action by solvent or medium. The geometrical arrangement of CHL and CAR in chloroplast lamellae may be controlled by lamellae structure. The occurrence of an intimate association of CHL and CAR in vivo was discussed by various workers[15-17]. The details in the nature of the binding interaction between CHL and CAR and in the mechanism of control of this interaction by solvent remain open. In order to make clear these points, further investigations are in progress.

Crystalizable CHL was extracted from spinach leaves and purified according to the procedure of PERKINS and ROBERTS[18]. CAR was obtained from MERCK. The absorption spectrum was recorded with a Hitachi Recording Spectrophotometer Model 356. A reaction vessel ($1 \times 1 \times 4$ cm^3) for the photochemical reaction was irradiated, at room temperature, by the light isolated from a 300 W xenon lamp with a suitable glass filter through a water layer of 6 cm thick. The amount of photobleached CHL was determined by the absorbance decrease of CHL red peak.[19].

Zusammenfassung. Das photoxytadive Ausbleichen des Chlorophylls a wird durch die Gegenwart des β-Carotins gehemmt. Die Hemmwirkung des β-Carotins und die Wechselwirkung zwischen Chlorophyll a und β-Carotin werden vom Lösungsmittel kontrolliert.

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Demonstration of Increased in vitro Autolytic Activity in a Denervated Muscle of Frog

Increased protein degradation is associated with the atrophy of the skeletal muscle[1]. The gastrocnemius muscle of frog[10], 1 month after sciatic denervation, atrophies to 28 ± 7.5%, while the proteolytic activity shows a significant elevation[2,3]. In this communication the autolysis of the water-soluble muscle proteins of the denervated frog is reported.

Chronic unilateral sciatic denervation for 1 month was carried out in the frog *Rana hexadactyla* as described earlier[4]. Only 1 leg of the animal was denervated and the muscle of the contralateral innervated leg served as control. The gastrocnemii were isolated after pithing the animal, immediately weighed, minced and homogenized. A 5% (Wt/Volume) homogenate of the muscle in icecold 0.25 *M* sucrose was prepared using an all-glass homogenizer and centrifuged for 10 min at 3000 *g* to collect the supernatant for experimentation. By this method, 0.25 *M* sucrose extracts most of the water-soluble proteins of the muscle, whereas the contractile proteins, being insoluble in sucrose medium, settle down as a precipitate. 1 ml of the supernatant was transferred into a series of 11 test tubes and incubated at 37°C (room temperature is 28 ± 2°C) in a water-bath. The protein from each of these samples was precipitated with equal volumes of 10%

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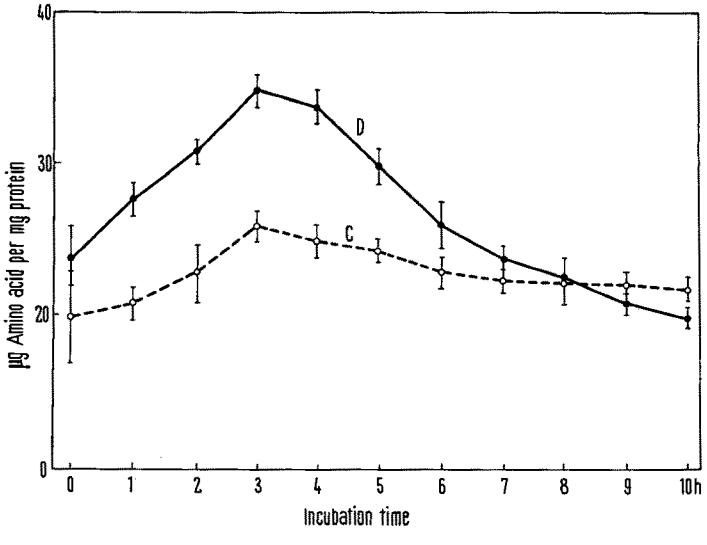

Fig. 1. Autolysis in the homogenates of gastrocnemius muscle of denervated frog. Plots are mean ± S.D.; $n = 10$; D, denervated and C, control muscle.

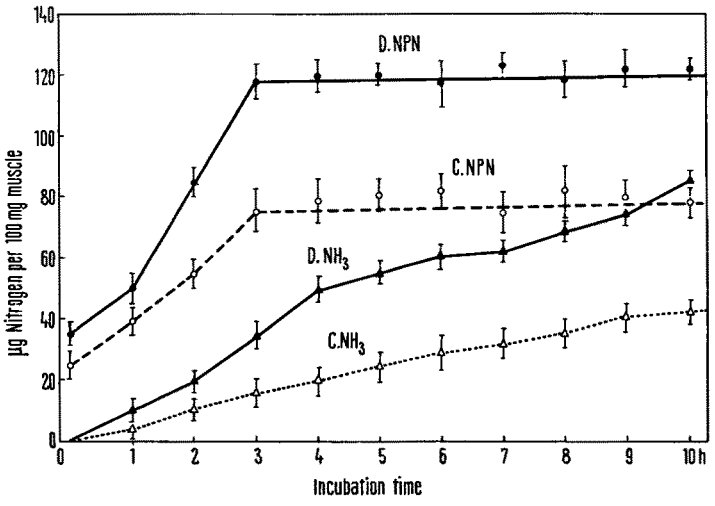

Fig. 2. The levels (mean ± S. D. plots, $n = 6$) of non protein and ammonia nitrogen in the autolyzing homogenates of control and denervated gastrocnemius muscle of frog. D. NPN and C. NPN are the nonprotein nitrogen in denervated and control muscles respectively. $D.NH_3$ and $C.NH_3$ are ammonia nitrogen in denervated and control homogenates, respectively.

trichloro-acetic-acid (TCA) at the end of hourly intervals of incubation over a period of 10 h. Samples in which protein was precipitated prior to incubation served as controls. The homogenate denatured by TCA was centrifuged and the supernatant was used for the estimations of total amino acids[5], ammonia (NH_3) and total non-protein nitrogen (NPN). NH_3 was estimated by nesslerization[6] and the NPN by the application of micro-Kjeldahl method[6]. The autolysis is expressed as µg total amino acids released during the period of incubation by 1 mg of initial homogenate protein, estimated by micro-Biuret method[7].

The Figures illustrate that the in vitro autolysis of the denervated and contralateral control muscles is influenced by the time of incubation. At 3 h incubation of the homogenate at 37°C the autolysis is maximum, but later it gradually decreases as the period of incubation increases. At all intervals of incubation, the denervated muscle shows relatively greater autolysis than the control. A 3 h incubation, where maximum autolysis was recorded by both the muscles, the denervated one shows about 40% higher activity than the control. The study of autolysis serves as an indicator of protein degradation[8]. The present results (Figure 1) may be due to the changes occurring in the cathespin activity and the probable endogenous protein substrates in the denervated muscle. There is evidence[4,9] that ^{14}C leucine incorporation rate increases in the denervated frog muscle. It is also known[3,10] that proteolytic activity increases on denervation in the same muscle. It could be conceivable, with this existing knowledge, that increased autolysis of the denervated muscle may be one of the causal factors for increased in vivo protein turnover in earlier studies[4,11].

Simultaneously, the NH_3 is found to increase along with the time of autolysis (Figure 2) in both the denervated and control muscles. The total NPN content, contrarily in both the muscles, increases up to 3 h incubation and the amino acids (the products of autolysis) continue to be degraded to NH_3, probably due to de-amination, thereby maintaining a steady NPN level. The slope of the curve for NH_3 is different for denervated muscle (Figure 2) which indicated a higher rate of de-amination which could be confirmed by the rapid disappearence (Figure 1) of amino acid in the denervated muscle homogenate.

The results (Figure 1) on autolytic rate are concomitent with the earlier findings[8,11] of other muscles. Plausible increase in de-amination of in vitro autolytically degraded amino acids of denervated muscles was not reported elsewhere and may have some significance in vivo for the metabolism and functional activity of the denervated muscle. Correlations between the increased proteolytic activity and functional activity of the denervated muscle have already been made by many investigators[1,11]. The increased protein metabolism of the denervated muscle, as evidenced by autolytic degradation (Figure 1) and protein synthetic rate[2,9], may have some significance in connection with increased fibrillation activity[12,13] of the denervated muscle[14].

Zusammenfassung. Es wird gezeigt, dass die erhöhte autolytische Abbaugeschwindigkeit im denervierten Muskel von Bedeutung für den Proteinumsatz ist und immer noch das zentrale Problem der Veränderungen darstellt, die bei Denervierung – hier Durchschneidung des Ischiadicus – auftreten.

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