If, instead of 90,000 Lux, lower light intensities are used in the same sequence of 2 min dark-light intervals, 'dark- and light adapted' animals behave totally differently (Figure 4). 'Light adapted' polyps do not show any reaction when exposed to 14,500 Lux while the dark adapted group clearly responds by contracting.

This marked difference between the 2 experimental groups also manifests itself when the sequence of dark and light periods is modified in the following manner: instead of using the same light intensity throughout a series we increased gradually the intensity in each subsequent light period starting from 250 Lux and ending up with the highest possible intensity of 90,000 Lux.

The comparison (Figure 5) between the behaviour of 'light- and dark adapted' animals under these conditions clearly shows that in dark adapted polyps the contractions already become evident when a light intensity of 3200 Lux is reached, whereas light adapted animals do not start reacting until hit by 90,000 Lux. Thus, the threshold for the response to white light is considerably lower in 'dark adapted' than in 'light adapted' animals.

This differential behaviour of polyps that had been kept in complete darkness and those subjected to continuous illumination can be interpreted as being a manifestation of an adaptation to particular light conditions. We have no information so far about the mechanism of adaptation and the level at which this adaptation takes place. Investigations about the possible role of the carotinoids are in progress.

Zusammenfassung. Die Kontraktionsintensität bleibt auch dann unverändert, wenn die Polypen von *Hydra attenuata* dem Dunkel-Hell-Wechsel (je 2 min) während 3 h ausgesetzt werden. Dunkeladaptierte Hydren reagieren auf Lichtreize auffallend empfindlicher als bei 3000 Lux helladaptierte Tiere.

P. TARDENT and E. FREI

Zoologisches Institut der Universität, 8006 Zürich (Switzerland), 29 November 1968.

Changes in Ascorbic Acid Content in Denervated Frog Gastrocnemius Muscle

It is shown that there is continuous local accumulation of ascorbic acid in the gastrocnemius muscle of rat after denervation[1]. In the present study, an attempt was made to examine certain causative factors for the increase of ascorbic acid content in the denervation atrophy of the muscle.

Unilateral denervation of the hind limb of the common Indian frog, *Rana hexadactyla*, was carried out according to KRISHNAMOORTHY and DAS[2]. The gastrocnemius muscles were excised, weighed and homogenized in ice-cold 5% metaphosphoric acid containing 1% $SnCl_2$[3]. The homogenates were centrifuged at 2000 rpm for about 20 min and the acid-soluble fraction was used for the assay of ascorbic acid. L-ascorbic acid (AsA), dehydro-L-ascorbic acid (DHA) and diketo-gulonic acid (DKA) were determined by the 2,4-dinitro-phenyl hydrazine method of ROE and KUETHER[3]. The same procedure was followed for the liver, kidney and adrenals after norit treatment[3] to remove interfering pigments. The blood was collected into a hypodermic syringe through the inferior vena-cava and pooled from 4–5 specimens for assay. The data were statistically analysed[4].

AsA, DHA and DKA concentrations were not changed in the kidney and adrenal tissues of frog after denervation (Table I), instead of an increase as in the gastrocnemius muscle. DHA and DKA were singularly absent in the

[1] G. L. A. GRAFF, A. J. HUDSON and K. P. STRICKLAND, Can. J. Biochem. *43*, 705 (1965).

[2] R. V. KRISHNAMOORTHY and A. B. DAS, Ind. J. exp. Biol. *6*, 4 (1968).

[3] D. GLICK, *Methods of Biochemical Analysis* (Interscience publishers, New York 1964), vol. 1, p. 132.

[4] F. E. CROXTON, *Elementary Statistics with Applications in Medicine and Biological Sciences* (Dover Publications, New York 1953).

Table I. The levels of catabolic products of ascorbic acid in the tissues of normal and denervated frog (period of denervation, 60 days)

Tissue	Catabolic product	Normal frog (mg %)	Denervated frog (mg %)	*t*-test value	Incidence of change on denervation
Serum	AsA	0.12 ± 0.007	0.11 ± 0.15	0.17	no change
	DHA	nil	nil	–	–
	DKA	nil	nil	–	–
Liver	AsA	80 ± 1.14	95 ± 3.00	7.80[a]	increase, $p = < 0.01$
	DHA	25 ± 1.94	24.5 ± 3.50	0.09	no change
	DKA	6 ± 1.23	7 ± 1.58	0.21	no change
Kidney	AsA	149 ± 8.35	157 ± 12.75	0.99	no change
	DHA	33 ± 3.24	35 ± 1.0	1.02	no change
	DKA	6 ± 1.58	7 ± 1.32	1.05	no change
Adrenal gland	AsA	98 ± 5.49	100 ± 5.83	0.25	no change
	DHA	24 ± 2.59	27 ± 4.66	0.97	no change
	DKA	7 ± 1.32	6 ± 1.93	0.74	no change

[a] Mean of 4 samples ± standard deviation.

Table II. Relative changes in AsA, DHA and DKA in denervation-atrophy of frog gastrocnemius muscle

Substance	Time after denervation	Weight change in muscle mass (% contra-lateral control)	No. of obser-vations	mg % of substance in normal muscle	mg % of substance in denervated muscle	Content of substance/whole denervated muscle (% contra-lateral control)	Incidence of change on denervation
AsA	5 min	100	5	39.2 ± 1.17	38.5 ± 0.81	100	no change $t = 0.92$
	68 days	69 ± 9.38	6	40 ± 11.49	81 ± 14.2	81 ± 6.3	increase $t = 5.021$, $p < 0.01$
DHA	5 min	100	5	30 ± 2.24	31 ± 2.65	100	no change $t = 0.57$
	68 days	69 ± 9.38	6	30.5 ± 11.2	60 ± 19.23	83 ± 11.5	increase $t = 4.11$, $p < 0.01$
DKA	5 min	100	5	7 ± 0.89	7.2 ± 0.63	100	no change $t = 0.46$
	68 days	69 ± 9.38	6	7.5 ± 3.25	13 ± 4.42	96 ± 2.2	increase $t = 2.42$, $p < 0.01$

Table III. Relative changes in the ascorbic acid catabolism of the frog gastrocnemius muscle during denervation (period of denervation = 68 days)

Ratio between the catabolic substance	No. observations	Normal muscle	Denervated muscle	Incidence of change on denervation
(1) AsA/DHA	6	1.35 ± 0.199	1.46 ± 0.28	$p > 0.05$, $t = 0.66$ no change
(2) DHA/DKA	6	4.51 ± 1.70	5.11 ± 2.07	$p > 0.05$, $t = 0.50$ no change

serum and their concentrations were steady in the liver of denervated frog. But the AsA has increased in the liver of denervated frog. No change was observed in the muscle 5 min after denervation (Table II); but 68 days after denervation, the muscle lost about 30% of its original mass; the AsA, DHA and DKA levels increased significantly per unit wet weight. When expressed per whole muscle mass (Table II), an apparent decrease in the levels of these products existed. However, this decrease is not as great as the loss in muscle mass. The net result of 68 days of denervation is the local accumulation of AsA in the muscle. The ratios of AsA/DHA and DHA/DKA were not altered significantly (Table III) after 68 days denervation, suggesting a steady catabolic pattern with reference to denervation.

The results presented in Table I agree with the view that the AsA is synthesized, transported through blood and accumulated in various organs. The increase of AsA in the liver of denervated frog is a noteworthy feature. Whether this increase is a resultant of active synthesis or active accumulation remains to be explained. However, the increased AsA in the liver of denervated frog must have significance, as AsA is known to offer good resistance to injury and trauma in animals[5].

There may be 3 reasons for the increased levels of AsA, DHA and DKA in the gastrocnemius muscle after 68 days of denervation. (a) The first possibility may involve a change in the cellular enzymes concerned with the synthesis and breakdown of AsA in the denervated muscle. This possibility is ruled out, for the results presented in Table III show that there is no change in the catabolic pattern of the AsA in denervated muscle. Furthermore, the muscle tissue is not reported to synthesize the AsA[6]. (b) The second possibility may lie in the transport of AsA through the circulation into the muscle from any store such as the liver. This possibility, however, is valid only when there is a change in permeability of the muscle membrane. (c) The change in the permeability of the muscle membrane due to denervation is thus the third possibility and the natural corollary of the second. Hence it is suggested that the increased levels of AsA and its catabolic products after denervation may be due to the change in the permeability of the muscle membrane which permits greater accumulation of these products[7].

Zusammenfassung. Nach Denervierung des Gastrocnemius beim Frosch waren die Konzentrationen der L-Ascorbinsäure, Dehydro-L-Ascorbinsäure (DHA) und Diketogguloniksäure (DKA) im Nieren- und Nebennierengewebe unverändert. DHA und DKA fehlten, während ihre Konzentrationen in der Leber trotz Ascorbinsäurezunahme gleichmässig verteilt waren.

R. V. KRISHNAMOORTHY[8] and P. SATYAM

Department of Zoology, Sri Venkateswara University and Department of Zoology, S.V. Arts College, Tirupati (Andhra Pradesh, India), 15 July 1968.

[5] P. D. F. MURRAY and E. KODICEK, J. Anat. *83*, 158 (1949).
[6] J. J. BURNS and A. H. CONNEY, A. Rev. Biochem. *29*, 413 (1960).
[7] The authors wish to thank Dr. K. S. SWAMI, Head of the Department of Zoology, Sri Venkateswara University, Tirupati for offering facilities. One of them (R.V.K.) is grateful to the Council of Scientific and Industrial Research of India for placing in the Scientists' pool.
[8] Present address: Department of Zoology, Bangalore University, Bangalore-1, India.