Die Proteine wurden in eine wasserlösliche und eine wasserunlösliche Fraktion zerlegt. Zu diesem Zweck wurden ganze Erlenmeyer-Kulturen abgenutscht, gewaschen und mit destilliertem Wasser in einem Glashomogenisator zerrieben. Die unlöslichen Anteile wurden abzentrifugiert und einmal mit Wasser ausgewaschen. In den vereinigten Überständen wurden die löslichen, im Sediment die unlöslichen Proteine durch Fällung mit Trichloressigsäure, Veraschen und Photometrieren mit Nessler-Reagens bestimmt[9]. Alle Werte sind Mittelwerte aus 5–6 Bestimmungen und wurden auf ganze Erlenmeyer-Kulturen bezogen.

Resultate und Diskussion. Wie aus Figur 1a hervorgeht, nehmen normale Kulturen zuerst bevorzugt Ammonium auf, was darin zum Ausdruck kommt, dass in den ersten 10 Tagen 80% des Ammoniums, aber nur 20% des Nitrats verbraucht werden. Erst nach fast vollständigem Verbrauch des Ammoniums wird vermehrt Nitrat aufgenommen, was zu einem sigmoiden Verlauf des Nitratgehalts in der Nährlösung führt. Ein Rest von ungefähr 5% Ammonium wird von diesen Kulturen anscheinend nicht mehr resorbiert. Eine bevorzugte Aufnahme von Ammonium neben Nitrat ist in vielen Pflanzen festgestellt[10] und auf eine Hemmung der Nitratreduktase durch Ammonium zurückgeführt worden[11].

In glucosestimulierten Kulturen werden, wie Figur 1b zeigt, sowohl Ammonium, als auch Nitrat schneller aufgenommen als in den Kontrollen. In bezug auf die Selektionierung zwischen Ammonium und Nitrat fällt jedoch auf, dass nach 3 Tagen bereits 20% des Nitrats aufgenommen sind, während im gleichen Zeitpunkt vom Ammonium erst 40% verbraucht sind. Dies bedeutet, dass in diesen Kulturen die Aufnahme von Nitrat gefördert wird. Ob Glucose selber diese Stimulierung hervorruft und ob sie, wie die meisten Regulatoren der Nitratassimilation[5], auf der Stufe der Reduktion des Nitrats zum Nitrit (Nitratreduktase) angreift, wird anhand weiterer Versuche abzuklären sein.

Der zeitliche Verlauf von Stickstoffaufnahme und Proteingehalt geht aus Figur 2 hervor. In normalen Kulturen (Figur 2a) erfolgt die Aufnahme des Stickstoffs aus der Nährlösung nahezu linear mit der Zeit. Vom aufgenommenen Stickstoff werden zum gleichen Zeitpunkt etwa 60% in den Proteinen wiedergefunden, wovon ungefähr $^2/_3$ auf die wasserlöslichen und $^1/_3$ auf die wasserunlöslichen Proteinfraktionen entfallen. Der Fehlbetrag in der Bilanz, der hier etwa 40% beträgt, dürfte zur Hauptsache auf resorbierte aber nicht metabolisierte N-Quellen, sowie auf niedermolekulare N-Verbindungen zurückzuführen sein.

In glucosestimulierten Kulturen (Figur 2b) folgt der Gesamtproteingehalt ebenfalls streng der Menge des aufgenommenen Stickstoffs. Allerdings erreichen hier beide Grössen nach 10 Tagen ein Plateau. Die unlöslichen Proteine nehmen über die ganze Versuchsdauer linear, aber rascher als in den Kontrollen, zu. Die Menge der löslichen Fraktion dagegen durchläuft nach einem anfänglichen steilen Anstieg ein Maximum und fällt nach dem 10. Tag wieder ab. Nach 20 Tagen liegt die Menge löslicher Proteine sogar unter derjenigen der unlöslichen, ein Zustand, der in normalen Kulturen nicht eintritt. Dies bedeutet, dass vom Moment an, da die Stickstoffquellen der Nährlösung erschöpft sind, das Verhältnis zwischen löslichen und unlöslichen Proteinen ständig abnimmt. Der Verlauf der Kurven lässt sogar vermuten, dass eine weitere Synthese von unlöslichen Proteinen durch einen Abbau löslicher Proteine wettgemacht wird. Bereits aus dieser groben Zerlegung der Zellproteine in zwei Fraktionen geht hervor, dass die einzelnen Proteine vom Stickstoffmangel sehr unterschiedlich betroffen werden, was eine quantitative Veränderung des Protein- und Enzymmusters dieser Pflanzen zur Folge haben muss. Da 70% der Zellproteine in den Plastiden lokalisiert sind[12], werden diese durch den Vorgang besonders stark betroffen. Der Abbau des Lamellarsystems ist eine sichtbare Folge davon und zeigt, dass auch Membranproteine davon erfasst werden. Wir schliessen aus diesen Ergebnissen, dass ein teilweiser oder vollständiger Verlust wichtiger Enzymproteine die primäre Ursache der strukturellen Veränderungen und der Einstellung der Photosynthesetätigkeit dieser Pflanzen während der Vergilbung darstellt.

Summary. The uptake of ammonium and nitrate by normal and glucose-stimulated *Spirodela oligorrhiza* plants was investigated. Nitrate is used more rapidly by plants grown on glucose than by controls, suggesting a stimulation of nitrate assimilation by glucose. In plants growing on a normal nutrient, the contents of both water-soluble and water-insoluble proteins linearly increase with time and nitrogen uptake. In glucose-stimulated cultures soluble protein increases rapidly during the first 10 days of cultivation, but declines thereafter, while insoluble protein rises continuously over the whole period of time. The decreasing ratio of soluble and insoluble protein reflects an alteration of the protein and enzyme pattern, which is suggested to be the primary reason for the change of plastid ultrastructure and the loss of photosynthetic activity.

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Succinic Dehydrogenase Activity During Starvation of a Terrestrial Pulmonate *Ariophanta* sp.

Metabolism of molluscan tissues during starvation and aestivation has been widely studied. These studies have revealed that many molluscs have lipid oriented metabolism, while others are carbohydrate-oriented. Terrestrial pulmonate *Helix pomatia*, for example, is carbohydrate-oriented[1]. Even though carbohydrate is the major energy source, considerable quantities of protein and lipid are utilized during starvation and aestivation. Similar observations have been made in fresh-water pulmonate *Planorbis corneus*[2], and in terrestrial snail *Ariophanta* sp.[3]. In *Thias lamellosa* there is an increase in oxygen consumption at 53 days of starvation[4]. Pulmonate snails, however,

show a decrease in oxygen consumption during starvation[5,6].

Decrease in the activity of a number of enzymes during aestivation has been reported by many workers. Decrease in succinic dehydrogenase, cytochrome oxidase, and ATPase in aestivating amphibious snail *Pila globosa* was observed[7,8]. Studies on *Pila virens* during aestivation have revealed the energy metabolism to be highly controlled and regulated[9,10]. Since the metabolism was found to be highly regulated during starvation as well in many cases[2,3], adaptive changes in the activity of a number of enzymes might be expected in order to regulate and conserve the energy utilization during starvation period. With this in view the present investigation was undertaken and the activity of succinic dehydrogenase was studied during different periods of starvation in *Ariophanta* sp. a terrestrial gastropod.

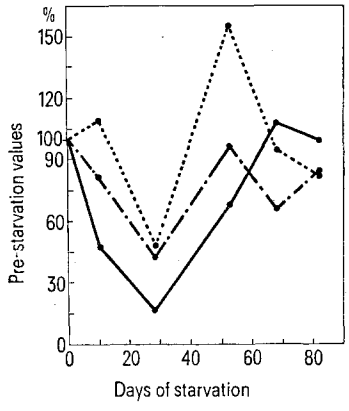
Materials and methods. *Ariophanta* sp. of uniform size were collected locally and stocked in glass containers in the laboratory. After one day a set of animals was sacrificed and the hepatopancreas, foot and the nerve ring were immediately isolated. Similarly the tissues were isolated from sets of snails after 10, 28, 52, 68, and 82 days of starvation.

The tissues were weighed and homogenized in cold sucrose solution (0.25*M*) to 10% W/V for hepatopancreas and foot and 5% W/V for nerve ring. The homogenates were centrifuged for 15 min at 3,000 rpm and succinic dehydrogenase activity was studied in the homogenate supernatants.

Enzyme assay. The succinic dehydrogenase activity in the supernatant was studied by *p*-Indophenyl-3-*p*-nitrophenyl tetrazolium chloride (INT) reduction method[11,12] with modification as follows. The incubation mixture contained 0.5 ml sodium succinate (0.06*M*), 1.0 ml of phosphate buffer of pH 7.4 (Sørensen) (0.06*M* succinate and 7.4 pH were found to give optimum activity in the tissues studied), 0.5 ml of 0.2% INT and 1.0 ml of homogenate supernatant. Controls contained, besides the reagents, 1.0 ml of heat-denatured homogenate supernatant. The incubation was carried out at 37 °C for 20 min for hepatopancreas and foot, for 8 min for nerve ring. The reaction was arrested by the addition of 6.0 ml of glacial acetic acid. Formazon formed was extracted in 5.0 ml of toluene at 0 °C overnight. The optical density of the toluene layer was measured in EEL photoelectric colorimeter using blue filter. The enzyme activity was expressed in µmoles of formazon per gram wet weight of tissue per hour.

Results. The Table shows succinic dehydrogenase activity in the tissues of *Ariophanta* sp. experssed in µmoles/g wet weight of tissue/h during different periods of starvation. A remarkable decline in the enzyme activity was noticed up to 28 days of starvation in all the tissues studied. Hepatapancreas showed more than 85% reduction in the activity, while in the foot and nerve ring it was nearly halved about the same period (Figure). After 28 days the nezyme activity showed a gradual rise throughout the starvation period suggesting an incrasing respiratory metabolism after 28 days. The data also indicate that during starvation the enzyme activity in the foot and nerve ring was higher compared to the activity in the hepatopancreas.

Discussion. The respiratory metabolism as revealed by succinic dehydrogenase activity shows a clear pattern of regulation in *Ariophanta* sp. There is a remarkable decrease in the enzyme activity in the first 28 days. This



Succinic dehydrogenase activity in the tissues of *Ariophanta* sp. expressed in % prestarvation values. ●—●, Hepatopancreas; ●---●, Foot; ●·····●, Nerve-ring.

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S. No.	Days of starvation	Number of animals	Hepatopancreas Mean ± S.D.	*p*-values	Foot Mean ± S.D.	*p*-values	Nerve-ring Mean ± S.D.	*p*-values
1.	Normal	10	201.6 ± 23.5	—	355.2 ± 38.3	—	730.0 ± 48.1	—
2.	10 Days	8	99.2 ± 23.4	S	388.9 ± 65.9	NS	586.0 ± 82.6	S
3.	28 Days	8	31.7 ± 6.3	S	175.3 ± 39.1	S	330.0 ± 75.0	S
4.	52 Days	10	138.8 ± 18.5	S	549.6 ± 24.9	S	705.0 ± 15.0	NS
5.	68 Days	8	218.0 ± 40.0	NS	336.0 ± 30.8	NS	476.2 ± 37.5	S
6.	82 Days	10	200.0 ± 53.5	NS	294.0 ± 50.9	S	620.0 ± 30.0	S

Succinic dehydrogenase activity in the tissue supernatants of *Ariophanta sp.* during different periods of starvation
Probability *p* values, S = significant; NS = not significant. Enzyme activity is expressed in µmoles/g wet wt./h.

decrease meansa decrease in the aerobic metabolism with consequent conservation of food stores. However, this conservation could not be continued for longer periods of starvation. A prolongation of starvation period resulted in a rapid decline in the major nutrient stores of the body[3]. The gradual elevation of succinic dehydrogenase activity from its lowest level at 28 days might therefore indicate a rapid breakdown of the metabolites and their utilization for sustenence during starvation period. The data also suggest that metabolism of hepatopancreas is comparatively highly regulated. Thus while there is nearly a 50% decline in the enzyme activity in the hepatopancreas after 10 days of starvation, it is maintained at more or less normal level in the foot; after 28 days there is more than 85% reduction in the enzyme activity while there is only a 50% decline in the foot. This clearly suggests that there is preferential breakdown and utilization of food reserves from foot. The enzyme activity in the nervering is reduced to half its original level after 28 days of starvation but later there is gradual increase which touches almost normal level of activity. This suggests that the activity in the nervous system does not show any marked decline during starvation[13].

Zusammenfassung. Nachweis, dass die Dehydrogenase-Aktivität im Hepatopankreas, Fuss und Schlundring bei der Lugenschnecke *Ariophanta* sp. eine ausgesprochene Enzymanpassung an die veränderten Stoffwechselbedingungen während langfristiger Hungerperioden zeigt und damit auf einen Überlebensmechanismus hinweist.

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Cholestanol and Chenodeoxycholic Acid: Metabolites of Injected Cholesterol-4-^{14}C in Pigeon Bile

Previous studies from this laboratory have shown that cholestanol (5α-cholestan-3β-ol) is present in high concentrations in tissues and feces of the White Carneau pigeon[1,2]. This stimulated us to determine whether this stanol is of endogenous origin in this species. Except for an in vitro study by SHEFER et al.[3] using rat and guinea-pig livers, others[4,5] could not completely rule out the contribution of intestinal bacterial flora in the conversion of mevalonate and cholesterol into cholestanol. We examined the possibility of the conversion of injected cholesterol into biliary cholestanol in pigeons with bile fistulas, which would eliminate the role of intestinal bacteria in this conversion. Attention was also given to the formation of biliary chenodeoxycholic and cholic acids, which are the major bile acids in pigeon bile[6]. Although this conversion of cholesterol to bile acids has been demonstrated in a number of animals[7], it has not been shown in the pigeon.

Material and methods. An albumin emulsion containing 1–2 μC cholesterol-4-^{14}C was injected i.v. into adult White Carneau pigeons after a cannula was inserted in the bile duct. The bile was collected for 6 to 8 h. Biliary steroids were extracted and the neutral products were separated from bile acids after saponification and extraction[8]. Cholestanol was separated from cholesterol by chromatography on $AgNO_3$-impregnated silica gel G[2]. Bile acids were resolved by thin-layer chromatography on silica gel G using the solvent system consisting of iso-octane : isopropyl ether : acetic acid, 50 : 25 : 40 (v/v/v) and eluted from the gel with methanol and assayed for radioactivity[8]. Identification of the bile acids as based on the behavior of bile acids on thin-layer and gas-liquid chromatography[8] and crystallization with authentic standards.

Results and discussion. The bile was collected for 6 to 8 h after the i.v. injection of cholesterol-4-^{14}C. Early samples of bile were used to determine the formation of labeled cholestanol to minimize the recirculation of cholestanol, if formed, in the intestinal tract by the labeled sterol excreted from the intestinal cell. The neutral biliary fraction (5.2% of total radioactivity) obtained after saponification was subjected to chromatography on $AgNO_3$-impregnated silica gel G to separate cholestanol from cholesterol. The cholestanol fraction was then assayed for radioactivity. An average of 2.13% of the label present in the neutral sterol fraction was contributed by cholestanol. Cholestanol fractions from a number of bile samples were pooled and the identity of this compound was established by chromatography on 2 solvent systems

Radiochemical purity of biliary cholestanol-4-^{14}C

Thin-layer chromatography (TLC)	Rf values	
	Standard	Sample
Silica gel G [a]	0.23	0.23
$AgNO_3$-impregnated silica gel G [c]	0.53	0.53
Specific activity determination [b]	dpm/mg	
First $AgNO_3$ (TLC)	168.5	
Second $AgNO_3$ (TLC)	176.3	
Third $AgNO_3$ (TLC)	170.8	

[a] Solvent system, heptane : isopropyl ether : acetic acid, 65 : 40 : 4 (v/v/v). [c] Solvent system, chloroform : methanol : acetic acid, 100 : 1 : 0.2 (v/v/v). [b] Carrier cholestanol added, 10 mg.

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