

show that on the side of the unilateral lesions, and on both sides in those rats with bilateral lesions, there was a very marked reduction in the cortical content of the noradrenaline metabolite.

Discussion. These results show that histologically confirmed lesions of the nucleus locus coeruleus cause a marked fall in the turnover of noradrenaline in the ipsilateral neocortex. This supports the suggestion arising from histochemical studies⁴ that a noradrenaline-containing system with a very wide distribution in the neocortex arises from a small nucleus in the mid-pons. This finding would be consistent with the view that electrical self-stimulation obtained from electrodes placed using the same stereotaxic co-ordinates, and with their tips close to the locus coeruleus, is due to the activation of a noradrenergic-system⁹, and this may provide a clue to its function¹⁰. It is also of interest that in the group of rats with bilateral lesions (but not in either of the other 2 groups) we observed a striking behavioural syndrome

of marked hyperkinesia and repeated jumping which appeared within 2 h of recovery from anaesthesia and lasted for 4 h. Lesions of catecholamine-containing pathways are probably followed by amine release from degenerating terminals¹¹. ANDEN described a form of hyperkinesia characterised by large jumps after administration of catapresan and apomorphine in rats and postulated that noradrenaline receptor stimulation was involved¹².

Résumé. Des lésions électrolytiques, soit unilatérales soit bilatérales, ont été pratiquées dans des cerveaux de rats, dans la région du locus coeruleus. Trois semaines après, la teneur en 3-méthoxy-4-hydroxyphénylglycol (MHPG) des deux cortices cérébraux a été analysée par chromatographie à gaz. Les lésions unilatérales ont réduit la teneur en MHPG du cortex ipsilatéral, et après les lésions bilatérales la réduction eut lieu dans les deux cortices.

G. W. ARBUTHNOTT, J. E. CHRISTIE, T. J. CROW,
D. ECCLESTON and D. S. WALTER

Electrical Ablation of locus coeruleus

	N	L. H. Cortex (ng)	R. H. Cortex (ng)
Control rats	6	45 ± 16	42 ± 12
Unilateral (L-sided) lesions	6	17 ± 9 ^a	47 ± 11
Bilateral lesions	4	20 ± 10 ^a	17 ± 4 ^b

^a $P < 0.005$ vs control. ^b $P < 0.01$ vs control. ^c $P < 0.025$ vs control.

Departments of Physiology and Mental Health, University of Aberdeen, Aberdeen AB9 1AS; and M.R.C. Brain Metabolism Unit, University, Department of Pharmacology, Edinburgh (Great Britain), 19 June 1972.

¹⁰ T. J. CROW, *Nature* 219, 736 (1968).

¹¹ U. UNGERSTEDT, *Acta physiol. scand. Suppl.* 367, 49 (1971).

¹² N.-E. ANDEN, in *Amphetamines and Related Compounds* (Eds. E. COSTA and S. GARATTINI; Raven Press, New York 1970), p. 447.

Increased Hexokinase Activity in Red and White Skeletal Muscle after Denervation

Red and white skeletal muscles exhibit differences in their metabolism and in their response to denervation. Oxidative enzymes predominate in red muscles while glycolytic enzymes predominate in white muscles. Denervation tends to eliminate these differences by causing a greater fall of the predominant group of enzymes in the involved muscle¹.

The initiation of glycolysis requires the phosphorylation of glucose by the enzyme hexokinase. It might therefore be expected that hexokinase activity would display a pattern similar to that of other enzymes associated with glycolysis, being more active in white than in red muscles and undergoing a decrease in activity after denervation. The opposite pattern for hexokinase activity was found

in the present study. No other enzyme associated with glycolysis has been reported to exhibit this behavior.

Methods. 24 male albino rabbits underwent unilateral transection of the sciatic nerve. Unoperated animals were used as controls. The soleus (a red muscle) and the gastrocnemius (predominantly white) were removed and homogenized at 4°C. Hexokinase activity was measured according to the method of SHARMA et al.² as modified by

¹ E. L. HOGAN, D. M. DAWSON and F. C. A. ROMANUL, *Arch. Neurol.* 13, 274 (1965).

² C. SHARMA, R. MANJESHWAR and S. WEINHOUSE, *J. biol. Chem.* 238, 3840 (1963).

Hexokinase activity in normal and denervated soleus and gastrocnemius muscles of the rabbit

Muscle type	Control	Denervated ^a Weeks after denervation		
		2	4	8
Soleus	987 ± 133	1,150 ± 137	1,390 ± 161	1,970 ± 188
Gastrocnemius	280 ± 42	470 ± 59	784 ± 89	1,330 ± 147

Values indicate mmoles of glucose-6-phosphate formed per kg of non-collagenous protein/h of incubation. The control and each of the denervated groups consists of 8 animals, and the values represent the means ± standard errors. ^aAll the differences between the denervated and the control muscles are significant for $p = 0.01$.

McLEAN and BROWN³. Non-collagenous protein was measured according to the method of LOWRY et al.⁴.

Results. Normal soleus exhibited 3 times as much hexokinase activity as the corresponding gastrocnemius muscle (Table). Denervation caused a progressive rise in this activity which, after 8 weeks, attained a 2-fold increase in the soleus and a 5-fold increase in the gastrocnemius, when using non-collagenous protein as a reference base. Mixing of different homogenates in various proportions consistently gave additive results, thus ruling out the presence of hexokinase inhibitors or activators to account for the differences found in enzyme activity.

Discussion. Hexokinase activity displays, both in control and in denervated muscles, just the opposite pattern of most enzymes associated with glycolysis. The connective tissue levels and enzymes of the pentose-phosphate pathway follow a similar pattern to that of hexokinase⁵. Previously, I have proposed that the pentose-phosphate pathway in skeletal muscles originates from their connective tissue, rather than from the muscle cells⁵. On the basis of a similar parallelism between connective tissue levels and the activity of hexokinase, it can be speculated that this enzyme is very active in the connective tissue of skeletal muscles.

Hexokinase occurs in multiple molecular forms, probably corresponding to different cellular or tissue compartments⁶. Two or more of these forms are present in muscles. On the other hand, at least 2 glucose-6-phosphate pools, exhibiting marked differences in their response to insulin, appear to exist in skeletal muscle⁷. The values in the present report probably reflect the combined presence of two or more molecular forms of

hexokinase, with connective tissue making a major contribution to the total hexokinase activity measured. It can be speculated that one of the enzyme forms exists within the muscle cells, in association with glycolytic enzymes and one of the glucose-phosphate pools, while another form resides in connective tissue elements and is associated the pentose-phosphate pathway and the other glucose-phosphate pool.

Resumen. La actividad de la hexoquinasa es mayor en el músculo rojo que en el blanco, y ésta aumenta como resultado de la denervación. Tales resultados son lo opuesto de los observados para la mayoría de las enzimas glicolíticas. Estos son explicables si una de las isoenzimas de la hexoquinasa se encuentra localizada dentro del tejido conjuntivo del músculo esquelético.

L. GARCÍA-BUÑUEL

Veterans Administration Hospital, and
University of Oregon Medical School, Sam Jackson Park,
Portland (Oregon 97207, USA), 23 May 1972.

³ P. McLEAN and J. BROWN, *Biochem. J.* **98**, 874 (1966).

⁴ O. H. LOWRY, N. H. ROSEBROUGH, A. L. FARR and R. G. RANDALL, *J. biol. Chem.* **193**, 265 (1951).

⁵ L. GARCÍA-BUÑUEL and V. M. GARCÍA-BUÑUEL, *Nature, Lond.* **213**, 913 (1967).

⁶ J. W. ANDERSON, R. H. HERMAN, J. B. TYRREL and R. M. COHN, *Am. J. clin. Nutr.* **24**, 642 (1971).

⁷ C. C. DULLY, R. M. BOCEK and C. H. BEATTY, *Endocrinology* **84**, 855 (1969).

Circadian-Rhythmik und Gruppenverhalten bei *Leucaspius delineatus* (Pisces, Cyprinidae)

Untersuchungen zur Gültigkeit der Circadian-Regel¹⁻³ an Fischen fehlten bisher. Daher haben wir mitteleuropäische Süßwasserfische auf die Abhängigkeit ihrer lokomotorischen Aktivität von unterschiedlichen Beleuchtungsstärken geprüft. In diesem Zusammenhang wurde nicht nur die circadiane Periodik eines aus dem sozialen Gruppen- bzw. Schwarmverband eliminierten Einzeltieres, sondern auch die von Fischgruppen, untersucht. Obwohl es für die gegenseitige Synchronisation in Populationen eine Anzahl von Hinweisen gibt^{4,5}, ist dieser Effekt im Labor unter Dauerbedingungen bisher nur selten nachgewiesen worden⁶⁻⁸.

Wir untersuchten den Süßwasserschwarfisch *Leucaspius delineatus* Heckel unter konstanter Beleuchtung von 4, 66 und 140 Lux, bzw. im Licht-Dunkel-Wechsel 12:12 Stunden, bei konstanter Temperatur. Die Registrierung der Schwimmaktivität erfolgte über Lichtschranken und Impulszähler^{9,10}. Vor Beginn der Versuche im November/Dezember mit konstanter Beleuchtung waren 9 Einzeltiere und 3 Gruppen ($n = 7$) mit einem 12:12 Stunden Licht-Dunkel-Wechsel ($L = 66$ Lux) synchronisiert. In den ersten Versuchstagen verhielten sich die Gruppen sehr einheitlich. Nach 1-2 Übergangsperioden, die unberücksichtigt blieben, setzte bei den Gruppen die Spontanperiodik ein, und an den darauffolgenden Tagen verschoben sich die Aktivitätsphasen nach links (vgl. Figur 1). Somit verkürzten sich bei den Tiergruppen die Periodenlängen. Durch die Verkürzung der Gesamtperiodenlänge im Dauerlicht von 66 Lux hat sich der Aktivitätsschwerpunkt¹⁰ vom 9. Nov.-21. Nov. gegenüber dem 12:12 Stunden-Tag bei Gruppe 5 um 7,5 h, bei Gruppe 4 um 8,5 h und bei Gruppe 2 um 8,2 h phasenver-

schohen. Die für jede Gruppe errechnete Steigung ist ein direkter Ausdruck der Frequenzabweichung gegenüber dem 24-Stunden-Rhythmus (Abszissen-Parallele) und zeigt, dass alle Versuchsgruppen von der 24-Stunden-Periode abweichen, also per definitionem eine Spontanfrequenz aufweisen¹¹. Die mittlere Periodenlänge der 3 untersuchten Gruppen beträgt $\tau = 22,33 \pm 0,03$ h.

Auch bei den Einzeltieren liess sich unter LL 66 Lux eine Spontanfrequenz in der gleichen Jahreszeit nachweisen. Die Periodenlänge betrug weniger als 24 h und war somit genau wie bei den Gruppen verkürzt. Im Unterschied zu diesen ist die Spontanfrequenz jedoch nur über einen kurzen Zeitraum von meist 2-3 Tagen ausgeprägt. Dann treten Auflösungserscheinungen auf. Die Tiere waren fast ständig aktiv und eine Periodik nicht mehr exakt festzustellen.

¹ J. ASCHOFF, *Z. Tierpsychol.* **15**, 1 (1958).

² J. ASCHOFF, *Circadian Clocks* (North-Holland Publ. Co., Amsterdam 1965).

³ R. WEVER, *Z. Tierpsychol.* **51**, 1 (1964).

⁴ J. ASCHOFF, *A. Rev. Physiol.* **25**, 581 (1963).

⁵ H. REMMERT, *Handbuch der Biologie* Akademische Verlagsgesell. Athenion, Frankfurt a. M. (1965), vol. 5, p. 1.

⁶ K. HOFFMANN, *Z. vergl. Physiol.* **43**, 544 (1960).

⁷ J. MEDOGORAG und M. LINDAUER, *Z. vergl. Physiol.* **55**, 450 (1967).

⁸ F. WEBER, *Zool. Jb. Physiol.* **72**, 136 (1966).

⁹ R. SIEGMUND, *Biol. Zentbl.* **88**, 295 (1969).

¹⁰ R. SIEGMUND und D. L. WOLFF, *forma et functio* **5**, 33 (1972).

¹¹ Zu freilaufenden Rhythmen bei Fischen unter natürlichen Lichtverhältnissen vgl. K. MÜLLER, *Naturwissenschaften* **55**, 140 (1968) und K. MÜLLER, *Oikos* **20**, 166 (1969).