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)

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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].

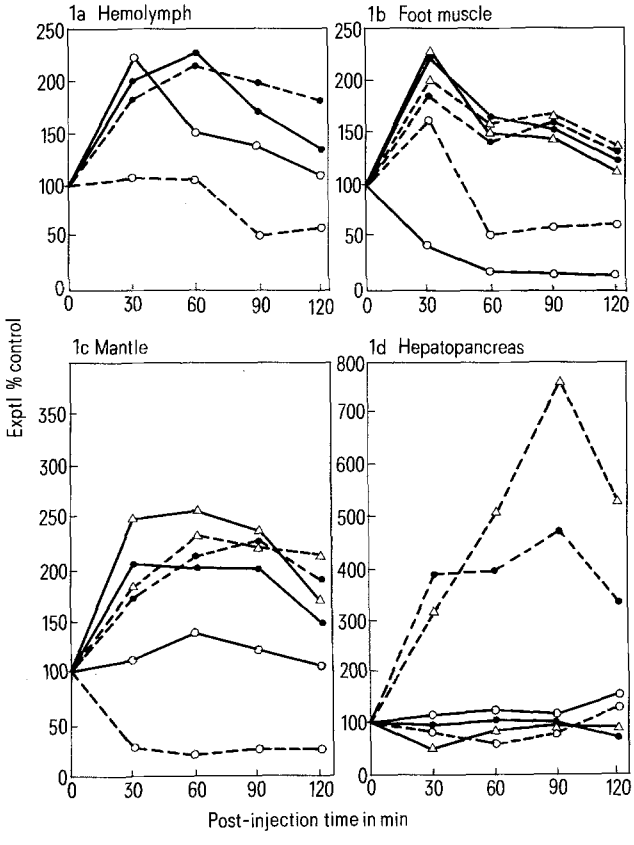
Partial characterization of the hyperglycemic factor of optic tentacles. The hemolymph TCHO of the snail was measured 30, 60, 90 and 120 min after injecting a) boiled extract, b) acid extract, c) alcohol extract, d) albumin extract and e) globulin extract of optic tentacles. The buffer extract was boiled 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

Table 1. Control levels of total carbohydrate (TCHO), glycogen and free amino acids (FAA) in the hemolymph, foot muscle, mantle and hepatopancreas of the snail *C. ligulata*. The values mg/100 ml for hemolymph, g/100 g dry wt for tissues are means ± SD of 10 determinations

	Tissues	TCHO	Glycogen	Free amino acids (mg)
Normal snails	Hemolymph	11.3 ± 3.9	–	2.1 ± 0.7
	Foot muscle	12.01 ± 4.0	6.0 ± 2.0	158.4 ± 53.8
	Mantle	14.06 ± 4.7	7.4 ± 2.5	576 ± 198
	Hepatopancreas	4.91 ± 1.7	1.75 ± 0.6	619 ± 208
Ablated snails	Hemolymph	23.5 ± 8.0	–	0.7 ± 0.2
	Foot muscle	28.52 ± 9.8	14.68 ± 5.0	939 ± 313
	Mantle	27.25 ± 9.2	13.35 ± 4.5	840 ± 283
	Hepatopancreas	27.2 ± 9.1	12.83 ± 4.3	665 ± 222



Effect of buffer extract of optic tentacles on total carbohydrates (TCHO), glycogen and free amino acid (FAA) levels in the hemolymph (i.e. blood), foot muscle (entire foot), mantle and hepatopancreas (digestive gland) of the ablated and normal snails, *C. ligulata*. ● = TCHO (—) = ablated snails, ○ = FAA (– – –) = normal snails, △ = glycogen.

total carbohydrates of blood and tissues but none in the hepatopancreas of the ablated snails. There is a decrease in glycogen in the hepatopancreas and a rise in other tissues of the ablated and normal snails. The ablation is followed by a decrease in free amino acids in the blood and rise in the tissues. It is reversed in the blood and foot muscle but not in the mantle and hepatopancreas by injection of the extract. Injection of extract into normal snails causes a significant decrease in free amino acids in the hemolymph, mantle and hepatopancreas, and an initial rise after 30 min followed by a fall in level in the foot muscle.

The significance of these changes is further enhanced by the experiments with various extracts. The buffer extract, alcohol extract, and albumin extracts cause a significant rise in total carbohydrates. The boiled, acid and globulin extracts have no significant effect (table 2).

Table 2. Effect of different extracts of optic tentacles on the hemolymph TCHO of the snail *C. ligulata*. The values mg/100 ml of blood are means ± SD of six determinations. Normal snails = 11.33 ± 3.92 (n = 10)

Type of extract	Time post-injection 30 min	60 min	90 min	120 min
1) Control Muscle extract	24.2 ± 11.0	18.0 ± 8.1	17.5 ± 7.9	16.5 ± 7.5
2) Experimental a) Buffer extract	44.5 ± 20.2*	39.1 ± 17.6*	34.9 ± 15.0*	30.2 ± 13.7*
b) Alcohol extract	41.6 ± 19.0*	52.8 ± 23.7*	57.9 ± 26.0*	50.9 ± 22.9*
c) Albumin extract	94.2 ± 42.1*	41.4 ± 18.5*	31.7 ± 14.2	18.4 ± 8.3

*p-values = < 0.01.

Discussion. It is apparent that ablation of optic tentacles is followed by a rise in total carbohydrates in the hemolymph and tissues, and synthesis of glycogen. Injection of tentacular extract too has a similar hyperglycemic effect. This is achieved, perhaps, by glyconeogenesis[10]. The alteration in the levels of free amino acids strongly indicate this possibility. The methods of extraction and characterization of the active principle followed here are very simple. Nevertheless, the presence in the optic tentacles of a hyperglycemic factor which is thermolabile, water-soluble and perhaps albuminous has been revealed.

When studying the constancy of blood constituents of *Helix pomatia*, Holtz and van Brand[15] suggested that there might be regulation. Closely-regulated carbohydrate metabolism during starvation and aestivation of several molluscs suggests possible endocrine involvement[16–19]. Goddard et al.[20] found that injection of albumin gland extract into *Helix aspersa* produced hyperglycemia. Even liver homogenate had a similar effect. The central ganglia of *Ariophanata* were found to produce hypo- and hyperglycemic factors[21]. The present investigation has revealed that the optic tentacles of *C. ligulata* produce a hyperglycemic factor, possibly a protein, involved in regulating carbohydrate metabolism.

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