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Determination of the Hepatic Galactose Elimination Capacity after a Single Intravenous Injection in Man

The reproducibility and the influence of uneven distribution

By

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Abstract

TYGSTRUP, N. *Determination of the hepatic galactose elimination capacity after a single intravenous injection in man. The reproducibility and the influence of uneven distribution.* Acta physiol. scand. 1963. *58*. 162—172. — The hepatic galactose elimination capacity was determined from the disappearance curve in blood after a single intravenous injection. Using conventional principles for the calculation the results in repeated experiments were reproducible within 10 per cent. Comparison of the results obtained by this method with those of an infusion method showed that the latter were consistently smaller. This difference, amounting to an average of 15 per cent, might be caused by uneven distribution of galactose between its intra- and extravascular volume of distribution. From theoretical considerations it was deduced that the uneven distribution might be corrected for by parallel displacement of the arterial concentration curve along the time axis. Experiments with continuous infusions at different rates indicated that on the average the curve of the mean concentrations in the body was delayed 7 min in relation to the arterial curve. When the single injection experiments were corrected for this delay, the difference between the results of single injection and infusion experiments disappeared.

The elimination rate of galactose by the liver is assumed to be independent of the concentration in the blood at concentration levels which are easily obtained in clinical galactose tolerance tests (TYGSTRUP and WINKLER 1954, WALDSTEIN *et al.* 1960). Under these conditions the galactose elimination rate may be of value as a liver function test.

When the hepatic galactose elimination rate is calculated from the disappearance curve in the blood after a single intravenous injection, the result may be influenced by the redistribution of galactose in the body and by the extrahepatic elimination. The renal excretion of galactose can be corrected for approximately (TYGSTRUP 1961). The aim of the present work was to study the reproducibility and to assess the effect of uneven distribution by comparison of experiments with single injection and infusion of galactose.

Methods

Experimental procedures

Most of the subjects studied had no evidence of liver disease, but some patients with cirrhosis of the liver were included for comparison. The experiments were performed in the morning, while the subjects were still lying in their beds. They were kept fasting for 15 hours, but were allowed to drink moderate amounts of water. No premedication was given, Galactose (MERCK, c. p.) was injected intravenously in aqueous solution, sterilized by filtration.

In experiments with *single injection* 100 ml of the solution was injected intravenously at a constant rate in the course of 6 min. Twenty minutes after the injection blood samples were drawn from a brachial or femoral artery, in subjects with normal liver function for 24 min at intervals of three minutes, and in patients with reduced elimination of galactose for 50 min at intervals of five minutes. The urinary excretion of galactose was determined in a sample voided some hours after the end of the blood sampling period.

In experiments with *continuous infusion* the solution was administered by calibrated, motor-driven syringes. The volume infused was between 0.5 and 2.5 ml per minute, the variation being less than 0.5 per cent. Arterial blood samples were drawn 20 to 30 min after beginning of the infusion, and at least 4, on the average 6, samples were taken at regular intervals during a period lasting for 20 to 60 min. With intervals of 15 to 30 min urine was collected through a bladder catheter.

The concentrations of galactose in plasma, urine, and the solutions injected were determined as described by TYGSTRUP *et al.* (1954). Plasma concentrations were converted to concentrations in plasma water by multiplication with the factor 1.05.

Calculations

Only plasma samples in which the concentration of galactose exceeded 500 mg/l were used for the calculations, since the hepatic galactose elimination rate usually falls at lower concentration levels (TYGSTRUP and WINKLER 1958). Only concentration-time curves which appeared rectilinear were used, and their slopes were calculated by regression.

In the single injection experiments the hepatic galactose elimination capacity (GE) and volume of distribution (V) were calculated by the equations given by TYGSTRUP (1961):

$$GE = (M - U_{\text{total}}) \,/\, t_{c\,=\,0}; \tag{1}$$

$$V = M/c_{t\,=\,0}; \tag{2}$$

where

M = the amount injected,

U_{total} = the amount recovered in the urine,

Table I. Reproducibility of the results in repeated experiments with single injections of galactose (500 mg per kg body weight)

a. Two successive determinations (A and B) on different days in 11 normal subjects and two patients with cirrhosis of the liver, 10 males and 3 females, age 16 to 66 years, body weight 48 to 82 kg.

		g (mg/l/min)	$c_{t=0}$ (mg/l)	$t_{c=0}$ (min)	V (l)	GE (mg/min)
Exper. A	Mean	42.0	2,470	61.0	13.8	505
$\frac{A-B}{A}$ 100	Mean	−1%	−1%	+ 1%	+ 3%	0%
	S. D.	8.3	6.3	3.3	6.1	6.9

b. Two successive determinations (A and B) on the same day in 5 normal subjects and two patients with cirrhosis of the liver, 4 males and 3 females, age 18 to 60 years, body weight 52 to 69 kg. The second injection was given 32 to 122 minutes after the first one, the residual concentration (19 to 1,110 mg/l) was subtracted from $c_{t=0}$ of experiment B.

		g (mg/l/min)	$c_{t=0}$ (mg/l)	$t_{c=0}$ (min)	V (l)	GE (mg/min)
Exper. A	Mean	44.5	2,450	59.5	13.3	512
$\frac{A-B}{A}$ 100	Mean	+ 5%	+ 3%	−1%	−3%	0%
	S. D.	13.3	11.3	8.2	7.8	7.7

c. Seven successive determinations on different days in the course of two weeks in one normal male, age 26 years, body weight 82 kg.

	g (mg/l/min)	$c_{t=0}$ (mg/l)	$t_{c=0}$ (min)	V (l)	GE (mg/min)
Mean	44.0	2,520	62.1	14.8	520
S. D.	7.44	336	3.53	1.83	42
Coeff. of variation.....	16.9%	13.3%	5.7%	12.4%	8.1%

Explanation of symbols: g = slope of the rectilinear part of the elimination curve in plasma, $c_{t=0}$ = the extrapolated concentration at zero time, $t_{c=0}$ = the extrapolated time at zero concentration, V = the volume of distribution, GE = the hepatic galactose elimination capacity.

$t_{c=0}$ = the extrapolated time at zero concentration of the rectilinear part of the elimination curve, and

$c_{t=0}$ = the extrapolated concentration at zero time.

From these equations *GE* and *V* are calculated on the average three and two per cent too high owing to incomplete correction for urinary loss.

Table II. Comparison between single injections and infusions

	Age (years)	Body weight (kg)	Single injection			Infusion		
			−g (mg/l/min)	V (l)	GE (mg/min)	I−U (mg/min)	−g (mg/l/min)	f
J. H.	17	73	−48.5	14.9	644	954	+ 31.8	0.86
I. R.	28	70	−55.2	13.5	642	585	− 0.9	0.91
S. C.	37	76	−50.5	13.1	590	603	+ 2.3	0.97
V. G.	47	65	−56.7	11.9	582	571	+ 4.4	0.90
B. E.	27	68	−51.1	12.7	574	558	+ 6.2	0.86
G. N.	42	55	−52.9	11.0	533	492	+ 9.5	0.77
Å. A.	39	65	−50.6	11.4	477	443	− 2.2	0.98
A. L.	48	65	−30.2	15.6	430	300	− 9.0	1.04
						296	− 10.7	1.12
G. P.	38	49	−26.5	15.2	381	551	+ 21.1	0.79
M. P.*	18	58	−36.6	11.0	354	265	+ 2.2	0.70
P. R.*	42	86	−16.7	19.6	251	216	+ 0.4	0.84

Initials marked with * designate patients with cirrhosis of the liver.
For explanation of g, V, and GE: see legend of Tab. I.
(Note: The slope of a falling curve is considered to be positive.)
I−U = infusion rate minus urinary excretion rate. $f = (I-U)/(-g \cdot V_{single} + GE_{single})$.

In infusion experiments with two infusions at different rates in the same subject, *GE* and *V* were calculated by

$$GE = \frac{g_B (I_A - U_A) - g_A (I_B - U_B)}{g_B - g_A} \quad (3)$$

$$V = \frac{(I_A - U_A) - (I_B - U_B)}{g_B - g_A} \quad (4)$$

where

g_A and g_B = the slopes of the arterial time-concentration curves in experiments *A* and *B*, (the slope of a falling curve is defined as positive)

I_A and I_B = the infusion rates, and

U_A and U_B = the mean urinary excretion rates in the intervals studied.

Equations (3) and (4) are based on the "series increment method" of Lewis (1950) and imply that *GE* and *V* are identical during different infusion experiments in the same subject, and that $I - U$ can be regarded as a constant in the interval used for determination of the slope. When more than two infusion experiments were performed in the same subject, *GE* and *V* were calculated by regression according to the same principle.

Results

In Table I are given the results of double determinations performed at an interval of one to 7 days (a), and on the same day (b). In (c) is shown the results from 7 determinations in one normal subject performed in the course of two weeks. It appears that in all three series the variation of the hepatic galactose

Table III. Comparison between single injections and infusions with different slopes

	Age (yrs)	Body weight (kg)	Single injection			Infusion			"Series increment"	
			−g (mg/l/min)	V (l)	GE (mg/min)	I—U (mg/min)	−g (mg/l/min)	f	V (l)	GE (mg/min)
E. J.	25	91	− 50.6	14.2	609	973	+ 37.0	0.86	12.3	509
						618	+ 10.6	0.81	(87%)	(84%)
						292	− 18.4	0.84		
H. I.	54	79	− 44.9	14.1	596	683	+ 16.1	0.83	11.4	499
						−13	− 45.1	−	(81%)	(84%)
V. C.	36	52	− 44.7	13.6	563	753	+ 37.3	0.70	8.9	420
						−67	− 54.6		(65%)	(75%)
B. A.	19	70	− 35.4	16.6	512	547	+ 7.0	0.87	12.9	456
						219	− 18.5	1.07	(78%)	(89%)
B. F.	43	45	− 56.9	10.1	505	649	+ 22.5	0.89	10.3	435
						369	− 9.5	0.90	(102%)	(86%)
						−83	− 48.6	−		
S. J.	23	62	− 39.0	12.1	407	431	+ 11.7	0.79	10.3	330
						267	− 9.5	0.91	(85%)	(81%)
						−56	− 36.1	−		
E. P.	30	62	− 30.8	14.3	392	554	+ 15.9	0.90	14.0	332
						298	− 2.4	0.84	(98%)	(85%)
P. N.	44	59	− 33.9	12.8	367	1,068	+ 78.6	0.78	9.7	249
						541	+ 40.3	0.61	(76%)	(68%)
						272	− 3.4	0.84		
						−59	− 32.0	−		
K. B.	55	59	− 31.7	12.9	341	374	+ 8.2	0.84	10.7	295
						186	− 11.8	0.98	(83%)	(87%)
						−49	− 31.5	−		
M. R.*	45	44	− 23.6	13.5	302	426	+ 18.9	0.77	8.3	270
						245	− 3.0	0.94	(61%)	(89%)
K. H.*	50	75	− 14.9	18.0	252	309	+ 9.8	0.72	13.5	177
						203	− 2.0	0.71	(75%)	(70%)

For explanation of symbols: see legend of Tab. I and II.

elimination capacity is about 10 per cent. The average difference between the double determinations in (a) and (b) is not statistically significant. In normal subjects and patients with cirrhosis of the liver there was no difference as to reproducibility, therefore they have been dealt with together in the tables.

The data from the experiments with single injections and infusions in the same subjects are given in Tables II and III, the latter including experiments with infusions at different rates.

In 6 experiments of Table III (characterized by negative values of I—U) the post-infusion slope on the average was one per cent (S.E. 6 per cent) smaller than the slope following a single injection.

In 10 experiments (6 from Table II and 4 from Table III) an approximately constant arterial concentration, i. e. between — 5 and + 5 mg/l/min, was obtained by infusion. The slope was on the average + 0.1 mg/l/min, the infusion rate minus urinary excretion rate 370 mg/min, and the hepatic galactose elimination capacity, calculated from the single injection experiments, 422 mg/min. Thus the true hepatic galactose elimination capacity is on the average about 88 per cent of the value calculated from a single injection experiment.

In subjects with two or more infusion experiments (Table III) the hepatic galactose elimination capacity calculated by the "series increment method" was found to be on the average 82 per cent of that obtained in the single injection experiments in the same patients. Similarly the volume of distribution was found to be 81 per cent.

This difference between the results of the single injection and the infusion experiments may be expressed by the factor $f_{GE} = GE_{\text{infusion}}/GE_{\text{single inj.}}$; and $f_V = V_{\text{infusion}}/V_{\text{single inj.}}$. The results obtained in Table III indicate that f_{GE} approximately equals f_V, and a common factor, f, can be calculated in each infusion experiment by

$$I - U = f(-gV_{\text{single inj.}} + GE_{\text{single inj.}}) \qquad (5)$$

The factor f calculated from all the infusion experiments of Tables II and III (except those where $I = 0$, in which the experimental error will have too great influence) and the corresponding single injection experiments is on the average 0.86 (S.D. 0.11, S.E. 0.019). Thus the hepatic galactose elimination capacity and the volume of distribution determined in the infusion experiments is on the average 86 per cent of that calculated from the single injection experiments. This value is not significantly different from that obtained by the "approximately constant concentration method" or by the "series increment method", but significantly different from 100 per cent ($p < 0.001$).

Discussion

It is conceivable that the difference between the elimination rates calculated from single injection and infusion experiments may be related to uneven distribution of galactose. When galactose is injected into an eviscerated animal from which no removal takes place, galactose will leave the intravascular compartment for about two hours (Levine *et al.* 1950). In the intact organism galactose moves from intra- to extravascular compartments during and for a short period following an intravenous injection. As the elimination by chemical transformation in the liver or by excretion in the kidneys takes place from the blood, the amount stored in the extravascular compartment during the initial period must re-enter the blood stream.

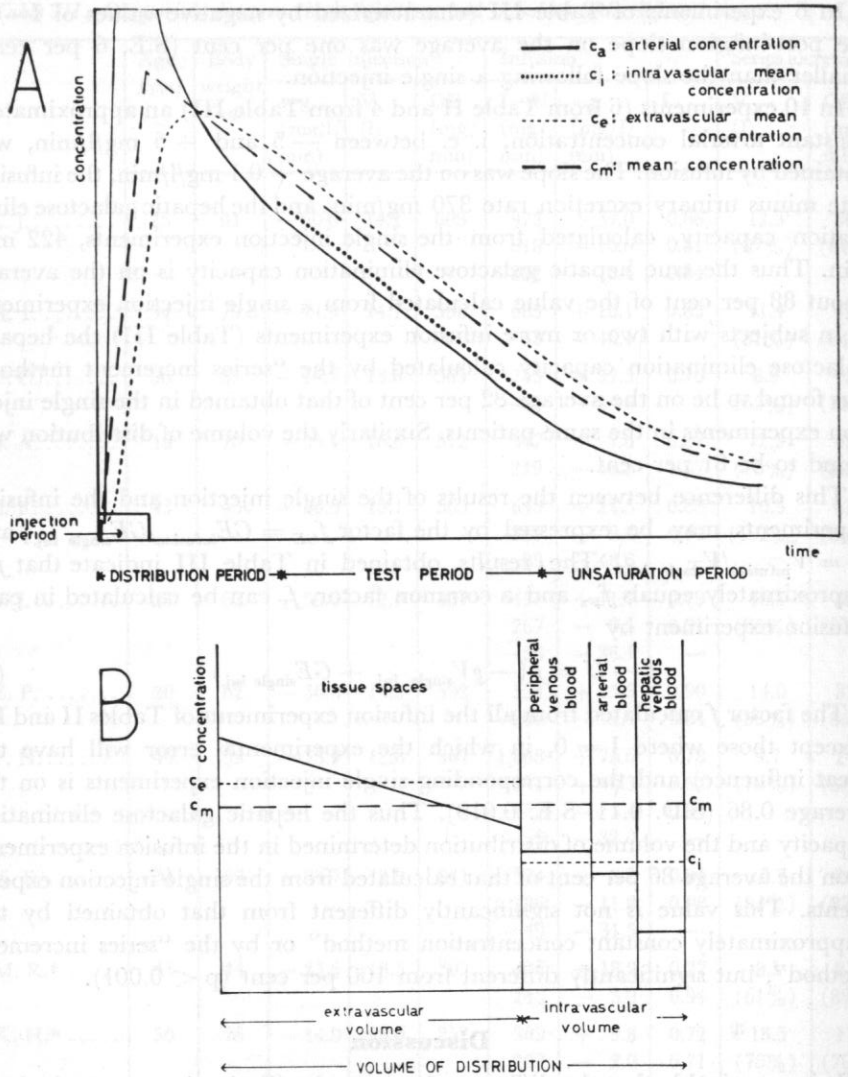

Fig. 1. A hypothetical single injection experiment with the concentrations in different compartments according to the model considered in the appendix. A. Time-concentration curves. B. "Cross section" of A at a given time during the test period.

Fig. 1 shows a hypothetical distribution of galactose during a single injection experiment when it is assumed that the elimination rate and the volume of distribution are constant. In this model (see appendix) uneven distribution results in parallel displacement of the arterial concentration curve relative to the curve of the mean concentrations in the system, the horizontal distance

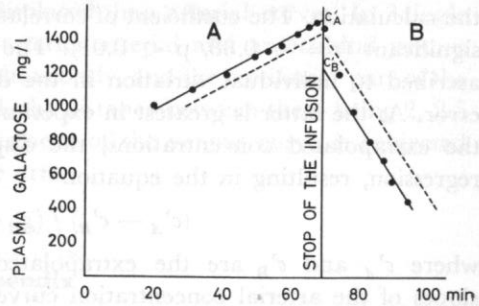

Fig. 2. Plasma galactose concentration curves during (A) and following (B) a constant intravenous infusion. The lines are drawn by rectilinear regression from the points situated at an approximately straight line, and extrapolated to the point at the time axis, where the infusion was stopped (c'_A and c'_B). The broken lines represent the theoretical mean concentrations in the volume of distribution. They are drawn parallel to and the same horizontal distance from the respective regression lines and intersect at the moment the infusion was stopped.

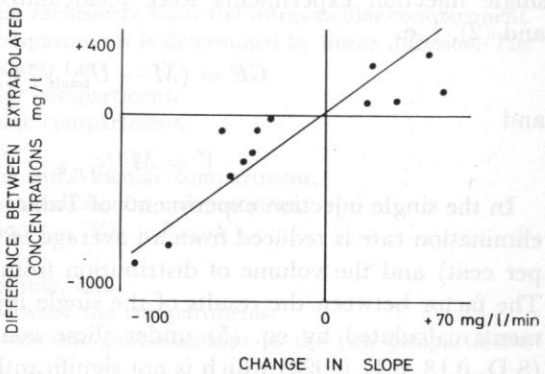

Fig. 3. Relation of the difference between the extrapolated concentrations of a rising and a falling plasma galactose concentration curve and the difference in slope of these curves. This ratio indicates the horizontal distance between the arterial and the theoretical mean concentration curves. The average ratio, calculated by rectilinear regression, was 7 min.

between the two curves being independent of their slope. In infusion experiments the results calculated from the arterial curve (eq. (3) and (4)) and the mean concentration curve will be identical. The extrapolated values of the arterial curve ($c_{t=0}$ and $t_{c=0}$), used for the calculation in the single injection experiments (eq. (1) and (2)) will be smaller than those of the mean concentration curve. Thus single injection and infusion experiments will give different figures for the hepatic galactose elimination capacity and volume of distribution, depending on the degree of displacement of the arterial curve.

The displacement of the arterial concentration curves in the organism may be assessed by extrapolation of the arterial curves in experiments in which the rate of infusion is changed. An experiment of this type is shown in Fig. 2. The displacement is calculated as the ratio of the difference between the slopes of the rectilinear part of the curves, or

$$K = (c'_B - c'_A) \,/\, (g_A - g_B);$$

(see appendix).

In 13 of the experiments shown in Table III the displacement could be calculated in this way. Fig. 3 depicts the relation between the data entering

the calculation. The coefficient of correlation between the values is statistically significant ($r = +0.88$, $p < 0.01$). The scatter of the points may partly be ascribed to individual variation in the displacement, partly to experimental error. As the latter is greatest in experiments with a small difference between the extrapolated concentrations, the displacement was calculated by linear regression, resulting in the equation

$$(c'_A - c'_B) \,/\, (g_B - g_A) = 7,$$

where c'_A and c'_B are the extrapolated concentrations, and g_A and g_B the slopes of the arterial concentration curves. Taking the mean value 7 min to be the parallel displacement of the arterial curve in all cases, the results of the single injection experiments were recalculated by modifications of eq. (1) and (2). i. e.

$$GE = (M - U_{\text{total}}) \,/\, t_{c=0} + 7)$$

and

$$V = M/\,(c_{t=0} - 7\ \text{g})$$

In the single injection experiments of Tables II and III the hepatic galactose elimination rate is reduced from an average of 468 mg/min to 410 mg/min (87 per cent) and the volume of distribution from 13.7 l to 12.2 l (88 per cent). The factor between the results of the single injection and the infusion experiments calculated by eq. (5) under these conditions is on the average 0.97 (S.D. 0.13, S.E. 0.023) which is not significantly different from 1.00. Thus the correction eliminates the difference between the two types of experiments. Better agreement between single injection and infusion experiments cannot be expected with the present method, as the coefficient of variation of the factor is of the same order of magnitude as that found in repeated single injection experiments (cf. Table I).

It may be questioned whether the assumption of a constant extravascular volume of distribution (see Fig. 1 and appendix) is justified. A continuous loss of galactose possibly takes place to compartments which equilibrate slowly, such as poorly vascularized areas (e. g. tendons (KRUHØFFER 1946)) or compartments with membranes less permeable to galactose (certain intracellular spaces (HUYCKE and KRUHØFFER 1955)). This loss cannot be measured from the present experiments, but is probably unimportant, since identical slopes were found after two single injections with short interval (Table I b) and after a single injection and the interruption of an infusion with rising concentrations (Table III, experiments with negative values of $I - U$).

The distribution of galactose in the body after a single intravenous injection must be very complex, being dependent on many uncontrollable factors as differences in the perfusion, the composition, and the permeability of various organs. The correction obtained by parallel displacement of the concentration curve in the blood therefore is an approximation. Concentration curves in peripheral venous blood will be less displaced than arterial curves. In 3 single injection experiments of the present series arterial and antecubital venous concentrations were determined simultaneously, and the rectilinear part of the two curves were found to be parallel, the distance between them being 2, 2.5 and 3.5 min. Presumably the displacement of the venous curve is subjected to variations in the blood flow of the arm.

Appendix

Consider a model with the following properties: It consists of two compartments, corresponding to the intra- and the extravascular volumes of distribution of galactose. Injection and elimination take place exclusively from the intravascular compartment. The exchange between the two compartments is determined by linear diffusion. The volume of the compartments is constant. Let

V_i = the volume of the intravascular compartment,
V_e = the volume of the extravascular compartment,
$V = V_i + V_e$,
c_i = the mean concentration in the intravascular compartment,
c_e = the mean concentration in the extravascular compartment,
c_m = the over all mean concentration $(= (V_i c_i + V_e c_e)/V)$,
I = the amount injected per minute,
E = the amount eliminated per minute, and
D = a constant for the diffusion between the compartments.

It follows from the assumptions that the concentrations in the two compartments have to satisfy the equations:

$$V_i\,(dc_i/dt) + V_e(dc_e/dt) = I - E, \tag{1}$$

$$dc_e/dt = D(c_i - c_e), \tag{2}$$

From the definitions and eq. (2) follows that

$$c_m = c_i - (dc_e/dt)\, V_e/DV. \tag{3}$$

Let I and E be constants, then the time course of c_i is rectilinear when a steady state is achieved. Thus

$$c_i = g_i t + b, \tag{4}$$

where g_i is the slope of the curve. From eq. (1) and (2) it appears that under these conditions

$$dc_e/dt = g_i, \tag{5}$$

and from eq. (3) and (5) that

$$c_m = c_i - Kg_i, \tag{6}$$

where K is V_e/DV which may be regarded as a constant for the model examined, representing the horizontal distance between the intravascular and the mean concentration curves.

The constant K may be determined by experiments with two infusions, A and B, at different rates, carried out in immediate succession (cf. Fig. 2). In period A the concentration course is rectilinear to the moment, the rate of infusion is changed, in the beginning of period B it is curvilinear, to become rectilinear when a new steady state is achieved. The rectilinear part of curves A and B are described by

$$c_{i,A} = g_{i,A}\,(t - t') + c'_{i,A} \tag{7}$$

$$c_{i,B} = g_{i,B}\,(t - t') + c'_{i,B} \tag{8}$$

where $c'_{i,A}$ and $c'_{i,B}$ are the extrapolated concentrations at the time t' where the rate of the infusion was changed.

As in Fig. 2 $c'_{i,A} \neq c'_{i,B}$. At t' the amount present in the model is $c'_m V$, thus $c'_{m,A} = c'_{m,B}$. From eq. (6), (7), and (8) the mean concentration curves in period A and B are described by

$$c_{m,A} = g_{i,A}\,(t - t' - K) + c'_{i,A} \quad (9)$$

$$c_{m,B} = g_{i,B}\,(t - t' - K) + c'_{i,B} \quad (10)$$

From eq. (9) and (10) follows that

$$K = (c'_{i,B} - c'_{i,A})/(g_{i,A} - g_{i,B}) \quad (11)$$

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Factors Determining the Circulatory Adjustments to Diving

I. Water immersion

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Abstract

ANDERSEN, H. T. *Factors determining the circulatory adjustments to diving. I. Water immersion.* Acta physiol. scand. 1963. *58.* 173—185. — The importance of water immersion *per se* for the circulatory adjustments to diving has been studied in the domestic duck. The heart rate has been used as an index of the cardio-vascular changes. The physiological reactions characteristic of diving were elicited upon submersion in ducks with free access to air through tracheal cannulas, just as well as in intact birds. Likewise, the adjustments to diving were maintained by emerging ducks after the nostrils were above the water surface, provided the rest of the beak was kept submerged. Also, when the lungs and air-sacs were ventilated at the normal rate during descent and submersion, the diving bradycardia was nevertheless elicited. By studying the heart rate during slow submersions of the beak and the head, it was found that the most marked cardiac slowing resulted from immersion of the level of the nares, whereas the conspicuous post-dive tachycardia was brought about during the first respiratory effort. It is concluded that the circulatory adjustments to diving are elicited by the actual water immersion, probably due to stimulation of peripheral receptors in the beak, especially in the region of the nostrils. The degree of distention of the lungs and the air-sacs, as well as variations in the venous return may also be important factors for the elicitation and abolition of the diving characteristics.

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