Effect of extracellular fluid volume expansion with isotonic saline in 9 sham operated and 8 acutely hypophysectomized dogs on arterial blood pressure (BP), urine output (V), urinary sodium concentration (U_{Na}) and excretion ($U_{Na}V$) and on glomerular filtration rate (GFR)

Period	Time (min)	I Non-hypox ($n = 9$)		II Hypox ($n = 8$)		P
BP (torr)						
1	0– 30	126.67 ±	2.50	113.13 ±	5.97	< 0.05
2	30– 60	123.89 ±	2.86	111.88 ±	7.13	ns
3	60– 70	127.22 ±	4.57	114.38 ±	7.82	ns
4	70– 80	126.11 ±	4.70	113.75 ±	8.22	ns
5	80– 90	121.67 ±	3.23	110.37 ±	9.15	ns
6	90–100	116.67 ±	3.54	112.86 ±	7.06	ns
7	100–110	115.56 ±	4.03	110.00 ±	7.94	ns
8	110–120	112.78 ±	4.09	105.71 ±	9.54	ns
9	120–130	113.57 ±	6.05	96.67 ±	8.63	ns
V (ml min^{-1})						
1	0– 30	0.25 ±	0.06	0.27 ±	0.07	ns
2	30– 60	0.31 ±	0.12	0.34 ±	0.10	ns
3	60– 70	3.81 ±	0.80	2.69 ±	0.46	ns
4	70– 80	11.34 ±	1.75	8.34 ±	1.76	ns
5	80– 90	14.23 ±	1.39	11.03 ±	1.92	ns
6	90–100	12.25 ±	1.33	9.32 ±	1.18	ns
7	100–110	8.92 ±	1.18	7.45 ±	1.01	ns
8	110–120	6.87 ±	0.84	6.22 ±	0.72	ns
9	120–130	4.99 ±	0.90	5.53 ±	0.79	ns
U_{Na} (mval l^{-1})						
1	0– 30	96.33 ±	29.65	18.00 ±	5.05	< 0.05
2	30– 60	100.78 ±	42.86	17.75 ±	5.90	ns
3	60– 70	133.00 ±	12.54	54.12 ±	14.55	< 0.01
4	70– 80	129.00 ±	7.07	69.00 ±	9.62	< 0.001
5	80– 90	109.89 ±	7.09	64.00 ±	8.49	< 0.001
6	90–100	107.89 ±	10.95	50.14 ±	9.49	< 0.01
7	100–110	108.78 ±	10.99	37.14 ±	8.97	< 0.001
8	110–120	115.67 ±	12.90	30.71 ±	7.01	< 0.05
9	120–130	111.57 ±	15.36	29.00 ±	9.92	< 0.01
$U_{Na}V$ (μval min^{-1})						
1	0– 30	28.44 ±	11.49	4.31 ±	1.33	ns
2	30– 60	39.22 ±	20.37	3.36 ±	0.83	ns
3	60– 70	506.33 ±	102.48	153.00 ±	59.71	< 0.05
4	70– 80	1423.78 ±	209.35	584.00 ±	175.13	< 0.01
5	80– 90	1585.67 ±	225.64	751.25 ±	183.80	< 0.05
6	90–100	1297.67 ±	200.51	493.71 ±	112.53	< 0.01
7	100–110	940.89 ±	171.77	295.29 ±	97.98	< 0.01
8	110–120	757.78 ±	133.53	192.29 ±	49.35	< 0.01
9	120–130	496.57 ±	69.98	162.83 ±	53.23	< 0.01
GFR (ml min^{-1})						
1	0– 30	53.12 ±	7.82	44.35 ±	12.92	ns
2	30– 60	50.11 ±	8.27	37.12 ±	8.75	ns
3	60– 70	119.58 ±	17.13	116.95 ±	18.41	ns
4	70– 80	78.80 ±	11.35	55.14 ±	4.41	ns
5	80– 90	69.47 ±	5.95	53.70 ±	6.69	ns
6	90–100	65.93 ±	4.30	54.06 ±	9.06	ns
7	100–110	62.71 ±	4.24	46.61 ±	5.70	< 0.05
8	110–120	61.46 ±	5.74	43.87 ±	4.47	< 0.05
9	120–130	56.73 ±	5.85	44.32 ±	4.10	ns

All values are calculated per 100 g of the kidney weight and are expressed as means ± SE. Expansion was completed in the 3rd and 4th periods. ns, non significant.

in the corresponding clearance periods between sham operated and hypophysectomized dogs were evaluated by the Student t-test.

Results and discussion. The results are summarized in the Table. Isotonic extracellular fluid volume expansion increased urine output and sodium excretion in both experimental groups; however, the peak natriuresis in the hypophysectomized animals represented only 50% of that in the control sham operated dogs due to the lower urinary sodium concentration. This difference was not accompanied by changes in either glomerular filtration rate or in blood pressure.

It is concluded that the pituitary plays a role in the mechanism of homeostatic natriuresis resulting from isotonic extracellular fluid volume expansion. Our previous suggestion that a pituitary natriuretic hormone might be involved[1–3] has subsequently been supported by the demonstration of natriuretic activity of neurophysin[4]. On the other hand, other investigators have recently isolated a natriuretic tri-dekapeptide from the posterior pituitary[5]. The role of a pituitary natriuretic hormone seems to be to decrease sodium reabsorption in the distal nephron[6–8], whereas on the basis of free-water clearance studies, it has been established that the decrease of proximal tubular reabsorption during extracellular fluid volume expansion is not dependent on any pituitary hormone[6–8] and its nature is still to be clarified.

Summary. The natriuresis following an i.v. isotonic saline loading corresponding to 10% of body wt. was markedly decreased after acute hypophysectomy, due to lowered urinary sodium concentration, in anaesthetized dogs. A role of the pituitary in such a homeostatic natriuresis is suggested.

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Circadian Rhythm of Bile Secretion in the Rat

Variations in bile flow during the day have been observed in man[1]. Moreover it is well established that, in the rat, body weight, liver weight, nucleic acids and protein contents, liver microsomal enzyme activity, oxygen consumption and mitochondrial activity, various enzyme activities (enzymes of amino-acid degradation, glycogenesis, carbohydrate and glycogen metabolism) follow a

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Body weight, stomach and intestine weight, liver weight, basal bile flow, bile acid excretion, estimated bile acid independent flow, blood sugar and electrolytes in bile in rats at 08.00, 17.00 and 24.00 h

11 rats in each group	Group I (08.00 h)	Group II (17.00 h)	Group III (24.00 h)
Weight (g)			
Body	190.18 ± 13.80[f]	187.73 ± 10.80[g]	206.45 ± 8.40[f,g]
Stomach + intestine	16.31 ± 2.51[e,f]	13.52 ± 6.49[e,g]	29.20 ± 3.11[f,g]
Liver	7.31 ± 0.85[e]	6.49 ± 0.40[e,g]	8.02 ± 0.59[g]
Basal bile flow			
$\mu l\ min^{-1}\ 100\ g^{-1}$	9.49 ± 1.80[e]	7.68 ± 0.94[e,g]	8.95 ± 1.25[g]
$\mu l\ min^{-1}\ g\ liver^{-1}$	2.46 ± 0.03	2.21 ± 0.03	2.29 ± 0.03
Basal bile acid excretion			
$nmol\ min^{-1}\ 100\ g$	269 ± 96	260 ± 76	241 ± 74
$nmol\ min^{-1}\ g\ liver^{-1}$	70 ± 23	75 ± 21	61 ± 18
Estimated BAIF[d]			
$\mu l/min^{-1}/100\ g^{-1}$	7.25 ± 1.65[a]	5.84 ± 1.30[a,c]	7.52 ± 1.42[c]
$\mu l/min^{-1}/g\ liver^{-1}$	1.89 ± 0.26	1.72 ± 0.25	1.92 ± 0.42
Blood sugar			
$g\ l^{-1}$	1.64 ± 0.22[f]	1.66 ± 0.33[g]	2.09 ± 0.41[f,g]
Electrolytes in bile			
meq. l^{-1} Na	143 ± 15	141 ± 17	140 ± 18
meq. l^{-1} K	5.5 ± 0.2	5.1 ± 0.3	5.1 ± 0.3
meq. l^{-1} Cl	90 ± 4	89 ± 5	88 ± 7

Values are mean ± 1 SD. [a] Significant difference between group I and II, $p < 0.05$. [b] Significant difference between group I and III, $p < 0.05$. [c] Significant difference between group II and III, $p < 0.05$. [d] BAIF = bile acid-independent flow. [e] Significant difference between group I and II, $p < 0.01$. [f] Significant difference between group I and III, $p < 0.01$. [g] Significant difference between group II and III, $p < 0.01$.

circadian rhythm[2-4]. The purpose of this study was to investigate whether bile flow in the rat follows a diurnal rhythm.

Materials and methods. 33 male Wistar rats (Evic-Ceba, 33 Blanquefort, France) were housed for 8 days before study in a dark room at constant temperature 22°C) and humidity. Rats were lighted with artificial light between 08.00 and 20.00 h and fed a standard diet from 20.00 to 24.00 h and water ad libitum. They were randomized in 3 groups of 11 rats: group I was studied at 08.00 h, group II at 17.00 h, group III at 24.00 h. The experiments were carried out in 2 days.

The animals were anesthetized with pentobarbital (Nembutal, Abbott). Body temperature was maintained between 37.5 and 38.5 °C on heating tables. The bile duct was cannulated with a polyethylene tube. Bile was collected at 10 min intervals. Basal bile flow and bile acid excretion was measured during the first 30 min following cannulation. Thereafter sodium taurocholate (Maybridge Biochemical Corporation, Tintagel, UK) was infused at a rate of 0.5 $\mu mol\ min^{-1}\ 100\ g\ bw^{-1}$ for 1 h. At the steady state, 2 consecutive 10-min samples of bile were collected. The regression line for bile flow vs bile acid excretion rate was established for each experiment. The bile acid independant flow (BAIF) was calculated as the intercept of the regression line with the vertical axis[5,6]. At the end of each experiment, arterial blood was sampled, the weight of the liver, the stomach and the intestine was measured. Bile acid concentration in bile was measured by an enzymatic technique[7], using 3 hydroxysteroid-dehydrogenase (Worthing Biochemical Corporation, Freehold, N.J. USA). Sodium and potassium concentration in bile were measured by flame photometry. Chloride concentration was measured in an automatic chloride titrator. Blood glucose concentration was measured using a standard glucose oxydase method.

Statistical analysis was performed using the Student's *t*-test, a value of $p < 0.05$ being regarded as significant.

Results. The results are given in the Table. The increase in body weight in group III was mainly due to an increase in the weight of stomach and intestine after food intake. Liver weight was significantly lower in group II than in groups I and III.

Basal bile flow was significantly lower at 17.00 h than at 08.00 h or 24.00 h; the excretion rate of bile acids did not differ significantly in the 3 groups. The relationship between bile flow and bile acid excretion rate for groups I and II is represented in the Figure. In the 3 groups the slopes were not significantly different. The estimated BAIF expressed in $\mu l\ min^{-1}\ 100\ g\ bw^{-1}$ was significantly lower in group II. Expressed in $\mu l\ min^{-1}\ g\ liver^{-1}$ the estimated BAIF was not significantly different in the 3 groups. The decrease in the BAIF between 24.00 h and 17.00 h (22%) paralleled the decrease in liver weight (19%). Blood sugar was significantly higher at 24.00 h than at 08.00 or 17.00 h. There were no significant differences in Na, K and Cl concentrations in bile.

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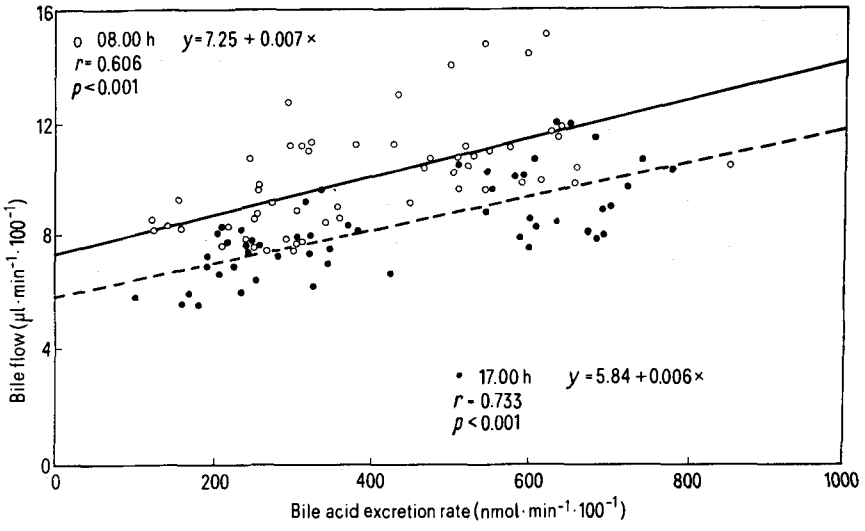

Relationship between bile flow and bile acid excretion. Group I is represented by the open circles, Group II by the dark circles. Each point corresponds to 1 measurement. The zero intercepts are significantly different, not the slopes (in order to simplify the figure, Group III has not been presented: $y = 7.52 + 0.005\,x$).

Discussion. In these experiments, the observed rhythm is not a natural one since, in addition to the lighting schedule, limited availability of food to a discrete portion of the dark period (from 20.00 to 24.00 h) was employed. This schedule was designed in order to avoid the individual variability in feeding behavior within a 12-hour period which may obscure the spontaneous oscillations in physiological activity[3].

Under these conditions, bile flow follows a diurnal rhythm with a lower bile flow at 17.00 h i.e. at the end of the light period and far from the feeding period. This decrease in bile flow can be explained by a decrease in BAIF. The estimation of the BAIF by the relationship between bile flow and bile acid excretion confirms this assumption. Erythritol clearance has not been measured, but it is generally accepted that, in the rat, bile acid independant flow is entirely of hepatocytic origin[5,6,8]; furthermore secretin injected intravenously does not increase bile flow[8]. BAIF decreases by 19% and liver weight by 22% at 17.00 h compared with the values obtained at the end of the food period during the darkness (24.00 h). A parallel decrease in liver weight and BAIF has also been reported in rats after a portocaval shunt[9,10]. Nevertheless a decrease in bile flow and in BAIF expressed in g liver^{-1} was found in rats after a 72-hour fast, a much longer period than in our experiments (20-hour fast)[11].

In bile fistula rats, kept in a restraining cage, with light between 09.00 and 19.00 h and food during the dark period, the biliary excretion of bile acids showed 2 peaks one at 01.00 h, the other about 06.00 h[12]. This rhythm paralleled the diurnal rhythm of the cholesterol-7α hydroxylase, the hepatic microsomal enzyme which is rate limiting in the rat for the bile acid synthesis from cholesterol[13]. However, in our experiments, no variations in bile acid excretion were found.

The rhythmic oscillations of biliary flow may be related to food intake and/or light-dark cycle. The variations in body weight, liver weight, protein, glycogen and enzyme content are mainly under the control of food intake, but these patterns can be strongly modified by the lighting schedule[3]. Nevertheless the rhythm of hepatocyte proliferation (and of DNA synthesis) may be synchronized by the light-dark rhythm which appears to act by controlling the feeding habits of animals under natural circumstances[14]. Food increases the blood level of several hormones which may enhance bile flow. In the rat, insulin[15] and glucagon[16] have been reported to increase BAIF.

It may be concluded that bile flow in the rat follows a diurnal rhythm with a decrease at the end of the light period and far from the feeding period. The decrease is due to a reduction of the BAIF. Parameters such as food, light and time schedule must be carefully checked in experiments concerning bile flow.

Summary. In rats the bile flow and the estimated bile acid independant flow (BAIF) were significantly lower at 17.00 h than at 08.00 and 24.00 h. The decrease in BAIF paralleled the decrease in liver weight. Bile acid excretion was not different.

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