difference for hybridization between the incubation at 24 and 37 °C. This suggests that self-association probably does not play an important role in apoA-I and apoA-II hybridization. It is of interest that the apoA-I did not associate with ^{125}I-labeled apoC-II and apoC-III. The apo C proteins in plasma are in equilibrium between HDL and triglyceride-rich particles. In vitro, the C proteins exchange rapidly between triglyceride-rich particles and HDL[16].
In vivo there is a net transfer of C proteins from HDL to triglyceride-rich particles but no transfer of A proteins[17] following a meal. Our finding that apoproteins C-II and C-III did not directly associate with apoprotein A-I suggests that apoprotein A-I in HDL may not play a direct role for the net exchange of apoproteins C between HDL and triglyceride-rich particles. Furthermore, it would be of interest to utilize anti-apoA-II antibodies as a reverse experiment to demonstrate if ^{125}I-labeled apoA-I would associate with apoA-II.

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Effect of 5-thio-D-glucose on blood glucose and glucose-6-phosphatase activity in mice

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Summary. Blood glucose was significantly elevated by 5-thio-D-glucose administration (25, 50, and 100 mg/kg). The rate-limiting enzyme glucose-6-phosphatase of liver was also increased. The elevation of blood glucose was due to the rapid glycogenolysis of liver.

5-Thio-D-glucose[2], a structural analogue of D-glucose causes an increase of blood glucose when it is administered i.p., i.v. and also p.o.[3,4]. The compound inhibits glucose uptake and glycolysis in liver, kidney and diaphragm[5]. The increase in blood glucose concentration from liver and kidney was believed to be controlled through glucose-6-phosphatase activity[6]. Since the compound increases blood glucose, the glucose-6-phosphatase activity was studied in intestines, liver and kidneys.

Materials and methods. Male albino mice weighing 25–30 g were used. The animals were maintained on the stock laboratory diet (obtained from Hindustan Lever, India) and water ad libitum. Glucose-6-phosphate (disodium salt) was obtained from Sigma Chemicals Co. (St. Louis, USA) and glucose oxidase from Koch-Light Laboratories Ltd (England). All animals were fasted for 24 h before the experiment and after a single gastric intubation of 5-thio-D-glucose (25, 50 and 100 mg/kg b.wt) were sacrificed by decapitation at various intervals (0, 30, 60, 90, 120, 150 and 180 min) for samples of blood and tissues, viz., liver, kidneys and intestines. Blood glucose was estimated by the method of Huggett and Nixon[7] as adopted by Krebs et al.[8]. Microsomes were prepared from the homogenates of liver, kidneys and intestines according to the method of Jorgensen[9] as described by Suketa et al.[10]. Glucose-6-phosphatase activity was estimated in the heavy microsomal fraction obtained by centrifugation (25,000 × g, 30 min) of the supernatant and sedimentation of the mitochondria at 10,800 × g for 30 min. All the preparations were carried out at 4 °C. The glucose-6-phosphatase activity was determined by the method of Yeung et al.[11]. The activity was expressed as units/g tissue. 1 unit of the enzyme releases 1 µmole of inorganic phosphate per min from glucose-6-phosphate at 30 °C.

Results and discussion. With graded doses of 5-thio-D-glucose (5 TG), i.e., 25, 50 and 100 mg/kg b.wt, there was an increase in blood glucose with time (fig. 1). With all the concentrations of 5 TG, blood glucose reached a maximum in 30 min and returned to near normal within 180 min. It was observed that the rise in blood glucose was proportional to the dose of 5 TG. The blood glucose was maximum in 30 min sample and then slowly decreased to almost normal in 180 min sample.

A continuous source of blood glucose is the hydrolysis in liver, kidneys and intestines of glucose-6-phosphate, which is derived either from glycogenolysis or other potential precursors[12]. Gluconeogenesis in liver[13] and kidney cortex[8], is well documented and both the tissues contain the enzymes of gluconeogenesis[14]. Hydrolysis of glucose-6-phosphate to glucose is effected by glucose-6-phosphatase and

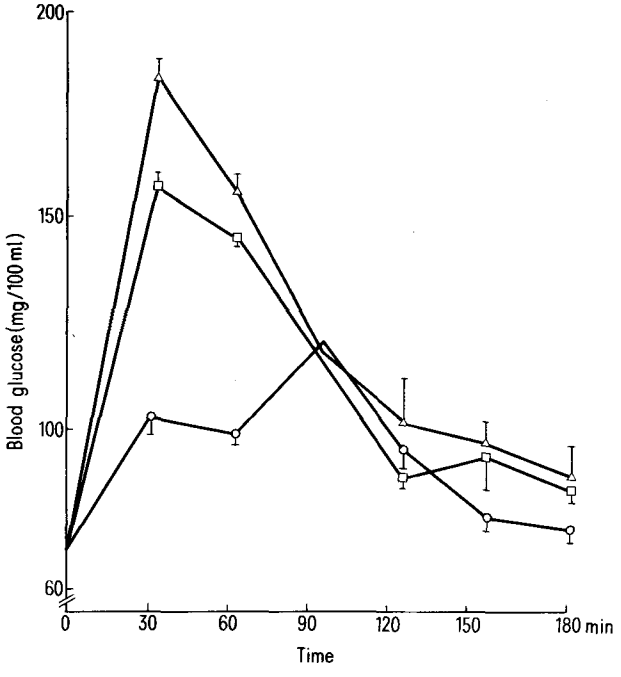

Figure 1. Effect of 5-thio-D-glucose (25 mg/kg, 50 mg/kg and 100 mg/kg b.wt) on blood glucose. O—O, 25 mg/kg; □—□, 50 mg/kg; and △—△, 100 mg/kg b.wt of 5-thio-D-glucose treatment. Values are mean ± SEM for 4 mice in each group.

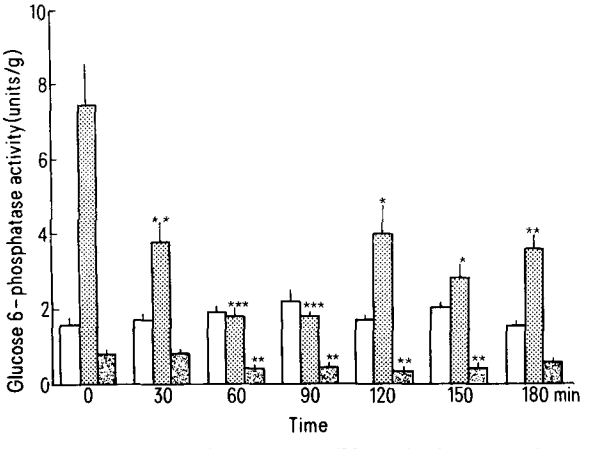

Figure 2. Effect of 5-thio-D-glucose (25 mg/kg b.wt) on glucose-6-phosphatase activity. □, Liver; ▨, kidney, and ■, intestine. Each point indicates mean ± SEM of 4 mice in each group. * $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$ and **** $p < 0.001$.

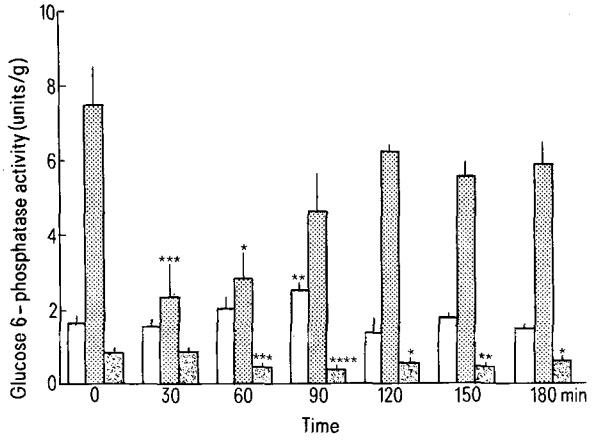

Figure 3. Effect of 5-thio-D-glucose (50 mg/kg b.wt) on glucose-6-phosphatase activity. □, Liver; ▨, kidney, and ■, intestine. Each point indicates mean ± SEM of 4 mice in each goroup. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

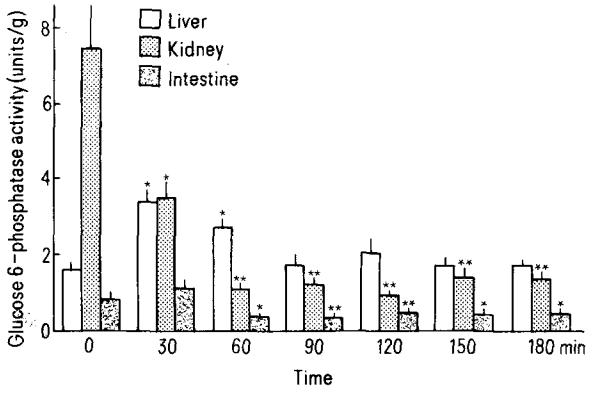

Figure 4. Effect of 5-thio-D-glucose (100 mg/kg b.wt) on glucose-6-phosphatase activity. Each point indicates mean ± SEM of 4 mice in each group. * $p < 0.01$ and ** $p < 0.001$.

the free glucose diffuses from the cell into the extracellular spaces including the blood thereby increasing blood glucose[13]. This enzyme is present in liver, kidneys and intestines; of these, liver and to a limited extent kidneys contribute to blood sugar[15]. As the blood glucose was increased by 5 TG, the rate-limiting enzyme glucose-6-phosphatase was estimated in the liver, kidneys and intestines of all the samples taken at different intervals of time.

In the liver, the enzyme activity was increased from 30 min sample to 90 min and then came back almost to normal in 180 min with all the doses of 5TG (figs 2–4). This suggests that either glycogenolysis or gluconeogenesis is increased in liver. But our earlier work showed that liver glycogen content was diminished by 50% after an administration of 5 TG[2]. Hence the increase of the enzyme could only be due to active glycogenolysis. Kidneys do not contain any significant glycogen. The activity of kidney glucose-6-phosphatase is chiefly concerned with gluconeogenesis[14,16], i.e., the release of glucose from glucose-6-phosphate. The renal enzyme is increased in conditions like diabetes[14,16], starvation[8,17] and acidosis[8,18], but decreased in alcohol-intake[19], thiamine deficiency[18,20], etc. Renal gluconeogenesis is normally increased when blood glucose is lowered[21]. In the present work, when the blood sugar is increased, possibly through hepatic glycogenolysis, there is no necessity for the kidneys to synthesize glucose through gluconeogenesis. Our finding of a significant lowering of glucose-6-phosphatase of the kidneys corroborates this conclusion (figs 2–4).

Intestines do not have any significant glycogen stores nor do they synthesize glucose by gluconeogenesis. Intestinal enzyme was not affected perhaps because, it has no significant role in either gluconeogenesis or glycogenolysis (figs 2–4).

It is therefore concluded that the hyperglycemia brought about by 5 TG is due to increased glycogenolysis in liver.

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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