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2 M.S. Feather and R.L. Whistler, Tetrahendron Lett. *1962,* 667.
3 D.J. Hoffman and R.L. Whistler, Biochemistry *7,* 4479 (1968).
4 M.J. Pitts, M. Chemielewski, M.S. Chen, M.M.A.Abd El.-Rahman and R.L. Whistler, Archs Biochem. Biophys. *169,* 384 (1975).
5 R.L. Whistler and W.C. Lake, Biochem. J. *130,* 919 (1972).
6 R.C. Nordlie, in: Control of Glucagon metabolism, p. 153. Ed. W.J. Whelan. Academic Press, New York 1968.
7 A.St.G. Huggett and Nixon, Lancet *2,* 368 (1957).
8 H.A. Krebs, D.A.H. Bennet, P.De Gasquet, T. Gascoyne and T. Yoshida, Biochem. J. *86,* 22 (1963).
9 P.L. Jorgensen, Biochem. biophys. Acta *151,* 212 (1968).
10 Y. Suketa, M. Sato and M. Kura, Experientia *36,* 438 (1980).
11 D. Yeung, R.R. Stanley and I. Oliver, Biochem. J. *105,* 1219 (1968).
12 A. White, P. Handler, E.L. Smith, R.L. Hill and I.R. Lehman, in: Principles of biochemistry, 6th edn, p.499. McGraw Hill Kogakusha Ltd, Tokyo 1978.
13 H.A. Harper, V.W. Rodwell and P.A. Mayes, in: Review of physiological chemistry, 17th edn, p.311. Lange Medical Publications, Maruzen 1979.
14 P.K. Joseph and K. Subrahmanyam, Indian J. Biochem. *7,* 45 (1970).
15 H.A. Krebs, Proc. R. Soc., B *159,* 545 (1964).
16 P.K. Joseph and K. Subrahmanyam, Biochem. J. *128,* 1293 (1972).
17 R. Sundaresan and K. Subrahmanyam, Indian J. Biochem. Biophys. *8,* 172 (1971).
18 S. Ramakrishnan, P.K. Joseph, R. Krishnamoorthy and K. Subrahmanyam, Indian J. Biochem. Biophys. *12,* 28 (1975).
19 S. Ramakrishnan, C.V. Prasanna, A. Balusubramanian and P.K. Joseph, Indian J. Biochem. Biophys. *11,* 61 (1974).
20 E.S.G. Baron, C.M. Lyman, M.A. Lipton and J.M. Goldinger, J. biol. Chem. *141,* 957 (1941).
21 P.B. Iyenedjian and G. Peters, Am. J. Phys. *226,* 1281 (1974).

Metabolism of the snail *Cryptozona ligulata* during regeneration of optic tentacles

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Summary. The biochemical changes in the hemolymph and tissues were followed during regeneration of the optic tentacles of the snail *Cryptozona ligulata* (Pulmonata-Stylommatophora). There is a remarkable increase in total carbohydrates in hemolymph and tissues and glycogen in tissues at the expense of free amino acids and fatty acids. It is clear that ablation of the optic tentacles stimulates carbohydrate synthesis through 'glyconeogenic' routes. The optic tentacles regenerate completely in 18–21 days.

The structure and regeneration of optic tentacles has been reported for a few gastropods[1], although not much is known about metabolism during their regeneration. It is well established that optic tentacles play a crucial role in the regulation of gametogenesis, oviposition, regeneration of gonads, and accumulation of galactogen in the albumen gland in some gastropods[2–9]. It is possible that the optic tentacles have an influence on general metabolism as well. This investigation presents the biochemical changes during the regeneration of optic tentacles of *C. ligulata.*

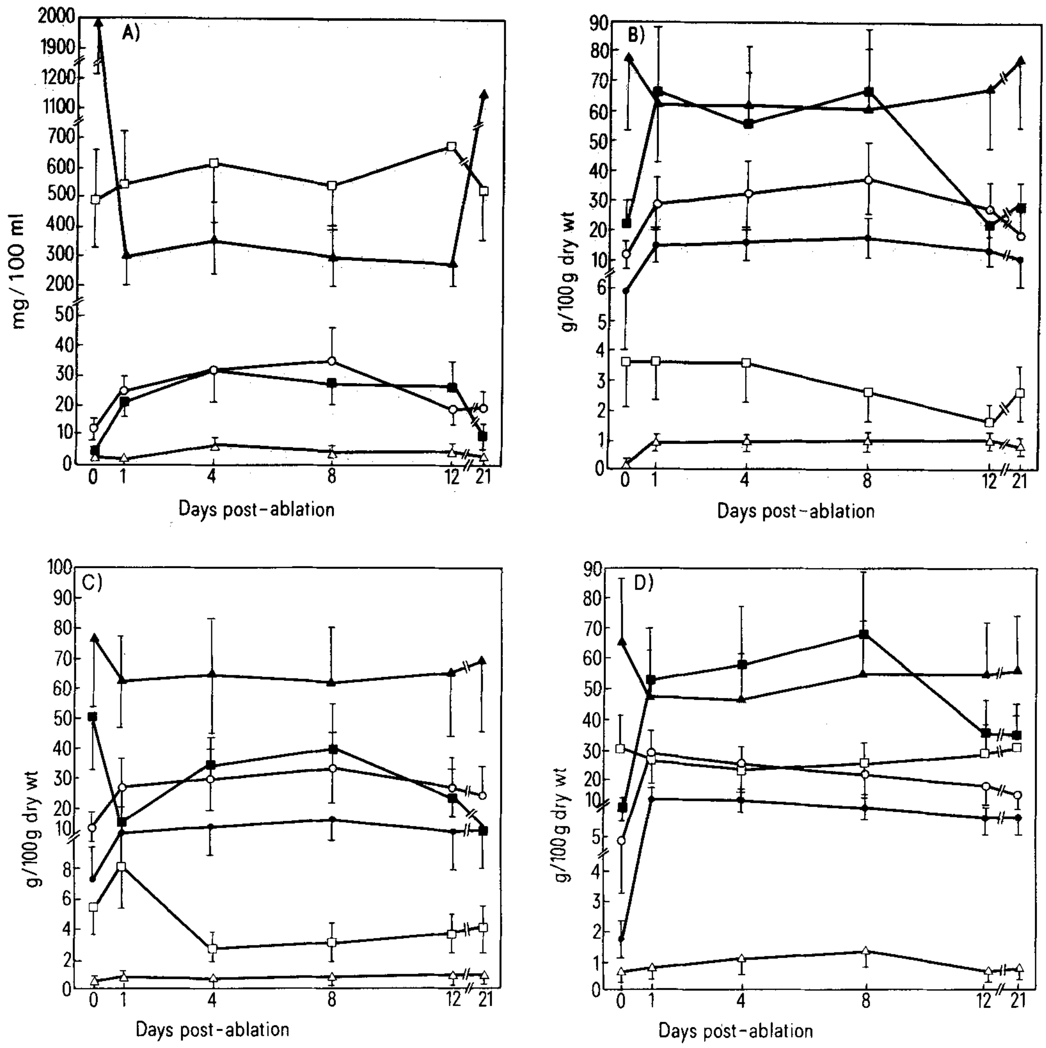
Materials and methods. Snails of uniform size collected locally were maintained on cabbage leaves over moist soil in vivaria. The optic tentacles of active snails were snap-cut and the ablated snails were maintained separately. Unoperated animals served as controls.

The normal and ablated snails were sacrified 1, 4, 8, 12, and 21 days after the operation. They were starved for 24 h prior to sacrifice. Foot muscle, mantle and hepatopancreas were isolated after collecting the hemolymph, and were dried at 80 °C in a hot-air oven to constant weight.

Total carbohydrates (TCHO) and total free amino acids in the trichloroacetic acid (TCA 5% w/v) supernatant of hemolymph were determined using anthrone and Folin-Ciocalteau reagent respectively[10,11]. Total hemolymph protein in the TCA precipitate was similarly determined using Folin reagent[12]. Total lipid in an aliquot of hemolymph was extracted and determined gravimetrically[13], and the percentage of free fatty acids in the lipid was determined by microtitration[14]. The dry tissues were repeatedly extracted with TCA (5% w/v) and total carbohydrates (TCHO) in the TCA supernatant and glycogen precipitated with methanol from the TCA supernatant were determined by the anthrone method[10]. Total protein in the TCA precipitate solubilized in 1 N NaOH and total free amino acids in the TCA supernatant were determined using the Folin-Ciocalteau reagent[11,12]. Lipid was extracted from the dry tissues and assayed gravimetrically[13], and the percentage of free fatty acids in the lipid fraction was determined by microtitration[14].

Results. The change in biochemical constituents in the hemolymph and tissues of the snail during regeneration of optic tentacles are given in figures a–d. There is a significant increase in TCHO in the hemolymph and tissues and glycogen in the tissues following ablation. The levels remain significantly high even at the end of regeneration period of 21 days. There is a significant decrease in the total protein level in the hemolymph but no remarkable change in the tissue during the regeneration period. There is a significant increase in total free amino acids in the tissues and this is maintained throughout the regeneration period. But in hemolymph there is a significant drop on the 1st day followed by a rise on the 4th day. This subsequently levels off, and a significant decline is obvious by day 21. There is an increase in total lipid in the mantle on the 1st day but a decrease to a subnormal level subsequently. There is no change in total lipid level in hepatopancreas, but in foot muscle the level reaches a significant low on the 8th and 12th day of regeneration. There is a significant fall in the percentage of free fatty acids in the mantle and a significant rise in hemolymph, foot muscle and hepatopancreas during the period of regeneration.

Discussion. The optic tentacles of *C. ligulata* regenerate in about 18–21 days and this period agrees with 20 days for *Ariolimax agrestis*[1]. The snails recover from surgical shock quickly, crawl about and feed normally. About the impact of ablation and regenerating optic tentacles on the snail's metabolism very little is known. It has been reported that ablation of optic tentacles in *Ariolimax columbianus* is followed by growth of the albumen gland and galactogen synthesis[9], suggesting that carbohydrate metabolism in this gland is under the control of the optic tentacles. A similar control perhaps operates in *C. ligulata* as well. The increase



Change in biochemical constituents in hemolymph (A); foot muscle (B); mantle (C); and hepatopancreas (D) of the snail *C. ligulata* after ablation of optice tentacles. ○, Total carbohydrate; ●, glycogen; ▲, total protein; △, total free amino acids; □, total lipids; ■, percent free fatty acids.

in glycogen in tissues and hemolymph-hyperglycaemia are perhaps associated with mobilization by glyconeogenic routes. The increase in free amino acids due to proteolysis in hepatopancreas and the rise in free fatty acids due to lipolysis in all the tissues suggests such a conversion.

It is possible that tissue metabolism of the snail is under the control of optic tentacles. Apart from classical histological evidence for neurosecretion in the optic tentacles of certain stylommatophoran pulmonates[15-17], there is abundant evidence to indicate that optic tentacles have a decisive influence on reproductive phenomena in many pulmonate snails[4-6,9,18]. Further evidence has been obtained to indicate that optic tentacles control carbohydrate synthesis by regulating gluconeogenesis[19]. It is not clear whether fully regenerated optic tentacles function in a manner similar to the original tentacles. Figures a-d suggest that although the optic tentacles are fully regenerated by the end of 21 days, all the metabolites do not reach the pre-ablation levels. However, a gradual tendency to return to pre-ablation levels after 21 days is clearly indicated.

1 L.H. Hyman, Invertebrates, Mollusca *1*, 615 (1967).
2 H. Gottfried and R.I. Dorfman, Gen. comp. Endocr. *15*, 101 (1970).
3 H. Gottfried, R.I. Dorfman and E. Forchielli, Gen. comp. Endocr. *9*, 454 (1967).
4 C. Wattez, Gen. comp. Endocr. *21*, 1 (1973).
5 C. Wattez and M. Durchon, C.r. hebd. Séanc. Acad. Sci., Paris *274*, 2328 (1972).
6 N. Takeda, Nature *267*, 513 (1977).
7 J. Bierbaur, Acta. biol. hung. *25*, 147 (1975).
8 L. Gomot, Archs Anat. Histol. Embryol. *56*, 131 (1974).
9 V.R. Meenakshi and B.T. Scheer, Comp. Biochem. Physiol. *29*, 841 (1969).
10 N.V. Carroll, R.W. Longley and J.H. Roe, J. biol. Chem. *220*, 583 (1956).
11 M.L. Anson, Experimental Biochemistry. Wiley, New York 1960.
12 CH. Lowry, N.J. Rosenbrough, A.D. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
13 J. Folch, M. Less and G.H. Stanesley, J. biol. Chem. *226*, 497 (1957).
14 L.V. Cocks and C. Van Rede, Laboratory handbook for oil and far analysts. Academic Press, New York 1966.
15 N.J. Lane, Q. J. microsc. Sci. *103*, 211 (1962).
16 N.J. Lane, Q. J. microsc. Sci. *105*, 31 (1964).
17 D.C. Rogers, Z. Zellforsch. *102*, 113 (1969).
18 E.M. Goudsmit, J. exp. Zool. *191*, 193 (1975).
19 O.V. Subramanyam, Doctoral thesis, S.V. University, Tirupati 1981.