

the plexus to the cauda epididymidis and epididymal vas deferens was not visible. Adrenergic nerves evidently reach the caput epididymidis by way of the superior spermatic nerve from the lumbar sympathetics in this species.

After castration, the adrenergic plexus was distributed to the smooth muscle in a manner that recalled the distribution of the cholinesterase reactive plexus of the rat epididymis<sup>16,17</sup>. In the cauda epididymidis, it appeared also to be confined mainly to the peripheral surface of the duct walls. In normal animals this is true for the more weakly innervated ducts of the upper cauda and lower corpus epididymidis. The effect was more readily apparent in the ChE-positive plexus than in the adrenergic one, where no clear reduction in fluorescence intensity could be recognized in individual axons. Absence of male sex hormones has only slight effects on the morphology of the adrenergic nerves to the male reproductive ducts, attributable mainly to the simultaneous atrophy of smooth muscle elements and associated tissue changes<sup>18</sup>.

**Résumé.** Après vasectomie complète, pratiquée sur des rats, la fluorescence des terminaisons nerveuses, ainsi que la substance transmettrice, disparaît le long du canal déferent et de l'extrémité de l'épididyme en deçà de la lésion. Chez les chats, les terminaisons nerveuses dans les

canalicules efférents et la tête de l'épididyme ne disparaissent pas après l'opération. Dans la direction de la glande et du ganglion prostatique, la matière fluorescente s'accumule dans les troncs nerveux, et les terminaisons nerveuses restent visibles. Après castration, quelques modifications compensatrices ont été observées.

K.-A. NORBERG, PAUL L. RISLEY  
and U. UNGERSTEDT

*Department of Histology, Karolinska Institute  
Stockholm (Sweden), 9th September 1966.*

<sup>16</sup> P. L. RISLEY and C. N. SKREPETOS, *Anat. Rec.* 148, 231 (1964a).

<sup>17</sup> P. L. RISLEY and C. N. SKREPETOS, *Anat. Rec.* 150, 195 (1964b).

<sup>18</sup> Acknowledgments: This work was supported in part by a research grant (No. B66-257) from the Swedish Medical Research Council. The second author was on leave from the Biology Department, University of Oregon, Eugene (Oregon, USA) aided by a special Research Fellowship granted by the National Council for Child Health and Human Development, National Institute of Health, USA Dept. of Health, Education and Welfare. Use of facilities of the Department of Physiology I of the Karolinska Institute with the permission of Prof. U. S. VON EULER and Research Docent R. ELIASSON is greatly appreciated. For skillful technical assistance, we are grateful to Mrs. ULLÅ FLYGER and Miss BERITH HANSON.

### **Influence of Hypercapnia on the Noradrenalin Content of Sympathetically Decentralized and Innervated Skeletal Muscle**

Hypercapnia results in increased sympathetic discharge with augmented catecholamine release from the sympathetic nerves<sup>1</sup> and the adrenal medulla<sup>2</sup>. EULER and HELLNER-BJÖRKMAN<sup>3</sup> found that increased sympathetic nerve activity had no influence on the noradrenalin content of cat spleen, heart, liver or kidney. This suggested that in these tissues noradrenalin synthesis in the adrenergic nerves keeps pace with release also during sustained hyperactivity.

Recent studies have shown that the noradrenalin content of certain sympathetically innervated tissues can be more easily altered by changes in sympathetic impulse activity than hitherto expected. Prolonged electrical stimulation of the sympathetic nerves to cat skeletal muscle<sup>4</sup> and rat salivary gland<sup>5</sup>, with frequencies considered to be within the physiological range, results in depletion of the noradrenalin content of these tissues.

The present study was undertaken to investigate whether an increased sympathetic activity induced by inhalation of high concentrations of CO<sub>2</sub> could affect the noradrenalin content of the adrenergic nerves in cat skeletal muscle.

Cats weighing 2.1–3.4 kg were anaesthetized with sodium pentobarbital i.v. and maintained on artificial respiration via a tracheal cannula. Gallamine HCl (50 mg/kg) was given to obtain complete muscle relaxation. One of the lumbar sympathetic chains was isolated by an abdominal approach and transected at the level of L4–L5. Gas mixtures containing 100% oxygen or 25% CO<sub>2</sub> in oxygen were administered by means of the respiration

pump for a period of 2 h, after which the gastrocnemius and tibialis anterior muscles from both legs were removed and placed in ice-cold 0.4 N perchloric acid. The muscles were homogenized with an Ultra-Turrax homogenizer. The noradrenalin content of the extracts was determined essentially according to HÄGGENDAL<sup>6</sup>.

**Results and discussion.** The presence of 25% CO<sub>2</sub> in the inhalation mixture did not significantly change the noradrenalin content in sympathetically decentralized gastrocnemius muscle ( $90 \pm 7$  ng/g in animals breathing 100% oxygen versus  $97 \pm 14$  in animals given 25% CO<sub>2</sub> in oxygen). On the other hand, there was a marked depletion of the noradrenalin content in the muscles with intact sympathetic outflow (Table). When compared on a % basis with the content in the contralateral decentralized muscles from the same animal, the difference was significant ( $p < 0.01$ ). Although the number of experiments on the tibialis anterior muscles is small, the data indicate the same changes as found in the gastrocnemius muscles. Also in animals breathing 100% oxygen, the noradrenalin values in the innervated muscle tended to be low, but the difference was not statistically significant.

<sup>1</sup> B. FOLKOW and B. UVNÄS, *Acta physiol. scand.* 75, 365 (1948).

<sup>2</sup> R. C. CANTU, G. G. NAHAS and W. M. MANGER, *Proc. Soc. exp. Biol. Med.* 122, 434 (1966).

<sup>3</sup> U. S. VON EULER and S. HELLNER-BJÖRKMAN, *Acta physiol. scand.* 33, Suppl. 118, 17 (1955).

<sup>4</sup> D. KERNELL and G. SEDVALL, *Acta physiol. scand.* 67, 201 (1964).

<sup>5</sup> B. FREDHOLM and G. SEDVALL, *Life Sci.* 5, 2023 (1966).

<sup>6</sup> J. HÄGGENDAL, *Acta physiol. scand.* 59, 242 (1963).

The present study shows that hypercapnia results in more than 50% depletion of the noradrenalin content of cat skeletal muscle. This depletion, which is dependent upon intact sympathetic outflow to the muscles, indicates that during the prevailing experimental conditions noradrenalin synthesis in the sympathetic nerves does not keep pace with impulse induced release. The phenomenon observed may indicate that adrenergic transmitter synthesis, during the initial phase of an abrupt increase in impulse activity, is only slowly and gradually stimulated. However, it is also conceivable that hypercapnia causes impairment of noradrenalin re-uptake and/or synthesis,

as such effects only become apparent during sustained transmitter release<sup>7</sup>.

**Zusammenfassung.** Aktivitätserhöhung im sympathischen Nervensystem bei Katzen wurde durch Inhalieren von 25% CO<sub>2</sub> in O<sub>2</sub> hervorgerufen. Es wurde der Einfluss erhöhter sympathischer Aktivität auf den Noradrenalin-gehalt sympathischer Nerven geprüft, welche zu motorischen Muskeln verlaufen. Hyperkapnia rief keine Veränderung im akut dezentralisierten M. Gastrocnemius hervor. Bei intaktem sympathischem Fluss zum Muskel bewirkte Hyperkapnia eine erhebliche Erniedrigung des Noradrenalin-gehaltes. Die Wirkung hängt somit von einem intakten sympathischen Ausfluss ab.

Noradrenalin content of sympathetically innervated muscles (values expressed as % of content in decentralized control muscles)

	100% O <sub>2</sub>		25% CO <sub>2</sub> + 75% O <sub>2</sub>	
	Mean	Range	Mean	Range
Gastrocnemius (6)	78	100-58	44*	81-26
Tibialis anterior (3)	85	100-75	42	55-33

\* Differs from control  $p < 0.01$ . Numbers within parentheses refer to number of cats used.

S. H. NGAI<sup>8</sup>, S. ROSELL  
and G. SEDVALL

Department of Pharmacology, Karolinska Institute,  
Stockholm 60 (Sweden), 4th November 1966.

<sup>7</sup> This investigation has been supported by Swedish Medical Research Council Projects Nos. W250 and 40X-667-01 and by the National Institutes of Health, USA (Grant No. 2Ti HE 5614-04).

<sup>8</sup> Present address: Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York City (USA).

## Spontaneous Activity of Rats on Diets Varied in Protein, Carbohydrate and Fat Content

Perusal of the literature reveals conflicting results on the relationship of spontaneous activity to diet. Some studies have indicated that low protein diets depress activity<sup>1,2</sup>. Other studies have suggested that dietary protein does not affect activity at all except at high protein levels, at which a decrease in activity occurs<sup>3,4</sup>. The ratio of protein to carbohydrate in the diet has also been investigated. Excitability level (number of standing up reactions) appears to be closely related to this ratio. As the level of proteins in the diet increases, the excitability level of rats decreases<sup>5</sup>. Activity level measured by the number of wheel turns has been claimed to decrease as the proportion of protein in the diet increased<sup>6</sup>. High fat diets have been said to depress activity initially at varying levels of dietary protein. However, upon prolonged administration, rats on high fat diets show an increase in the level of spontaneous activity above that for rats on low protein diets<sup>7</sup>.

To try to clear up these contradictions, 6 male and 6 female Charles River albino rats were fed from weaning diets where the proportion of carbohydrate, protein and fat were varied systematically (Table I). The protein was casein, the carbohydrate sucrose, the saturated fat lard, and the unsaturated fat corn oil. The usual mineral and vitamin supplements were included. The animals were matched by sex and age at the time of running (28-82 days old). Each rat was placed in a stabilimeter cage for 6 consecutive days with free access to food and water. The cages were wire mesh with a closed top, 22.5 cm in diameter and 20.5 cm high, with a 6 cm diameter, 5 cm high food cup in the middle. Sawdust was placed in the

bottom pan which rested on 4 switches which counted every time 2 switches were closed. A water bottle spout entered through an opening in the cage. The apparatus was turned off once daily, when counters were read and rats weighed.

The results showed a progressively stabler daily activity over the 6 day period for each group. The mean activity level was obtained by averaging activity scores

Table I. % calories of protein, carbohydrate and fat

Foodstuff	Diet				
	HP	SC	LP	HUF	HSF
Protein	59.8	24.5	9.8	24.5	24.5
Carbohydrate	19.1	64.5	79.2	-	-
Saturated fat	4.4	4.4	4.4	11.0	68.9
Unsaturated fat	6.6	6.6	6.6	64.5	6.6

<sup>1</sup> F. A. HITCHCOCK, Am. J. Physiol. 84, 410 (1928).

<sup>2</sup> J. R. SLONAKER, Am. J. Physiol. 96, 547 (1931).

<sup>3</sup> T. S. HAMILTON, J. Nutr. 17, 565 (1939).

<sup>4</sup> E. A. SMITH and R. M. CONGER, Am. J. Physiol. 142, 663 (1944).

<sup>5</sup> J. LAT, Physiologia bohemoslov. 5, 38 (1956).

<sup>6</sup> G. H. COLLIER, R. L. SQUIBB and F. JACKSON, Psychonomic Sci. 3, 173 (1965).

<sup>7</sup> S. FRANKOVA, Activitas nerv. sup. 4, 471 (1962).