nylylation number (E_n^-) of approximately nine. In this respect GS I from strain UMKL 20 is similar to the GS from *Rhizobium japonicum* 61A96 and GS from other bacteria in being subjected to regulation by adenylylation and deadenylylation[1,3]. A similarly high degree of adenylylation has also been reported for the GS I from *Rhizobium japonicum* and is common for cultures grown under conditions where oxygen may be limiting[3].

Apparent K_m values of GS I calculated from linear double reciprocal plots for ammonia and glutamate were 5.6 and 15.2 mM, respectively (data not shown). No inhibition of the enzyme by ammonia was detected in the presence of up to 25 mM ammonium chloride. However, in whole cells, a decrease in the biosynthetic activity of GS I was reported in *Rhizobium* cultures subjected to ammonia shock[3]. The effects of various end products of glutamine metabolism on the activity of GS I were also studied to determine whether the enzyme is subjected to regulation by the effectors. Table 2 shows that pyrophosphate at 5 mM had a very strong inhibitory effect on GS I. The other effectors were only moderately effective in affecting the activity of GS I. Such inhibitory patterns are similar to the GS from *E. coli* and many bacteria[1] which are subjected to regulation by feedback inhibition. These results showed that GS I from *Rhizobium* sp. UMKL 20 has similar characteristics to the GS from *E. coli* and many other microorganisms. Such characteristics would indicate that it is possible to use techniques established for the *E. coli* enzyme in detailed studies of the GS from *Rhizobium*.

Table 1. Purification of glutamine synthetase I from *Rhizobium* sp. UMKL 20

Protein fraction	Specific activity[a]	Yield (%)
Crude extract	0.47	–
Polyethylene glycol precipitation	2.20	53.5
Acetone precipitation	18.10	15.0

[a] The specific activity, expressed in units per mg protein, refers to the transferase activity at pH 7.25 (see Methods section).

Table 2. Effectors of glutamine synthetase I from *Rhizobium* sp UMKL 20

Coumpound (5 mM)	% activity
None	100
Glutamine	77.5
Glycine	68.2
α-Ketoglutarate	68.1
Aspartate	66.7
AMP	53.8
ADP	27.9
Pyrophosphate	< 5

All compounds were added at a final concentration of 5 mM to the standard reaction mixture for the biosynthetic assay as described in the Methods section. Mg : ATP was maintained at 1 : 1.

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Reversal of net secretion to net absorption of potassium in rat large intestine by dietary potassium depletion

E. Scharrer[1]

Institut für Veterinärphysiologie der Universität Zürich, Winterthurerstrasse 260, CH-8057 Zürich (Switzerland), and Institut für Physiologie, Physiologische Chemie und Ernährungsphysiologie der Universität München, D-8000 München (Federal Republic of Germany), 6 April 1984

Summary. Feeding rats a diet low in potassium and high in sodium for 2 weeks led to a reversal of net potassium secretion to net potassium absorption in ligated segments of distal large intestine (colon descendens and rectum) under in-vivo conditions. This change in the direction of net potassium transport is probably important for the maintenance of potassium homeostasis.
Key words. Rat intestine; diet, low potassium, high sodium; potassium homeostasis; potassium transport.

Sodium absorption and potassium secretion by the large intestine appear to be influenced by diet. Thus, it is well known that sodium absorption by the colon is enhanced in animals on a low sodium diet[2-5] and potassium secretion is stimulated in animals fed a high potassium diet[6]. It was the aim of the present short study to investigate the effect of potassium depletion on potassium, sodium and chloride transport by rat distal large intestine (colon descendens and rectum). An in vivo sac technique was employed[7].

Methods. Adult male Sprague-Dawley rats (initial mean b.wt 233 g), which were housed in a colony room illuminated from 07.00 to 19.00 h, were fed for about 2 weeks either a normal potassium (= NK) or a low potassium (= LK) diet. To obtain a low potassium diet the K_2HPO_4 of the mineral mixture used for the diets was replaced by Na_2HPO_4. Thus, only traces of potassium (0.04%) were present in the LK-diet. The potassium content of the NK-diet was 0.45%. The sodium content of the diets was 0.72% (LK-diet) and 0.45% (NK-diet). Both diets contained the same basic components (13% casein supplemented with 1%, D,L-methionine; 76.7% corn starch; 3.3% soybean oil; 2% vitamin mixture; 5% mineral mixture). The composition of the mineral mixtures used for both diets is shown in table 1.

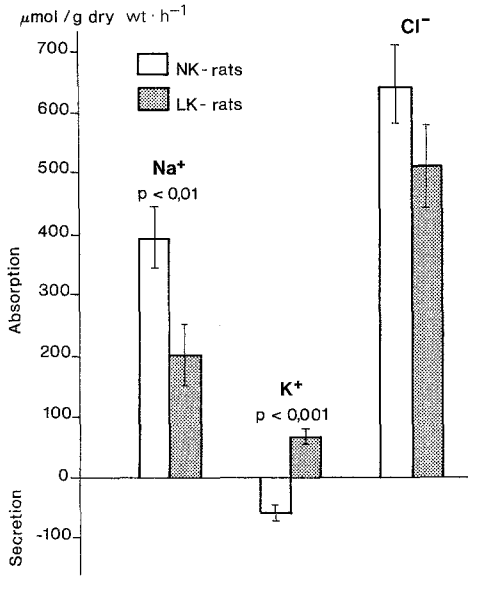
Food and water were offered to the rats all the time. The weight gain of the LK-rats was significantly lower than that of the NK-rats (table 2) reflecting a small depression of food in-

take during potassium depletion. We measured food intake for 3 days beginning on the 6th day of feeding the experimental diets. During this period mean daily food intake in group LK (15.0 ± 1.1 g) was somewhat lower than in group HK (16.7 ± 0.6). The difference, however, was not significant. It is very unlikely that the small group difference in food intake had any affect on the transport values.

Net absorption (or secretion) of electrolytes (Na^+, Cl^-, K^+) was determined in anesthetized animals (Xylazin-hydrochloride, Bayer, Leverkusen, FRG, 7 mg/rat, i.m. and Ketamin-hydrochloride, Park, Davis & Company, München, FRG, 37.5 mg/rat i.m.) after introducing Krebs-Henseleit bicarbonate buffer (composition: Na^+ 144; K^+ 5.9; Ca^{++} 2.5; Mg^{++} 1.2; Cl^- 128; HCO_3^- 25; $H_2PO_4^-$+HPO_4^{--} 1.2; SO_4^{--} 1.2 mmoles/l; pH 7.4) into the cleaned, ligated colon descendens and rectum (length: 5 cm). One ligature was placed about 0.5 cm proximal to the anus and the 2nd one about 5 cm more proximal. Absorption (or secretion) was measured over 1 h and was expressed as the quantity lost from (or having appeared in) the lumen per g dry-weight. ^{3}H-labeled polyethylenglycol (mol.wt 4000, Amersham Buchler GmbH, Braunschweig, FRG) was used as an unabsorbable marker, allowing calculation of volume changes of the colonic fluid from the increase of ^{3}H-concentration. The recovery of PEG was 98%. ^{3}H-activity was determined, using liquid scintillation counting. The fluids were assayed for Na^+, K^+, (Instrumental Laboratory Flame Photometer, model 243) and Cl^- (Instrumental Laboratory Chloride Analyzer, model 279).

Results. Table 2 shows that there was no significant difference in the dry weight of the colon descendens and rectum (length: 5 cm) between NK- and LK-rats. The sodium concentration of the plasma was also similar in both groups, whereas plasma potassium concentration was about 40% lower in the LK-rats compared with the NK-rats. Plasma chloride concentration was also somewhat lower (ca 7%) in LK-rats.

Na^+ and Cl^- were absorbed from the intestinal lumen and Na^+ absorption related to intestinal dry weight was significantly lower in LK-rats compared with NK-rats (fig.). Net water absorption (ml/g dry weight per h) did not differ significantly between group LK (1.03 ml) and group NK (1.37 ml). Interestingly, a net secretion of K^+ occurred in NK-rats, while there was a net K^+ absorption in LK-rats (fig.). K^+ concentration of the intestinal fluid increased from 5.9 mmoles/l to 10.6 mmoles/l during the experiments in group NK and decreased from 5.9 mmoles/l to 2.4 mmoles/l in group LK (table 2). Thus, in contrast to group NK, K^+ appeared to be absorbed against a concentration gradient in group LK, because the mean K^+ plasma concentration was 3.3 mmoles/l in this group. Final Na^+ concentration of the intestinal fluid was lower in group NK than in group LK (table 2), whereas Cl^- concentration did not differ significantly.



Net absorpotion (or secretion) of sodium, potassium and chloride from (into) the ligated segments of colon ascendens + rectum in rats fed a normal potassium (= NK-rats) or a low potassium (= LK-rats) diet.

Discussion. The most interesting result of this simple short study is the reversal of net potassium secretion to net potassium absorption in the rat distal large intestine (colon descendens and rectum) caused by feeding a diet low in potassium and high in sodium. This change in the direction of net potassium transport is probably due to potassium depletion, because alterations in sodium intake appear to affect only the size of net potassium secretion but do not alter the direction of net potassium transport[3]. As it is well established that sodium absorption from the colon is reciprocally related to dietary sodium intake[2–5], the difference in net sodium absorption between group LK and group HK might be attributable to the group difference in sodium intake.

There is one other study on the effect of dietary potassium depletion on potassium transport by rat distal colon[8]. However, in this study the rats were potassium-deprived for only 3 days. Net potassium secretion was observed in the control animals and fell to zero in the potassium-depleted animals in this study, when the intestinal lumen was perfused with an electrolyte solution of a similar composition to our electrolyte solution. A net absorption of potassium was only observed when the distal colon was perfused with a low sodium electrolyte solution (sodium concentration: 20 mmoles/l). The discrepancy between those results and ours is probably due to a different degree of potassium depletion.

Net potassium transport by the large intestine probably de-

Table 1. Minerals present in 50 g mineral mixture*

	NK-diet	LK-diet
$CaCO_3$, g	12.50	12.50
$Ca_3(PO_4)_2$, g	11.70	11.70
K_2HPO_4, g	9.80	—
Na_2HPO_4, g	5.10	14.90
NaCl	6.70	6.70
$MgSO_4 \times 7H_2O$, g	3.00	3.00
Trace elements**	1.20	1.20

* 1 kg diet contained 50 g mineral mixture. ** Fe-II-fumarate 369 mg, $MnSO_4 \times H_2O$ 296 mg, Zn-acetate × $2H_2O$ 322 mg, Cu-II-acetate × H_2O 45 mg, KJ 4 mg, NaF 13 mg, $CoSO_4 \times 7H_2O$ 6 mg, $Na_2SeO_3 \times 5H_2O$ 2 mg, MgO 143 mg.

Table 2. Body weight and plasma electrolyte concentrations of rats, dry weight of intestinal segments, and final intestinal fluid electrolyte concentrations

	NK-rats (n = 8)		LK-rats (n = 7)
Final body weight (g)	297 ± 5*	p < 0.05**	277 ± 7
Intestinal dry weight (mg)	65 ± 6	n.s.***	55 ± 4
Plasma Na^+ conc. (mmol/l)	137 ± 1	n.s.	139 ± 1
Plasma K^+ conc. (mmol/l)	5.5 ± 0.4	p < 0.01	3.3 ± 0.3
Plasma Cl^- conc. (mmol/l)	95.4 ± 0.6	p < 0.001	89.2 ± 1.1
Intestinal fluid			
Na^+ conc. (mmol/l)	123 ± 5	p < 0.05	139 ± 3
K^+ conc. (mmol/l)	10.6 ± 0.9	p < 0.001	2.4 ± 0.6
Cl^- conc. (mmol/l)	93 ± 5	n.s.	100 ± 5

NK = normal potassium diet; LK = low potassium diet. * Values are means ± SEM. ** Statistical evaluation with Mann-Whitney-U-test. *** Not significant.

pends on the luminal sodium concentration, because the efficiency of the Na, K-pump in the antiluminal membrane of the epithelial cell depends on sodium uptake into the epithelial cell through the luminal membrane[14] and active potassium secretion is driven by the Na, K-pump[6].

There is some evidence that the distal large intestine has the capacity to secrete and to absorb potassium by active mechanisms requiring metabolic energy[9-13]. It could therefore well be that potassium depletion associated with a high sodium intake reduces potassium secretion to support maintenance of potassium homeostasis. In this case an absorptive process for potassium, which is normally hidden by potassium secretion, might become apparent.

However, our results might be also explained by a stimulation of active potassium absorption induced by potassium depletion.

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Regulation of carbohydrate metabolism by the optic tentacles of the garden snail *Cryptozona ligulata* (Gastropoda – Stylommatophora)

O.V. Subramanyam

Department of Zoology, Silver Jubilee Government College, Kurnool, Andhra Pradesh (India), 12 April 1984

Summary. The presence of a hyperglycemic factor in the optic tentacles of the snail *C. ligulata* is reported here. A preliminary characterization based on crude extracts indicates the factor to be water-soluble, heat labile and to be an albumin. The ablation of optic tentacles and injection of optic tentacle extract into operated and normal snails caused a rise in blood sugar, total carbohydrate and glycogen in the foot muscle and mantle and a decrease in hepatopancreatic glycogen. The ablation also caused a fall in blood free amino acids and a rise in the tissues, which was reversed in the blood and foot muscle by injection of the extract. Possible conversion of amino acids to total carbohydrates and glycogen by gluconeogenesis is suggested.

Key words. Snail; *Cryptozona ligulata;* optic tentacles; hyperglycemic factor; free amino acids; total carbohydrates; carbohydrate metabolism.

The role of the optic tentacles of pulmonate gastropods in regulating metabolism is little understood, although there are reports of the occurrence of neurosecretory cells in them[1-4]. However, the tentacular control of reproduction and aestivation of a few snails has been described[5-9]. The role of optic tentacles of *C. ligulata* in regulating carbohydrate metabolism is discussed in the present investigation.

Materials and methods. The collection of snails, their maintenance and ablation of optic tentacles have been described elsewhere[10]. The extract of optic tentacles was prepared by homogenizing the optic tentacles in phosphate buffer (pH 7.4) and centrifuging at 2000 rpm for 10 min to remove cellular debris. The extract was prepared so that 0.2 ml contained active substance equivalent to the quantity present in two optic tentacles. 0.2 ml of the extract was injected into normal snails (with optic tentacles intact) and experimental snails (24 h after removing the optic tentacles). Control snails received 0.2 ml of foot muscle extract prepared likewise. The snails were sacrificed 30, 60, 90 and 120 min after injection. The foot muscle, mantle and hepatopancreas were isolated after collecting the blood, and dried at 80 °C to constant weight. Total carbohydrates and total free amino acids in the blood and tissues, and glycogen in the tissues were determined[11,12]. The data were subject to statistical analysis using Student's t-test[13].

Partial characterization of the hyperglycemic factor of optic tentacles. The hemolymph TCHO of the snail was measured 30, 60, 90 and 120 min after injecting a) boiled extract, b) acid extract, c) alcohol extract, d) albumin extract and e) globulin extract of optic tentacles. The buffer extract was boiled for 15 min in a water bath, cooled, and the clear supernatant obtained after centrifugation was used as boiled extract. The optic tentacles were homogenized in ice-cold acetone and filtered through Whatman paper 1. After repeated washings with acetone, the cold-dried residue was extracted with 0.1 N HCl and used as acid extract. The alcohol extract was prepared as a clear supernatant after centrifugation of the optic tentacular homogenate prepared in absolute ethanol.

The proteins were fractionated in the cold from the buffer extract by ammonium sulphate precipitation[14]. The albumins and globulins obtained as major fractions were dissolved separately in 0.1 N HCl and used as separate extracts. All the extracts were prepared in such a way that 0.2 ml contained active principle equivalent to the quantitiy in two optic tentacles. The optimum dose and time course were determined prior to experimentation. The methodology followed here, although very simple and basic, has yielded highly significant and promising results.

Results. The normal levels of total carbohydrate, glycogen and free amino acids in the tissues of the snail, *C. ligulata* are presented in table 1. The effect of optic tentacle extract on glycogen in the tissues, TCHO and FAA in the blood and tissues is shown in the figure, 1a–1d. Both ablation and injection of extract into ablated and normal snails cause a significant rise in