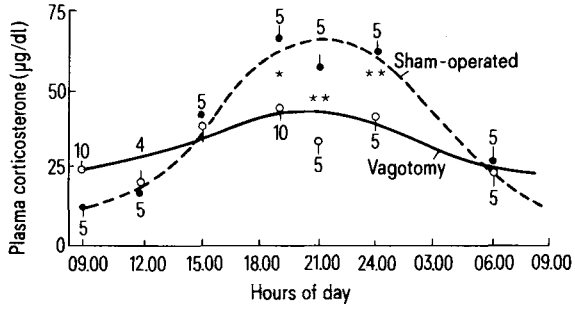
operated ones 67.2 ± 3.15 µg/dl at 19.00 h and 11.3 ± 3.60 µg/dl at 09.00 h, respectively. As seen in the figure, the corticosterone levels of the vagotomized rats were significantly lower at 19.00, 21.00 and 24.00 h, while higher at 09.00 h as compared with corresponding levels in the sham-operated ones. When computed results for the vagotomized group were compared with those of the sham-operated one, the mesor and acrophase were similar in both groups, but a large difference was seen in the amplitude. It was 28.4 µg/dl in the sham-operated group, but only 9.5 µg/dl in the vagotomized one, as shown in the table and the figure.

Discussion. A number of studies have demonstrated that a restricted feeding and/or watering schedule are capable of entraining the circadian rhythm of plasma corticosterone[1-6]. In these reports a very rapid fall in the plasma levels of corticosterone was observed following presentation of food and water. There might be some inhibitory pathways in the central nervous system to suppress ACTH secretion in relation to feeding and watering, but the mechanism of the inhibitory processes are not yet known[9,10]. In this respect, indirect influence of gastrointestinal activity cannot be excluded, since subdiaphragmatic vagotomy was found to cause a marked reduction in the amplitude of plasma corticosterone rhythmicity. The fact suggests that decreased secretion and movement of the gastrointestinal tract induced by ablation of the vagus causes a reduction of rhythmic variation of ACTH secretion, or that afferent impulses from the gastrointestinal tract to the central nervous system via the vagus are involved in the circadian rhythm of the pituitary-adrenal system. The possibility that a lack of vagal mediated insulin secretion plays a role in the reduced circadian rhythmicity should also be considered. However, so far as we examined it, no difference was observed in blood sugar levels between the 2 groups of rats at 09.00 and 19.00 h. Therefore, decreased insulin secretion would not be the main cause of the reduced circadian rhythmicity.



Circadian rhythm of plasma corticosterone in sham-operated (●---●) and vagotomized (○——○) rats. Vertical lines indicate SEM. Numbers in the figure are the number of rats. *$p < 0.05$, **$p < 0.01$.

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The plasma volume of the Wistar rat in relation to the body weight

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Summary. The plasma volume of 43 male Wistar rats, weighing between 140 and 350 g, was determined. A close linear relationship between plasma volume and body weight was found: plasma volume (ml) = 0.0291 × body weight (g) + 2.54.

Knowledge of the plasma volumes of experimental animals is of practical importance for many investigators, for instance those studying the clearance of injected proteins from blood[2,3]. A number of authors[4-11] have determined the plasma volumes of rats by measuring the plasma concentration of an i.v. injected indicator, e.g. a radioiodinated protein or the dye Evans' Blue (also known as T-1824). Plasma volumes have been determined either from a single sample taken shortly after injection, or by extrapolating the plasma clearance curve. Values for the plasma volume of the rat given in the literature concern groups of animals within a more or less narrow range of body weights; the relation between plasma volume and body weight has (with 1 exception, see below) not been investigated. We have now determined this relation in normal male Wistar rats weighing between 140 and 350 g. The results are compared with data from the literature.

Materials and methods. Male rats of an inbred Wistar strain (T.N.O., Zeist, The Netherlands), weighing between 140 and 350 g, were used. The animals were fed ad libitum on a complete laboratory diet (Hope Farms, Woerden, The Netherlands) and received water ad libitum. All experiments were done on anaesthetized animals. Anaesthesia was induced and maintained with Fluothane (I.C.I., Macclesfield, Cheshire, G.B.) in a mixture of NO and O_2. Bovine serum albumin (Sigma, St. Louis, Mo, USA), labelled with 125-iodine as described by Kooistra et al.[2], was used as indicator. In order to remove any traces of rapidly cleared material, the labelled albumin (10 µCi ^{125}I/mg of protein) was screened before use. Screening was done by injecting 20 mg labelled albumin dissolved in 0.50 ml phosphate buffered saline (6 mM sodium phosphate buffer, pH 7.35, containing 0.15 M NaCl) in a rat of 250 g. After 1 h the animal was killed and the plasma collected. This plasma, containing screened radiolabelled albumin, was used for plasma volume determinations after 3-4-fold dilution with phosphate buffered saline. Solutions used for injection were always centrifuged at 15,000 × g for 30 min immediately before injection in order to remove any traces of insoluble material.

Rats were injected via the penile vein, with 0.20 ml labelled plasma (containing about 1 µCi ^{125}I) per 100 g b.wt. Mixing

Survey of plasma volumes from the literature

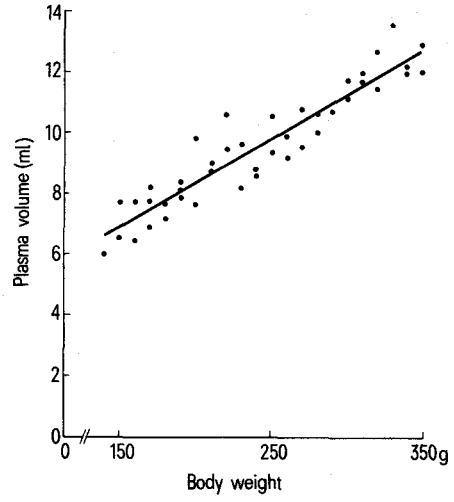
	Strain	Sex	Number of rats	Mean body weight(g)	Haematocrit (%)	Indicator used	Plasma volume (ml)	Values from this study (ml)	Literature reference
A)	Wistar	m	9	332	42.9±0.9	RISA	12.57±0.50	12.20±0.27	4
	Wistar	m	16	223	47.8±0.4	T-1824	8.38±0.09	9.03±0.16	5
	Wistar	m	10	225	43.0±1.3	^{125}I-IgM	6.53±0.24	9.07±0.16	6
	Sprague-Dawley	m	12	189	40.9±0.4	RISA	9.16±0.47	8.04±0.20	7
	Sherman	m+f	50	305	47.8	T-1824	11.56±0.14	11.42±0.22	8
B)	Wistar	m	35	393	46.1±0.7	T-1824	12.29±0.45	b.r.	9
	Sprague-Dawley	m	24	441	47.2±0.5	RISA	14.11±0.40	b.r.	10

Results of studies on groups of rats with mean body weight in (A) or beyond (B) the range of this study are cited. Values are means±SEM. RISA, radioiodinated serum albumin; m, male; f, female; b.r., beyond the body weight range of this study.

of the tracer with plasma was complete within 30 sec. Blood samples of about 0.15 ml were obtained from the orbital plexus 2, 20, 40 and 60 min after injection, and collected in heparinized tubes. From these samples, 50 µl aliquots were used for haematocrit determinations and the remainder was centrifuged at 10,000×g for 10 min. Duplicate samples of 20 µl of plasma were used for radioactivity measurements. Radioactivity was assayed as described by Kooistra et al.[2] in vials of the type described by Ashcroft[12]. Haematocrit values were read after centrifugation for 60 min at 1500×g and corrected for trapped plasma.

Results and discussion. During the 1st h after injection of the screened albumin, radioactivity in the plasma disappeared in an exponential way. The concentrations of acid-precipitable radioactivity in plasma (less than 1% of the plasma radioactivity was acid-soluble at all times) were plotted against sampling time in semi-logarithmic diagrams. The decrease in plasma concentration with time (apparent half-life 2.86±0.15 h; mean±SEM, 43 animals) can be ascribed to redistribution of the injected albumin between the vascular and extravascular spaces[13].

Plasma volumes were obtained by dividing the injected dose by the concentration of the tracer at the time of injection, found by extrapolation of the regression lines. In the figure, plasma volumes obtained from 43 rats, weighing between 140 and 350 g (mean b.wt 241 g), are plotted against their body weights. In this range a close linear relationship (correlation coefficient 0.937) was found between the 2 parameters: plasma volume (ml) = 0.0291 × body weight (g) + 2.54. The SE of estimate is 0.67 ml.



Plasma volumes of male Wistar rats of varying body weight. The regression line (r=0.937) can be described by the equation: plasma volume (ml)=0.0291×body weight (g)+2.54.

From this value, the standard errors and confidence limits for the individual body weights can be calculated by use of the appropriate statistical formulae[14].

The mean haematocrit was 46.1±0.4% (mean±SEM; 43 animals). Haematocrit and body weight were not correlated ($p < 0.01$). Unlike Garcia[15], we did not observe lower haematocrit values in animals with body weights up to about 200 g. Strain differences (Garcia worked with Long-Evans rats) might explain the difference between his data and ours.

In the table our results are compared with values obtained by others from groups of rats with more or less narrow ranges of body weight. The values are generally in good agreement with our data (with the possible exception of the relatively low value found by Rasmussen[6], using labelled macroglobulin). The only other study that directly correlates body weight and plasma volume as determined by means of a plasma tracer is that of Ivarsson et al.[11]. These authors found a somewhat different relationship between the 2 parameters: plasma volume (ml)=0.026×body weight (g)+0.92. In their study rather heavy (>400 g) Sprague-Dawley rats and rats of a selected Wistar strain showing no anaphylactoid reaction[16] were used.

Results by Garcia[15] and Tribukait[17] cannot directly be compared with ours. These authors have estimated plasma volumes by measuring the red cell mass and the venous haematocrit. Since the venous haematocrit differs from the body haematocrit[17] this method does not give absolute values.

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