KINETICS OF THE DISAPPEARANCE OF GALACTOSE FROM THE PLASMA AFTER A RAPID INTRAVENOUS INJECTION

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In studies of the intermediary metabolism of galactose the presence of a simultaneous galactosuria has been an obstacle to the interpretation of several experimental results (Deuel, 1936). In order to avoid this obstacle investigators have either used an animal species with kidneys slightly permeable to galactose or resorted to a nephrectomized animal. The results of these procedures, however, cannot be immediately extended either to other animal species or to the intact animal.

There is need, therefore, for a method of calculating 1, the amount of galactose present in the body at any given time during the experiment, and 2, the rate at which galactose is removed from the plasma by processes other than excretion. The first depends on the existence of a volume of distribution, that is, a fixed volume of body fluids which hold galactose in equilibrium with the plasma (Dominguez, 1934). The second requires an analysis of the changes in the concentration of galactose in the plasma. Such an analysis incidentally determines the volume of distribution.

In general, the equation of the rate of change of the concentration in the plasma is quite complex, for it contains both the rate at which the substance enters the plasma and the rate at which the substance leaves the plasma. This complex situation can be simplified by injecting the substance as rapidly as possible into the vein. Under these conditions, both the time taken by the injection and the phenomena occurring in this interval can be neglected. The change in the concentration of the substance in the plasma will then reduce simply to a fall in concentration, and the equation of the rate of fall in concentration will contain only the rates of the processes that produce this fall.

In the particular case of galactose this fall in concentration is brought about by 1, diffusion from the plasma into other body fluids; 2, excretion, and 3, chemical transformation. Diffusion, a purely physical phenomenon, needs no special comment. In regard to excretion, only renal excretion need be considered because galactose is not excreted in significant amounts by channels other than the kidney. As to the chemical transformation, whether oxidation, polymerization, direct conversion into glucose, etc., only the rate of the first step of this transformation is required. This rate, that is, the rate at which galactose is removed from the plasma by or for conversion, will be referred to as the rate of utilization.

It is the purpose of this paper to analyze the fall in the concentration of galactose in the plasma into its different components and, from data on both excretion and plasma concentration, to determine the volume of distribution, the

rate of utilization, and the relation of the rates of excretion and utilization to the plasma concentration.

It is hoped that the results of this study will be of use to investigators of galactose metabolism. Moreover, because of the generality of the reasoning on which the analysis of the plasma curve is based, the method given here is expected to be applicable to other substances and to other processes.

EXPERIMENTAL PROCEDURE. The experiments were carried out in five female mongrel dogs weighing from 11.4 to 22.7 kgm. The routine diet of these dogs consisted of Purina Dog Chow and occasional bones. No food was given to the animals the day before the experiment and during the experiment. In order to ensure adequate diuresis, water was available to them at all times and, in most instances, was given by stomach tube during the experiment.

A weighed amount of *d*-galactose (C. P., Pfanstiehl), varying between 3.56 and 65.46 grams, was dissolved in freshly distilled water and the solution sterilized by filtering through a Seitz filter. The solution was injected into a vein as rapidly as possible. The quantity of fluid injected varied between 19.9 and 217 cc. and the concentration of the solution between 15.4 and 47.8 grams of galactose per 100 cc. of solution. In 20 of the 23 experiments the time consumed by the injection varied between $\frac{1}{2}$ and 5 minutes; in one experiment it took 7 minutes (2 injections); in another $9\frac{1}{2}$ minutes (2 injections); and in still another 19 minutes (2 injections). In most experiments a jugular vein was used for the injection. The only ill effects, observed especially with the larger doses, were tremor of the whole body and flush of the skin and of the mucous membranes. If they occurred at all, both effects appeared during or shortly after the injection but disappeared in twenty minutes or less.

The samples of blood were withdrawn from a jugular vein at varying times after the injection. The times were arranged so that the intervening concentration could be obtained by linear interpolation with an error not larger than the error of the chemical method.

The bladder was catheterized with a no. 14 coude catheter. The urine was collected directly into graduated cylinders and the volume read at room temperature. When the volume of urine was small, the washings of the catheter were also analyzed. The intervals of collection were selected so as to justify the use of the mean rate of excretion in a given interval as the rate of excretion at the middle of the interval.

A number of samples of blood and urine were analyzed before and between the experiments in order to determine the concentration of endogenous reducing substances given by the chemical methods employed.

Chemical methods. The blood was withdrawn in amounts varying from 10 to 20 cc. and was collected in bottles containing approximately 30 mgm. of dried potassium oxalate. The plasma was separated by centrifugation usually within one or two hours after the blood withdrawal. In a few experiments the late blood samples were kept in the refrigerator (6°C.) overnight, before centrifugation, but repeated checks showed no essential change in the concentration of reducing substances of the plasma. For the determination of the reducing substances of

the urine and the non-fermentable reducing substances of the plasma, the method of Folin and Wu (1920) was used, with *d*-galactose standards of appropriate strength. The yeast-fermentable reducing substances of the plasma were removed by the method of Blanco (1928). The Klett Biocolorimeter was used for the color matching. The determinations were made in duplicate, sometimes in triplicate, on samples renumbered by an independent worker. In some of the later experiments the Klett readings were checked with the Evelyn or the Klett-Summerson photoelectric colorimeter.

TABLE 1

Protocol of experiment 5, dog 1

URINE			PLASMA	
Interval of collection (9:30 a.m.–3:43 p.m.)	Volume	Concentration of reducing substances	Time of blood withdrawal	Concentration of non-fermentable reducing substances
	cc.	*mgm./100 cc.*		*mgm./100 cc.*
9:30 - 9:47	219.5	3393	9:51 a.m.	997
9:47 -10:11.5	181.0	4800	10:16	687
10:11.5–10:35.5	114.5	5575	10:39	551
10:35.5–10:57	80.5	5075	11:01	406
10:57 -11:20	53.7	6518	11:23	332
11:20 -11:48.5	41.8	7093	11:51.5	234
11:48.5–12:21	65.0	3755	12:28 p.m.	138
12:21 - 1:13.5	139.0	1411	1:18	63.3
1:13.5- 1:52	23.7	2923	1:56	37.7
1:52 - 2:27	7.0	2285	2:31	14.1
2:27 - 3:11	97.2	116	3:13	13.3
3:11 - 3:43	44.0	75.2	3:46	9.5

Food was withdrawn 24 hours before the experiment. From 9:26.5 to 9:29 a.m., 91.3 cc. of a 36.2 per cent solution of *d*-galactose (Pfanstiehl) was injected into a leg vein, and from 9:35 to 9:36 a.m., 68.2 cc. of the same solution was injected into a jugular vein. Flush and trembling developed in the course of the injections. Trembling had ceased by 9:40, and flush by 9:50 a.m. A small amount of urine was lost accidentally at the first catheterization. At 8:31, 11:25 a.m. and 1:59 p.m. water was given by stomach tube, 400 cc. each time. The animal was fed at 3:20 p.m. when the experiment was practically over. At 8:08 the following morning, the urine voided during the night and washings of the cage floor (290 cc.), together with 35 cc. of catheterized urine, were pooled and analyzed. The concentration of reducing substances in this sample was 209 mgm. per 100 cc. and the rate of excretion in this long interval 0.69 mgm. per minute.

Data. An illustrative protocol is reproduced in table 1. The weights of the dogs on the day of the experiment, the quantities of injected galactose, and the amounts of galactose excreted in the indicated intervals of time are given in table 2. In order to save space, the calculated amount of excreted galactose and the per cent difference between the excreted and calculated amounts are also given in table 2.

The endogenous values of both the non-fermentable reducing substances of the plasma and the reducing substances of the urine are essentially those given

in another paper (Dominguez, Goldblatt and Pomerene, 1937). The mean values for the plasma, expressed in milligrams of galactose per 100 cc., are the following: dog 1, 10.6; dog 2, 13.6; dog 3, 9.2; dog 4, 8.0; and dog 5, 9.6. The

TABLE 2

Quantities injected and amounts excreted

DOG NO.	EXPT. NO.	WEIGHT	QUANTITY INJECTED G	AMOUNTS EXCRETED IN INDICATED TIMES				PER CENT DIFFERENCE $100 \times \frac{E-I}{E}$
				Found		Calculated		
				t	E	t_1	I	
		kgm.	*gm.*	*min.*	*gm.*	*min.*	*gm.*	
1	1	13.9	3.56	98.4	1.034	99.1	1.075	−3.95
	2	13.6	6.82		2.327			
	3	11.3	7.83		2.258			
	4	13.0	10.65	160	4.189	155.5	4.375	−4.45
	5	14.1	57.74	341	38.235	314	40.493	−5.9
	6	13.6	59.71	1505	33.410	∞	33.600	−0.57
	7	22.7	63.79	374	40.144	366.6	40.671	−1.31
2	1	16.6	4.20		0.987			
	2	16.8	10.12	228	3.884	227.4	3.595	+7.45*
	3	16.8	17.62		5.560			
	4	16.3	31.34	284.5	15.884	284.5†	15.193	+4.35
	5	16.8	36.64	356.5	19.858	356.5†	20.087	−1.15
	6	16.6	65.46	421	43.971	431.0	42.960	+2.35
3	1	15.7	13.40	205.5	6.448	182.7	6.448	0
	2	16.3	50.14	304	32.032	284.4	37.515	−19.02*
4	1	11.6	24.98	282	17.464	297.8	18.224	−4.35
	2	11.4	38.42	370	27.656	372.4	29.949	−8.3*
	3	11.3	42.68		30.885			
5	1	15.9	3.96	115	0.839	109.4	0.896	−6.7
	2	12.0	28.68	307	16.218	282.6	17.437	−7.5
	3	17.7	39.14		22.31			
	4	15.4	55.34		38.25			
	5	15.4	55.49		36.67			

* In these experiments there is a large discrepancy between the amount excreted in the first interval of collection and the amount calculated for this interval. If this interval is omitted, the per cent differences become: +0.22 (expt. 2, dog 2), −3.58 (expt. 2, dog 3), and −3.50 (expt. 2, dog 4).

† The times t_1 of experiments 4 and 5, dog 2, are 466 and 472 minutes, respectively. However, the integration cannot be carried to these times because the catheterization of the bladder was stopped at the times given.

mean values for the rate of excretion, in milligrams per minute, are, in the same order: 0.56; 0.58; 0.79; 0.48; and 0.47. In this paper the mean values of the blanks will be treated as constants.

Rate of excretion. If the rates of excretion and the plasma concentrations

are plotted on ordinary co-ordinate paper with the times as abscissae and if the points of each respective set are joined by segments of straight lines, the resulting curves fall regularly from the beginning to the end of the experiment. By graphic linear interpolation the rates of excretion y can be paired with the corresponding plasma concentrations x. If the data (y, x) are now plotted in the ordinary way, the relation between these quantities is, in most instances, approximately linear from the endogenous level up to the highest plasma concentration reached in these experiments. As an example, we have plotted the points obtained from four experiments (fig. 1). The linear relation between the plasma concentration x and the rate of excretion y is evident.

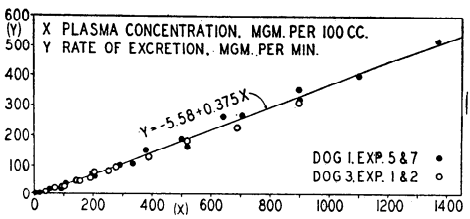

Fig. 1

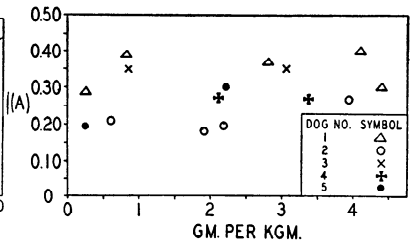

Fig. 2

Fig. 1. Relation of the rate of excretion of reducing substances to the plasma concentration of non-fermentable reducing substances. Forty-two pairs of observations from four experiments on two dogs have been plotted. Below $x = 60$, ten points cannot be seen distinctly because of overlapping. The line was fitted by the method of least squares. The standard error of the slope is 0.00476. This line does not fit well the mean endogenous point of dogs 1 and 3. The line passing through this mean and the centroid of all the other points is $y = -2.95 + 0.366\ x$. However, the difference between the slopes of these two lines is 0.009, and, lying within twice the standard error of the slope of the first line, cannot be considered significantly different.

Fig. 2. Relation of the excretion constant A to the quantity injected per kilogram of body weight. In most of the experiments the excretion constant A (table 3) is independent of the quantity injected. The slight correlation in the experiments on dog 2 and the apparently greater correlation in the experiments on dog 5 are probably due to the effect of diuresis.

Since the endogenous pair is used in the calculation of the slope, we can make this pair the origin of co-ordinates and write the relation between the rate of excretion of galactose η and the plasma concentration of galactose ξ in its simplest form,

$$\eta = A\xi. \tag{1}$$

In this equation, the excretion constant A can be interpreted as the rate of excretion of galactose per unit plasma concentration of galactose.

Strictly speaking, A is constant only when diuresis is constant. However, when water is given in several doses during the experiment, a systematic variation in diuresis is prevented, and very low diureses are not likely to occur. Under these circumstances, a mean value of A is maintained throughout the experiment

and the slope of the line is constant (fig. 1). On the other hand, when water is not given during the experiment (as happened inadvertently in expts. 3, 4 and 5, dog 5), diuresis falls continuously, the value of A diminishes, and the relation between the rate of excretion and the plasma concentration ceases to be linear.

Restricting ourselves to the fifteen experiments in which A is constant, we can see in figure 2 that there is no correlation between the excretion constant and the dose (data of tables 2 and 3).

Equation of the plasma concentration curve. As stated in the introduction, the rate at which galactose disappears from the plasma is equal to the sum of the rates of diffusion, excretion and utilization. After equilibrium between the plasma and the tissues is reached, the rate of disappearance is equal to the sum of only the rates of excretion and utilization. If we call ζ the quantity of galactose present in the body at a given time, η the rate of excretion and u the rate of utilization, we have, after equilibrium,

$$-\frac{d\zeta}{dt} = \eta + u. \tag{2}$$

The quantity ζ is equal to the product of the plasma concentration ξ and the volume of body fluids which hold galactose in equilibrium with the plasma. If we call this volume V, then $\zeta = V\xi$. Since the volume V can be assumed to remain constant during the experiment, equation 2 becomes

$$-V\frac{d\xi}{dt} = \eta + u. \tag{3}$$

Inserting in equation 3 the expression for η from equation 1 we get, finally,

$$-V\frac{d\xi}{dt} = A\xi + u. \tag{4}$$

This equation shows the relation that exists between the curve of the plasma concentration ξ, the slope of this curve $\frac{d\xi}{dt}$, and the rate u at which galactose is removed from the plasma by processes other than excretion.

The rate u can now be determined in two ways: 1, assume a mathematical expression for u and test, after integration of equation 4, how closely the plasma data are satisfied, or 2, assume a mathematical expression for the plasma curve and, after performing the operations indicated in equation 4, compute u at a series of points. The first method was used to give the rate u from the initial value of the plasma concentration down to small values of this concentration—20 mgm. per 100 cc. or less. The second was used to show the trend of the curve u at the low values of the plasma concentration.

The steps in the calculations can be simplified by the following considerations.

In the first place, the plot of log ξ and the time (fig. 3, upper right) shows that, in the beginning, when the difference in concentration between the plasma and the tissues is greatest, there is no initial drop in concentration which is due to

diffusion. In order to determine the early stages of this diffusion it would be necessary to make observations within the first five or ten minutes after the injection. For the purpose of the present study, however, this small interval of time

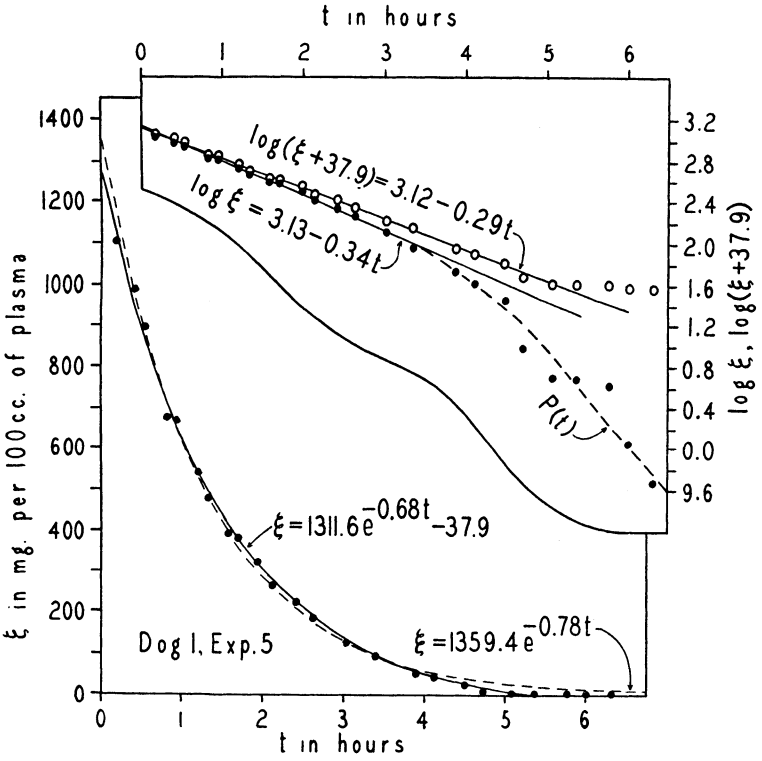

Fig. 3. Time curve of the plasma concentration ξ and rate of excretion η of galactose in experiment 5 on dog 1. The plasma concentrations have been plotted after subtraction of the endogenous blank. The rates of excretion, after subtraction of the blank, have been multiplied by $1/A$ (equation 1) in order to bring them to the plasma level. These two sets of points have been drawn without distinction. The simple exponential curve (broken), which fits the points down to the time $t = 4$, passes systematically above the points beyond $t = 4$. This situation is better demonstrated in the upper right corner of the figure, where the logarithms of the same experimental data have been plotted. The first part of the plot (black circles) follows a straight line down to $t = 3.2$. From $t = 3.2$ to $t = 6.2$ the points follow the broken curve $P(t)$. This curve represents a polynomial of the fourth degree in t, fitted to the mean position of these points. In this experiment, the constant p of equation 6 is equal to 37.9. The straight line of log $(\xi + 37.9)$ and the curve of ξ corresponding to equation 6 (continuous) follow the points down to $t_1 = 5.2$.

can be neglected. Therefore, equation 4 can be assumed to hold from the beginning of the experiment.

In the second place, the same plot of figure 3 shows that the logarithms of ξ do not decrease linearly throughout the experiment. This non-linearity means, of course, that the rate of utilization is not proportional to the plasma concentration, except perhaps in the early part of the experiment. A closer approximation

is obtained by assuming for the rate of utilization a linear function of the plasma concentration, namely,

$$u = a + B\xi. \tag{5}$$

Substituting this expression for u in equation 4 and integrating, we get

$$\log [a + (A + B)\xi] = \log [a + (A + B)\xi_0] - \frac{A + B}{V} \cdot \log e \cdot t,$$

or, more simply,

$$\xi = qe^{-kt} - p, \tag{6}$$

where

$$q = \xi_0 + p, \tag{7}$$

$$k = \frac{A + B}{V}, \tag{8}$$

$$p = \frac{a}{A + B}, \tag{9}$$

$$V = \frac{G}{\xi_0}, \tag{10}$$

and where e is the base of natural logarithms, t the time, and, as before, ξ the plasma concentration and V the volume of distribution. The initial plasma concentration ξ_0 means the concentration at the time $t = 0$. In these experiments, the time zero has been identified with the beginning of the injection. As far as the plasma concentration is concerned, this identification is arbitrary, because during the injection and even for a short time after the injection the concentration of the substance in the plasma varies greatly in different parts of the circulatory system. However, as long as observations are made on excretion, it is better to consider that the experiment begins at the beginning of the injection.

Computations. The constants q, k and p in equation 6 were computed by the method of least squares as modified by Scarborough (1930). The values of the constants are given in table 3.

The degree of approximation obtained was ascertained both by examining the closeness with which the calculated curves follow the data and by comparing the amount actually excreted with the amount calculated. The curves fit the data down to small values of the plasma concentration, below 20 mgm. per 100 cc. An example of the fitting is shown in figure 3. The amounts calculated are given in table 2. Even though the curves were not fitted down to $\xi = 0$, the integration was carried to the time t_1 corresponding to zero plasma concentration ($t_1 = \frac{\log q - \log p}{k \cdot \log e}$) because the error thus committed is negligible. The mean difference between the amount found and the amount computed, expressed in per cent of the former, is -3.46, and the average difference, irrespective of sign,

±5.48. The largest single errors are found in the first fifteen minutes of the experiments (table 2).

The difference between the amount excreted in the time t_1 (table 2) and the amount excreted in 24 hours, expressed in per cent of the former, varies between −5.02 (expt. 1, dog 1) and +2.44 (expt. 2, dog 2), with a mean of −1.21 and an average of ±2.07.

TABLE 3

Numerical values of the constants in the equations of the rate of excretion η, plasma concentration ξ, and rate of utilization u

DOG NO.	EXPT. NO.	$\eta = A\xi$	$\xi = qe^{-kt} - p$				$u = a + B\xi$	
		A	q	k	p	V	a	B
		Excretion constant				Volume of distribution		Utilization constant
		100 × cc. per minute	*mgm. per 100 cc.*	*1/hr.*	*mgm. per 100 cc.*	*liters*	*mgm. per minute*	*100 × cc. per minute*
1	1	0.288	143.26	1.878	6.44	2.60	5.25	0.527
	4	0.390	252.70	0.961	20.98	4.60	15.44	0.346
	5	0.401	1349.56	0.677	37.91	4.53	19.40	0.111
	6*	0.302	1461.05	0.885	0	3.64	0	0.235
	7	0.369	1570.87	0.821	10.42	4.09	5.83	0.190
2	2	0.219	291.57	0.919	8.95	3.58	4.91	0.330
	4	0.181	805.77	0.490	18.03	3.98	5.85	0.144
	5	0.196	864.37	0.416	32.68	4.24	9.61	0.098
	6	0.269	1444.43	0.454	55.39	4.71	19.75	0.087
3	1	0.347	359.84	0.846	27.35	4.03	15.53	0.221
	2	0.349	1388.91	0.636	68.15	3.80	27.41	0.053
4	1	0.272	1057.98	0.884	13.14	2.39	4.63	0.080
	2	0.270	1500.78	0.773	12.37	2.58	4.11	0.063
5	1	0.194	141.38	0.991	23.19	3.35	12.83	0.360
	2	0.325	793.22	0.783	19.85	3.71	9.60	0.159

* In this experiment the value of p cannot be distinguished from zero. Because the plasma data fall as in the other experiments, we believe that this value of p is due to the chance distribution of errors.

In view of these facts we consider equation 5 a satisfactory representation of the rate u, except at low values of the plasma concentration.

We have not attempted to give a mathematical expression to the whole curve of the rate u because of the difficulties encountered in the integration. But we have constructed this rate at low levels of the plasma concentration by fitting a polynomial in the time t to the terminal part of the curve of log ξ (fig. 3, upper right) and, from equation 4, determining u at a number of points. The details of this construction cannot be given here.[1]

[1] The computations used in this paper are quite laborious. For some of the applications, however, the values of the computed quantities can be obtained with sufficient approxima-

RESULTS. The foregoing analysis of the plasma curve answers the problems stated in the introduction.

1. The volume of distribution has been computed (equations 7 and 10). This volume varies between 2.39 and 4.71 liters of water (table 3). The mean value, 3.72 liters, with a standard error of 0.189, is significant. In per cent of the body weight, the volume varies between 18.0 and 35.4, with a mean of 24.9. Also in per cent of the body weight, the mean volume V of each dog is, in the order of table 3, 25.2, 24.8, 24.4, 21.6 and 25.2. In comparison with the large variation in both the quantity injected and the plasma concentration, the variation of the volume V is quite small (table 3). In fact, the coefficient of variation of the volume V is 0.190, a coefficient slightly larger than that of the body weight, 0.183, but considerably smaller than that of the excretion constant, 0.239.

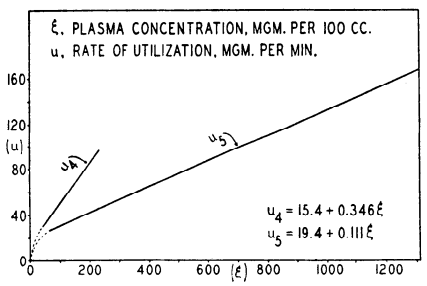

Fig. 4

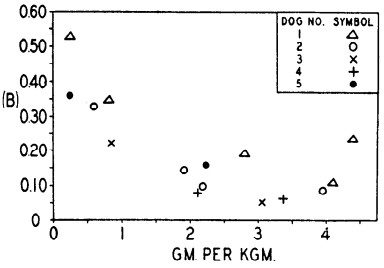

Fig. 5

Fig. 4. Relation of the rate of utilization of galactose to the plasma concentration. Because the lines are computed from experiments in which the plasma concentration falls continuously from the beginning of the experiment, the diagram is to be thought of as representing the change of the rate of utilization from the higher to the lower concentrations, that is, from the right to the left of the figure. The subscripts 4 and 5 in both the equations and the lines refer to the experiments 4 and 5 on dog 1, respectively. The lines u_4 and u_5 were computed by fitting equation 6. The interrupted curve from the left end of the line u_5 to zero was obtained from equation 4 by the construction outlined in the text.

Fig. 5. The utilization constant B depends on the quantity of galactose injected. In each dog, the larger the quantity injected, the smaller B. An exception occurs in experiment 6, dog 1, the experiment with the largest dose.

Therefore, the quantity of galactose present in the body at any time during the experiment can be determined by multiplying the concentration of galactose in the plasma by the volume of distribution.

2. The rate of utilization, that is, the rate at which galactose is removed from the plasma by or for conversion, has been determined in the presence of a simultaneous rate of excretion. From the initial plasma concentration down to

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tion by means of graphic analysis. After smooth curves have been drawn through the points representing the data on plasma concentration and rate of excretion, the quantities required in equation 4 for computing the rate of utilization can be estimated graphically. The only quantity which offers some difficulty is the slope of the tangent $\left(\frac{d\xi}{dt}\right)$ of the plasma curve. Adequate methods for drawing tangents are described in Daniels (1928).

values of this concentration that vary in different experiments, but are usually below 20 mgm. per 100 cc., the rate of utilization diminishes linearly with the plasma concentration (fig. 4). For values below this concentration, the rate of utilization diminishes rapidly and not linearly toward zero (fig. 4). The values of the constants a and B of the linear part (equation 5), computed from equations 7 to 10, are given in table 3.

Contrary to the slope A of the rate of excretion, the slope B of the linear part of the rate of utilization depends on the quantity injected. Thus, in figure 4, the slope of the line u_4 (10.65 grams injected) is three times as great as that of the line u_5 (57.74 grams injected). With the exception of experiment 6, dog 1, the plot of the slope B (table 3) and the dose in grams per kilo of body weight

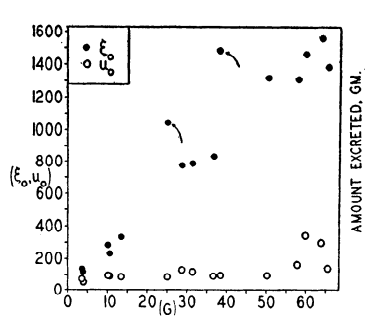

Fig. 6

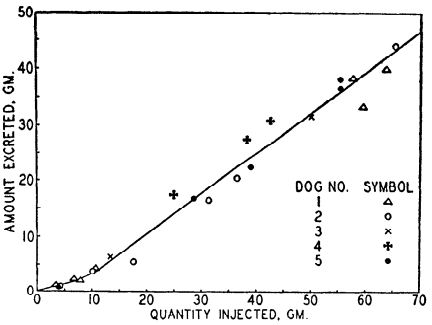

Fig. 7

Fig. 6. The initial plasma concentration ξ_0 is given in milligrams per 100 cc., the initial rate of utilization u_0 in milligrams per minute, and the quantity injected G in grams. In dogs of comparable size, the initial plasma concentration is proportional to the quantity injected (equation 10). The arrows point to the larger initial plasma concentrations of dog 3, the smallest dog. In experiment 6, dog 1 ($G = 59.7$), and in experiment 7, dog 1 ($G = 63.8$), the initial rates of utilization are exceptionally high.

Fig. 7. Relation of the amount of galactose excreted to the quantity injected. The curve following the mean position of the points rises at first with a slope not higher than 0.25, then, from about 4.0 to 10 grams, it bends upward, and, from a dose of about 10 grams up to the largest dose, 65.46, it rises linearly with a slope of 0.72.

(table 2) shows that, in each dog, the larger the quantity injected, the smaller the slope (fig. 5).

Although not established by these experiments, this systematic variation in the slope B points toward the existence of an upper limit to the initial rate of utilization. From the data of tables 2 and 3 it can be shown that the quantity injected, the initial plasma concentration, and the initial rate of excretion vary as 1:18, 1:13 and 1:25, respectively, but the initial rate of utilization varies only as 1:6. It is likely that beyond some dose, larger than any used here, there will be no further rise in the initial rate of utilization (fig. 6).

As a consequence of the effect of the dose on the rate of utilization, the proportion of excreted galactose is not constant. The amount excreted represents 22 to 30 per cent of the quantity given when the latter is less than 4.2 grams and 56

to 72 per cent when the quantity injected is more than 38 grams. This variation is illustrated in figure 7.[2]

DISCUSSION. Although no particular end product of conversion is being considered here, the calculated rate of utilization assigns an upper limit to the rate of conversion as a whole, for, if all the non-excreted galactose is converted into, let us say, glycogen, it is clear that the rate at which glycogen is formed from galactose cannot be greater than the rate at which galactose is removed from the plasma by or for the purpose of glycogenesis. If not all the non-excreted galactose is converted into glycogen, the proportion which is converted can be determined by comparing the excess of glycogen formed in a given time with the integral of the rate of utilization in the same time. By extending these investigations to other sugars, their efficiency for glycogen formation can be assessed.

If figure 4 is completed with the lines of the rates of utilization in all fifteen experiments, it will be seen that the path outlined by the initial values of the rate of utilization lies above the entire set of straight lines. This situation is true for the fall in plasma concentration after a rapid intravenous injection. If, on the other hand, the concentration in the plasma is made to rise slowly, what will be the course followed by the rate of utilization? Will it follow one of the lines already determined or will it follow the upper path outlined by the initial rates of utilization? If the concentration of galactose in the plasma rises at first and then falls, as in *oral* experiments, will the rate of utilization follow an upper path during the rise in concentration and a lower path during the fall in concentration? Until these points are settled for galactose and other glycogen-forming carbohydrates, it cannot be concluded that differences in glycogen formation are due to differences in either their glycogenetic efficiency or their rate of absorption from the intestine.

With respect to the variation in the proportion of excreted galactose, it should be recalled that, under the same experimental conditions, the proportion of excreted xylose is constant. This constancy is due to the fact that the rate of utilization of xylose is proportional to the plasma concentration and that the constant of proportionality is independent of the dose (Dominguez, Goldblatt and Pomerene, 1937). The metabolism of these two sugars is therefore quite different.

Finally, concerning the volume of distribution, there is no doubt that this volume represents a real partition of the body fluids even though the anatomical site of this partition is not specified. From the standpoint of the method used

[2] For the kinetics of elimination following a rapid intravenous injection, Teorell (1937) has proposed an equation identical in form to our equation for xylose (Dominguez, Goldblatt and Pomerene, 1937). Teorell obtains this equation in a theoretical way by assuming 1, Fick's diffusion law for the rate of diffusion into the tissues; 2, Fick's diffusion law for the rate of excretion, and 3, a chemical reaction of the first order for the rate of "inactivation". The second assumption is inadmissible. The third assumption is not sufficiently general. As shown here, the part of galactose which disappears from the plasma for conversion does not disappear at the rate of a monomolecular reaction.

here, this volume can be looked upon as a characteristic of any substance under investigation. This volume might be expected to be different with different substances. On the contrary, many substances, as dissimilar in chemical structure as in physiological properties, distribute themselves in volumes of quite comparable magnitude. Thus, sulfate, sucrose, chloride, xylose and thiocyanate—to mention only the substances recently compiled by Adolph (1943)—are distributed in volumes which do not differ much from the volume of distribution of galactose. Even creatinine, a substance which occupies eventually a volume two and a half times as large as that of galactose, enters first, and almost immediately, into a volume about equal to that of galactose (Dominguez, Goldblatt and Pomerene, 1935). These facts should be taken into consideration by physiologists interested in the anatomical interpretation of these volumes.

SUMMARY AND CONCLUSIONS

1. The disappearance of galactose from the plasma after a rapid intravenous injection has been studied in five dogs.

2. The data consist of the rate of excretion at consecutive intervals, the plasma concentration at selected times, and the endogenous blanks.

3. During and shortly after the injection, galactose diffuses into a volume of body fluids equivalent to about 25 per cent of the body weight. The concentration of galactose in this volume is in equilibrium with that of the plasma virtually from the beginning of the experiment. Hence, the amount of galactose present in the body at any time during the experiment can be calculated by multiplying the plasma concentration by the volume of distribution.

4. The subsequent fall in the concentration of galactose in the plasma is due only to renal excretion and chemical transformation. Diffusion takes no part in this fall.

5. The part excreted is lost at a rate (rate of excretion) which is proportional to the plasma concentration. The constant of proportionality is independent of the quantity injected.

6. The part transformed is removed from the plasma at a rate (rate of utilization) which is not proportional but is linearly related to the plasma concentration. The linear relation holds from the initial plasma concentration to concentrations of about 20 mgm. per 100 cc. or less.

7. The slope of the linear part of the rate of utilization depends on the quantity injected. In general, the larger the dose, the smaller the slope.

8. The initial rate of utilization does not rise in proportion to the quantity injected. To an 18-fold variation in the latter there corresponds only a 6-fold variation in the former. It is probable that the initial rate of utilization reaches a stationary value.

9. The plasma concentration can be represented by the equation

$$\xi = qe^{-kt} - p,$$

where ξ is the plasma concentration, t the time, and e the base of natural logarithms. The constants q, k, and p are defined in terms of (1) the volume

of distribution and (2) the constants relating the rates of excretion and utilization to the plasma concentration.

10. The equation of the plasma concentration is checked both by fitting the data and by calculating the amount excreted. The mean difference between the amount actually excreted and the amount calculated, expressed in per cent of the former, is about 3.5. The largest single error is found in the amount excreted in the first fifteen minutes after the injection.

11. From the relation of the rate of utilization to the dose it follows that the amount excreted is not proportional to the quantity injected. The amount excreted is less than 30 per cent of the quantity injected when the latter is less than 4.2 grams, and 56 to 72 per cent when the quantity injected is more than 38 grams.

12. The significance of the calculated rate of utilization in studies of the intermediary metabolism of carbohydrates is discussed.

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