance of galactose from the plasma during the test period is exclusively determined by hepatic elimination and renal excretion, and if hepatic elimination rate and renal clearance are presumed to be constant in this interval, the curve is described by the equation

$$\frac{dc}{dt} = -\frac{GE}{V} - \frac{Cl \times c}{V} \qquad (1)$$

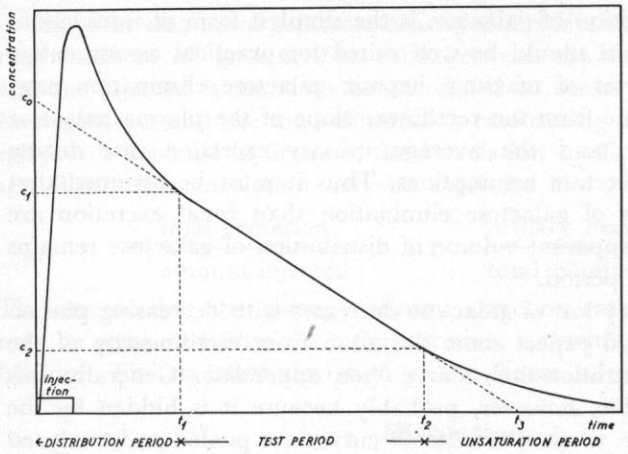


Fig. 5. Theoretical plasma galactose concentration-time curve following a single intravenous injection of 500 mg/kg body weight (TYGSTRUP and WINKLER 1954). The broken lines show the extrapolations and projections used for the calculations.

where c is concentration, t is time, GE is maximal hepatic galactose elimination rate, Cl is urinary clearance, and V is volume of distribution. Therefore

$$c = \left(c' + \frac{GE}{Cl}\right) \times e^{-\frac{Cl}{V} \times (t - t')} - \frac{GE}{Cl} \qquad (2)$$

where e is the base of natural logarithmus, and c' and t' are corresponding values of concentration and time, *e. g.* c_1 and t_1 in Fig. 5.

The shape of the curve decribed by equation (2) depends on the relative magnitude of the constants. In order to evaluate the deviation of this curve from rectilinearity in normals and cirrhotics, the curves of Fig. 6 were constructed. The constants used for the special solutions depicted in the figure were obtained by calculating average values of c_1, GE, Cl, and V from the material of group A and group C in Table I (curve I and curve II, respectively). Curve I therefore may be taken to represent an "average" normal subject, and curve II an "average" patient with cirrhosis of the liver. A number of concentrations were calculated for values of t greater than 20 min and c greater than 400 mg/l, *i. e.* covering the usual test period. These concentrations, with the standard deviation of the analysis, are plotted in Fig. 6. From each series of points the best fitting straight line was computed by regression.

Fig. 6 shows that in both cases examined the deviation of the straight line from the calculated concentrations is smaller than the standard deviation of the analytical procedure. For practical purposes it therefore is warranted to regard the total elimination rate in this period as constant.

Making use of this approximation equation (1) may be written

$$g = -\frac{GE}{V} - \frac{U_2}{(t_2 - t_1) \times V} \qquad (3)$$

where g is the slope of the best fitting straight line during the test period, and U_2 is the amount excreted in the urine in this period.

The volume of distribution (V) may be calculated from the concentration after distribution has taken place (c_1) and the amount present in the body at that time (t_1) (*cf.* Fig. 5).

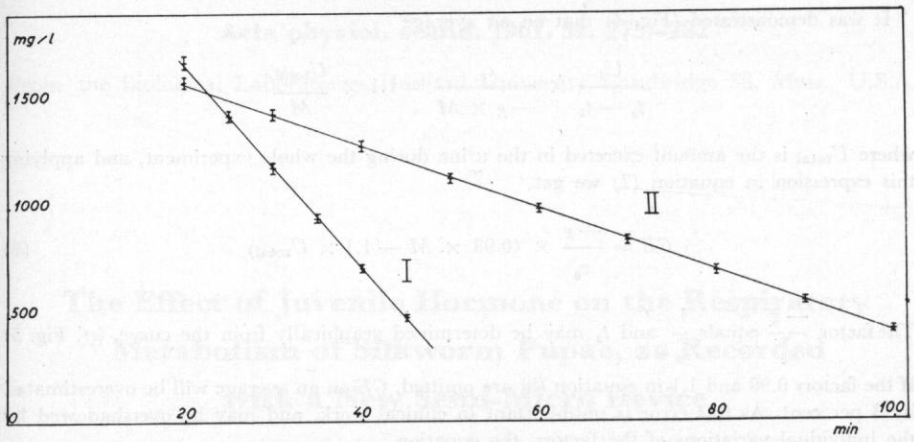


Fig. 6. The influence of the kinetics of urinary excretion of galactose on the plasma concentration-time curve in an "average" normal subject (curve I) and in an "average" patient with cirrhosis (curve II). The concentrations shown (+) were calculated from equation (2), assuming the urinary excretion rate to be proportional to the plasma concentration, and the straight line was calculated by regression from the same concentrations, assuming the urinary excretion rate to be constant according to equation (3). The deviation of the straight line from the calculated concentrations is seen to be smaller than the standard deviation of the analytical procedure, depicted by horizontal bars. (S. D. = 0.01 × c + 10 mg/l.)

$$V = \frac{M - GE \times t_1 - U_1}{c_1} \tag{4}$$

where M is the amount injected, and U_1 is the amount excreted in the urine in the distribution period. Eliminating GE from equation (3) and (4) we get

$$V = \frac{M - \left(U_1 - \frac{U_2}{t_2 - t_1} \times t_1\right)}{c_1 - g \times t_1} \tag{5}$$

where the denominator $c_1 - g \times t_1$ equals c_0 (*cf.* Fig. 5).

From the material of Table I it was calculated that on an average

$$U_1 - \frac{U_2}{t_2 - t_1} \times t_1 = 670 \text{ mg or } 0.02 \times M,$$

and applying this value equation (5) may be written

$$V = \frac{0.98 \times M}{c_0} \tag{6}$$

From equations (3) and (6) it appears that

$$GE = -g \times \frac{0.98 \times M}{c_0} - \frac{U_2}{t_2 - t_1} \tag{7}$$

It was demonstrated (Fig. 4) that on an average

$$\frac{U_2}{t_2 - t_1} \times \frac{c_0}{-g \times M} = 1.1 \times \frac{U_{\text{total}}}{M}$$

where U_{total} is the amount excreted in the urine during the whole experiment, and applying this expression in equation (7) we get

$$GE = \frac{-g}{c_0} \times (0.98 \times M - 1.1 \times U_{\text{total}}) \tag{8}$$

The factor $\frac{-g}{c_0}$ equals $\frac{1}{t_3}$ and t_3 may be determined graphically from the curve. (*cf.* Fig. 5)

If the factors 0.98 and 1.1 in equation (8) are omitted, *GE* on an average will be overestimated by 3 per cent. As this error is unimportant in clinical work, and may be overshadowed by the individual variations of the factors, the equation

$$GE = \frac{M - U_{\text{total}}}{t_3} \tag{9}$$

may be preferred for this purpose.

References

Bollman, J. L., F. C. Mann and M. H. Power, The utilization of galactose following complete removal of the liver. *Amer. J. Physiol.* 1935. *111.* 483—491.

Dominguez, R. and E. Pomerene, Kinetics of the disappearance of galactose from the blood after a rapid intravenous injection. *Amer. J. Physiol.* 1944. *141.* 368—381.

Gammeltoft, A. and K. Kjerulf-Jensen, The mechanism of renal excretion of fructose and galactose in rabbit, cat, dog and man. *Acta physiol. scand.* 1943. *6.* 368—384.

Önen, K. H., Renal haemodynamics in hepatic cirrhosis. *Lancet* 1960. *i.* 203—204.

Tygstrup, N., K. Winkler, E. Lund and H. C. Engell, A clinical method for determination of plasma galactose in tolerance tests. *Scand. J. clin. Lab. Invest.* 1954. *6.* 43—48.

Tygstrup, N. and K. Winkler, Kinetics of galactose elimination. *Acta physiol. scand.* 1954. *32.* 354—362.

Tygstrup, N. and K. Winkler, Galactose blood clearance as a measure of hepatic blood flow. *Clin. Sci.* 1958. *17.* 1—9.

Waldstein, S. S., L. A. Greenburg, A. D. Biggs and L. Corn, Demonstration of hepatic maximum removal capacity (L_m) for galactose in humans. *J. Lab. clin. Med.* 1960, *55.* 462—475.