anoestrus values is the frequent presence of 'shoulders' or frank double peaks of secretion during the oestrous phase of the annual cycle. 21 out of 29 profiles showed such evidence of a bimodal secretion pattern, whereas during anoestrus only 1 out of 16 24-h profiles appear to have a 2nd peak. Although clearly the presence of more peaks cannot be excluded with the sampling times used, it is theoretically possible to detect up to 3 peaks in December and January and 2 peaks in May, August and September. A similar bimodal pattern of melatonin secretion has been previously reported in 6 out of 8 castrated ram-lambs in 12L 12D sampled at frequent intervals[9].

A previous report by Rollag et al.[15] of plasma melatonin variations at different stages of the annual reproductive cycle in the ewe indicated that no overall statistical differences were present with regard to melatonin secretion, with the exception that the duration of elevated melatonin concentration corresponded to length of photoperiod and hence was longest during oestrus. Our experimental conditions differed from those of Rollag et al.[15], inter alia in that sheep were both maintained and sampled in natural light, as opposed to being sampled in artificial light. The melatonin assay employed here appears to give lower basal values in sheep than that of Rollag et al.[13,15]. The data reported by Rollag et al., particularly for ewes in luteal phase in November (mid-oestrus)[15] is nevertheless suggestive of a bimodal pattern of secretion, although values from individual sheep are not shown.

Both unimodal and bimodal patterns of physiological phenomena such as activity and hormone concentrations have been frequently observed and may depend on photoperiod length[3,18-20]. Melatonin secretion in the ewe is likely to depend principally on photoperiodic conditions: experiments in castrates would however be essential to differentiate photoperiodic effects from any effects due to gonadal steroids.

Whether, in natural conditions, melatonin secretion is an important determinant of reproductive activity in the ewe remains an open question, however, the association of double peaks or extended secretion with reproductive activity suggests a possible mechanism for photoperiodic control. Assuming a single, short-duration, dark-phase peak of melatonin secretion, corresponding to short nights, is inhibitory to reproductive function, and that melatonin receptors in the sheep are subject to reduced sensitivity after exposure to melatonin (or 'down-regulation') as recent evidence suggests in the hamster[21,22], then the presence of 2 peaks, or extended secretion, indicative of long-nights, could hypothetically cancel the inhibitory effect of 1 peak of short duration, allowing reproductive resurgence or restraining reproductive atrophy. This theory would predict that melatonin administration in late light-phase in anoestrus would induce early oestrus and that inhibition of melatonin secretion in early dark-phase in oestrus would induce early anoestrus, although clearly allowance must be made for a possible underlying endogenous annual rhythm of reproductive competence.

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2 We are grateful for the excellent technical assistance of A. Marston and J. Bradtke.
3 W.B. Brown and J.M. Forbes, J. Endocr. *84*, 91 (1980).
4 G.K.B. Barrell and K.R. Lapwood, J. Endocr. *80*, 397 (1979).
5 G.A. Lincoln, J. Endocr. *82*, 135 (1979).
6 J.F. Roche, F.J. Karsch, D.L. Foster, S. Takagi and D.J. Dziuk, Biol. Reprod. *2*, 251 (1970).
7 J. Herbert, J. Endocr. *55*, 591 (1972).
8 I. Nir, R.J. Reiter and R.J. Wurtman, eds, Proceedings International Symposium on the Pineal Gland. J. neural Transm., suppl. *13*. Springer, Berlin 1978.
9 J. Arendt, J.M. Forbes, A. Marston and W.B. Brown, J. Endocr. *85*, 1P (1980).
10 G.A. Lincoln, O.F.X. Almeida and J. Arendt, J. Reprod. Fert., in press (1981).
11 F. Turek, C. Desjardins and M. Menaker, Science *190*, 280 (1975).
12 L. Tamarkin, W.K. Westrom, A.I. Hamill and B.D. Goldman, Endocrinology *99*, 1534 (1976).
13 M.D. Rollag and G.D. Niswender, Endocrinology *98*, 482 (1976).
14 D.J. Kennaway, E.G. Frith, G. Phillipou, C. Matthews and R.F. Seamark, Endocrinology *101*, 119 (1977).
15 M.D. Rollag, P.L. O'Callaghan and G.D. Niswender, Biol. Reprod. *18*, 279 (1978).
16 J. Arendt, L. Wetterberg, T. Heyden, P.C. Sizonenko and L. Paunier, Hormone Res. *8*, 65 (1977).
17 A.M. Symons, J. Endocr. *56*, 327 (1973).
18 J. Aschoff, Handb. Zool. *8*, 1 (1962).
19 G.A. Lincoln, A.S. McNeilly and C.L. Cameron, J. Reprod. Fert. *52*, 305 (1978).
20 J.-P. Ravault and R. Ortavant, Annls Biol. anim. Biochim. Biophys. *17*, 459 (1977).
21 E.L. Bittman, Science *202*, 648 (1978).
22 H.J. Chen, G.C. Brainard and R.J. Reiter, Neuroendocrinology *31*, 129 (1980).

Effect of diet on osmotic water flow across rat colon mucosa[1]

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Summary. Osmotic water flow across colon mucosa was increased in rats adapted to a high protein diet (HP) compared to rats fed a high carbohydrate diet (HC). The diet-induced change of the osmotic permeability of the colon is probably a manifestation of a regulatory mechanism controlling intestinal water absorption.

We have shown in former work[3,4] that Na, Cl and water absorption from the colon ascendens is elevated in rats adapted to a high protein diet (HP) compared to rats fed a high carbohydrate diet (HC). Moreover in these experiments the ratio between net water absorption and net absorption of solutes was higher in HP-rats than in HC-rats, indicating that the osmotic permeability of the colon epithelium might be affected by the diet. In the present study we have therefore investigated whether the osmotic water flow across colon epithelium is influenced by feeding

rats a high protein diet. The experiments were performed with ligated colon segments filled with a hypotonic mannitol solution.

Material and methods. Adult male Sprague-Dawley rats (initial b.wt: 200–220 g) were fed for about 4 weeks either a high carbohydrate (HC, 13% casein) or a high protein (HP, 88% casein) diet. The diets were isocaloric. The exact composition of the diets is described elsewhere[4,5]. Osmotic water flow across colon epithelium was determined in anesthetized animals (xylazin-hydrochloride, Bayer, Leverkusen, 7 mg/rat, i.m., and ketamin-hydrochloride, Parke Davis, Munich, 37.5 mg/rat, i.m.) after introducing 2 ml of hypotonic mannitol solution (107 mosmol/kg) into the cleaned, ligated colon ascendens (length: 5–6 cm, luminal hydrostatic pressure: about 6–7 cm H_2O). Osmotic flow was measured over 1 h and was expressed as the volume lost from the lumen per g dry weight and per cm^2 macrosurface. ^{3}H-labelled polyethyleneglycol (mol.wt ~ 4000, Amersham Buchler GmbH, Braunschweig) was used as unabsorbable marker, allowing calculation of volume changes of the colonic fluid from the increase of the ^{3}H-concentration. The recovery of the PEG on the average was 99.4% (n = 7). The ^{3}H-activity was measured using liquid scintillation counting, and the osmolality was determined with a Knauer osmometer. The colonic fluid was also assayed for Na^+, K^+ (Instrumental Laboratory Flame Photometer, model 243) and Cl^- (Instrumental Laboratories Chloride Analyzer, model 279).

Results. The data in table 1 show that the size of the colon segments did not differ significantly between both groups of animals. However, the final osmolality of the colonic fluid was significantly higher in group HP than in group HC. Table 1 also shows that in both groups at the end of the experimental period, Na, Cl and K were present in the colonic fluid in low concentrations, indicating that these ions had diffused from the blood into the colon lumen during the experiment to a small extent. As shown in table 2 the osmotic water flow from the colon lumen to the blood in group HP exceeded that in group HC significantly. From the osmotic flow and the osmotic gradient, plasma-to-lumen, the osmotic water permeability of the colon was calculated. The osmotic gradient (= Δ mosmol) was defined as the difference between plasma osmolality and the average osmolality of the colonic fluid:

$$\Delta\text{osmol} = \frac{C_i + C_f}{2} - P$$

where C_i and C_f is the initial and final osmolality of the colonic fluid, and P, the osmolality of the plasma (mosmol/kg). The mean value for the plasma osmolality[4] in HP-rats (299 ± 2 mosmol/kg) was slightly higher than that in HC-rats (290 ± 2).

As shown in table 2, the osmotic permeability of the colon in group HP was about twice as high as in group HC.

Discussion. The results presented demonstrate for the first time that the osmotic permeability of the colon mucosa can be changed by diet. Other investigators have also studied the osmotic permeability of the large intestine in cases where Na absorption was increased by dietary manipulations. Thus, Skadhauge and Thomas[6] could observe no change in osmotic permeability of the lower intestine of Na^+-depleted hens, in which Na^+ absorption was enhanced dramatically[7]. Our data indicate that the increased solute-coupled water absorption from the colon ascendens of HP-rats demonstrated in a previous study[3,4] is due to an increased osmotic permeability of the colon mucosa. It is at present unclear whether differences in pore size of the epithelial membranes or the tight junctions account for the different osmotic permeability in HP- and HC-rats.

In our experiments a linear dependence of osmotic flow upon the osmolality difference between the plasma and the luminal fluid has been tacitly assumed. According to experiments of Miller et al.[8], who also used hypotonic mannitol solutions for studying in vivo osmotic flow across the intestine, this assumption seems to be adequate.

It is well established that the water requirement of mammals fed a high protein diet is increased, probably because large amounts of urea must be excreted via the urine[9–12]. The diet-induced change of the osmotic permeability of the colon could therefore be a manifestation of a regulatory mechanisms controlling intestinal water absorption.

Table 1. Size and final luminal osmolality and electrolyte concentrations of ligated colon segments in HC- and HP-rats

	Group HC (n = 7)	Group HP (n = 11)
Wet weight (mg)	699 ± 48*	686 ± 18
Dry weight (mg)	173 ± 17	181 ± 8
Macrosurface (cm^2)	12.6 ± 0.3	12.3 ± 0.6
Osmolality (mosmol/kg)**	186 ± 4	208 ± 4
	$p < 0.01$***	
Na^+ concentration (mmoles/l)	18.7 ± 2.2	17.9 ± 0.7
Cl^- concentration (mmoles/l)	16.1 ± 2.3	14.7 ± 1.2
K^+ concentration (mmoles/l)	4.4 ± 0.3	4.9 ± 0.3

* $\bar{x}$ ± SEM; ** initial osmolality: 107 mosmol/kg; *** Mann-Whitney U-test.

Table 2. Osmotic water flow and osmotic water permeability determined in colon segments of HC- and HP-rats

	Group HC (n = 7)		Group HP (n = 11)
Osmotic flow			
$ml/cm^2 \cdot h$	0.025 ± 0.003		0.049 ± 0.003
		$p < 0.01$	
$ml/g^* \cdot h$	2.0 ± 0.3		3.3 ± 0.2
		$p < 0.05$	
Osmotic permeability			
$\mu l/cm^2 \cdot h \cdot mosmol$	0.18 ± 0.03		0.35 ± 0.02
		$p < 0.01$	
$\mu l/g^* \cdot h \cdot mosmol$	13.7 ± 2.3		23.4 ± 1.8
		$p < 0.05$	

* Dry weight.

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3 E. Scharrer and Ö. Katona, in: Intestinal Ion Transport, p. 398. Ed. J.W.L. Robinson. MTP Press Ltd, Lancaster 1976.
4 E. Scharrer and M. Hosser, Pflügers Arch. *388*, 165 (1980).
5 E. Scharrer and H. Zucker, Z. Ernähr. Wiss. *9*, 312 (1969).
6 E. Skadhauge and D.H. Thomas, Pflügers Arch. *379*, 237 (1979).
7 D.H. Thomas and E. Skadhauge, Pflügers Arch. *379*, 229 (1979).
8 D.L. Miller, S.A. Hamburger and H.P. Schedl, Am. J. Physiol. *237*, E389 (1979).
9 E. Scharrer, J. Lutz and E. Boehncke, Ernähr.-Umsch. *18*, 248 (1971).
10 E. Scharrer, Thesis. University of Munich, Munich 1965.
11 H. Zucker and A. Steger, Wien. tierärztl. Mschr. *62*, 338 (1975).
12 H. Meyer and M. Pferdekamp, Zentbl. VetMed. A *27*, 746 (1980).