metric measurements, was 11.2 ± 3.6% larger than in the controls 3 days after the operation. The effect of tendon shortening is also apparent in denervated muscles (Table). The relative hypertrophy of stretched and denervated muscles, when compared to their denervated controls, is actually due to a transient delay in denervation atrophy induced by tendon shortening (Figure).

The findings appear to indicate that an increase in passive tension can produce hypertrophy of mammalian skeletal muscles through a direct effect not mediated by nerves. The transient nature of the hypertrophic process in this experimental system is presumably due to softening of the tendon tissue at the site of the suture and consequent release of tension. Sarcomere length in the plantaris muscle 3 days after the operation was in fact, on the average, 2.34 ± 0.06 μm, i.e. not significantly different from control[8].

Riassunto. L'accorciamento del tendine distale del muscolo plantare del ratto produce una transitoria ipertrofia muscolare con un massimo aumento in peso a tre giorni dall'operazione. L'accorciamento tendineo combinato con sezione del nervo risulta in un temporaneo ritardo nella comparsa dell'atrofia da denervazione.

S. SCHIAFFINO[8]

National Research Council Unit for Muscle Biology and Physiopathology, Institute of General Pathology, University of Padova, Via Loredan 16, I–35100 Padova (Italy), 11 March 1974.

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Retention of Noradrenaline-^{3}H in Brain and Preferential Extraction of Labeled Metabolites by Glutaraldehyde Fixation

All radioautographic investigations aimed at the ultrastructural examination of noradrenaline-^{3}H (NA-^{3}H) uptake and storage sites, in peripheral or central nervous tissue, have relied on the use of primary fixation with glutaraldehyde to preserve the tracer in situ[1]. Thus, selective accumulations of radioactivity have been found characteristic of catecholaminergic nerve cell bodies and axon terminals in the adult rat brain, following intracerebral administration of NA-^{3}H[2–4]. Moreover, such reactions could be specifically attributed to the presence of NA-^{3}H rather than labeled metabolites, provided that monoamine oxidase was inhibited[3].

In these and other studies, prevailing experimental conditions precluded the conclusive answering of several outstanding questions: 1. what is the proportion of brain NA-^{3}H actually retained in tissue after primary fixation with glutaraldehyde? 2. are some metabolites of NA-^{3}H also bound to brain tissue by this fixative? 3. is there any validity in the assumption that NA-^{3}H metabolites might be preferentially extracted in the course of glutaraldehyde fixation?

These problems were tackled by simple experiments on newborn rats, where it was deemed feasible to: a) obtain reproducible measurements of NA-^{3}H and labeled metabolites from brains of different animals by introducing NA-^{3}H via the bloodstream[5]; b) alter the respective brain contents of NA-^{3}H and labeled metabolites to a significant degree, using enzymatic inhibitors[6–8]; c) estimate the retention of radioactivity in nervous tissue, and extraction by the fixative[9], following immersion of brain slices in glutaraldehyde.

Material and methods. 23 newborn Sprague-Dawley rats of both sexes were each given 100 μCi of NA-^{3}H, within 24 h after birth (DL-noradrenaline-7-H^3, specific activity: 9.6–20 Ci/m*M*, obtained chromatographically pure from Amersham/Searle). The tracer, contained in 50 μl of aqueous solution, was injected in 15 sec into an exposed jugular vein. 1 group of rats (11) also received a combined pretreatment with both an inhibitor of monoamine oxidase (IMAO: Catron, 10 mg/kg s.c., 45 min before NA-^{3}H) and catechol-*O*-methyl transferase (ICOMT: Pyrogallol, 200 mg/kg s.c., 45 min before NA-^{3}H and hourly thereafter).

All animals were decapitated 3 h after administration of the tracer. Their entire brains were rapidly removed and frozen on dry ice. Several specimens from control and pretreated rats were homogenized in perchloric acid (0.4 *N*), and measured for total radioactivity (^{3}H) and NA-^{3}H contents, using liquid scintillation spectrometry before and after chromatography on aluminium oxide respectively[10]. The other brains were cut into 1 mm-thick slices, fixed for 1 h by immersion in four 15 min baths of 12.5 ml of 3.5% glutaraldehyde in 0.05 *M* phosphate buffer (Sorensen sol. A) at room temperature, and dissolved in Soluene. The amounts of ^{3}H retained in tissue and extracted by the fixative were then measured by scintillation. All values were corrected for quenching, with internal standards, and expressed per mg wet weight of tissue.

Results and discussion. The main results of these experiments are summarized in the Table. 3 h after i.v. injection of NA-^{3}H in newborn rats, radioactivity is found in appreciable quantity within the brains of animals pretreated or not with enzymatic inhibitors. In pretreated rats, total brain ^{3}H is significantly elevated, amounting to 1.6% of the initial dose administered compared to 0.7% in control rats, presumably due to preservation of higher levels of the exogenous amine in the bloodstream[11,12].

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Extraction of brain radioactivity (^{3}H) by glutaraldehyde fixation 3 h after intravenous injection of tritiated norepinephrine (NA-^{3}H) in newborn rats pretreated or not with enzymatic inhibitors

	Total ^{3}H (dpm/mg)	NA-^{3}H (%)	^{3}H extracted by fixative (%)
Controls	5,717 ± 457 (12)	4.5 ± 0.5 (3)	70.2 ± 2.6 (9)
IMAO + ICOMT	13,265 ± 1,144 (11)	72.6 ± 1.3 (4)	44 ± 3.5 (7)

Mean values ± S.E.M. Number of rats in each group given in brackets. All differences between control and pretreated rats significant with $p < 0.001$ (Student's test).

Estimations of NA-^{3}H in unfixed brains indicate that the amine itself accounts for 72.6% of the radioactivity in pretreated rats, whereas, in control rats, less than 5% of total brain ^{3}H persists as NA-^{3}H. The extraction of radioactivity during immersion of brain slices in glutaraldehyde, taking place almost entirely within the first two baths of fixative, is significantly different in the two groups: it represents 44% versus 70% of total brain ^{3}H, in pretreated and control rats respectively.

In control rats, however, despite the fact that 95% of the radioactivity is in the form of labeled metabolites of NA-^{3}H, the extraction by the glutaraldehyde fixative amounts only to 70% of total brain ^{3}H. This confirms that glutaraldehyde has a capacity to bind some metabolites of NA-^{3}H to nervous tissue, as previously revealed by radioautographs of the rat brain, which exhibited a diffuse reaction 3 h after intraventricular administration of normetanephrine-^{3}H[3]. Conversely, in pretreated rats, slightly more radioactivity is removed from brain slices than can be accounted for by the labeled metabolites alone. Such a small loss of NA-^{3}H could have been due to slow penetration of glutaraldehyde, during a fixation procedure carried out by immersion rather than vascular perfusion[13].

From these results, it may be inferred that at least 75% of NA-^{3}H is retained in brain tissue after glutaraldehyde fixation. This figure is in agreement with previously reported estimates derived from experiments where the exact nature of the radioactivity present in nervous tissue could not be precisely determined at the time of fixation[14, 15]. The data also emphasize the value of glutaraldehyde as a primary fixative for the preservation of NA-^{3}H, as opposed to paraformaldehyde or ice cold $KMnO_4$ solutions, which extract much greater proportions of this tritiated amine from nervous tissue[15–17,3].

The preferential extraction of NA-^{3}H metabolites by glutaraldehyde fixation, demonstrated in the present investigation, had already been suspected in earlier radio-isotopic[9] and radioautographic[18,14] studies. It is likely that deaminated metabolites constitute the main component of the extractable radioactivity, since they lack the amino group presumably responsible for the binding of catecholamines to glutaraldehyde in vitro[19].

Since the greater fraction of NA-^{3}H removed from nervous tissue during the elaborate preparative sequences required for electron microscopy or high-resolution radioautography appears to be lost in the primary fixative[9, 15], two properties of glutaraldehyde fixation will concur to the in situ preservation of this exogenous amine within brain: retention of a major proportion of NA-^{3}H itself, and preferential extraction of its labeled metabolites[20].

Résumé. Trois heures après une injection i.v. de noradrénaline tritée (NA-^{3}H) chez le rat nouveau-né, moins de 5% de la radioactivité mesurée dans le cerveau correspond à cette amine marquée. Par contre, lorsque la monoamine oxydase et la catéchol-*O*-méthyl transférase sont inhibées, la NA-^{3}H constitue 73% de la radioactivité cérébrale. Dans ces conditions, l'extraction de la radioactivité par la fixation au glutaraldéhyde 3.5% dans le tampon phosphate diffère également de façon significative et indique qu'une fraction majeure de la NA-^{3}H est liée au tissu par le glutaraldéhyde, tandis que les métabolites marqués sont préférentiellement extraits.

L. Descarries and J. C. Dupin

Centre de recherche en sciences neurologiques, Université de Montréal, BP 6208, Montréal H3C 3T8 (Québec, Canada), 2 May 1974.

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Stereospecificity of Oxotremorine Antagonists

Previous work in our laboratories has shown that a number of N-(*tert*-aminoalkynyl)-substituted succinimides and pyrrolidones are rather potent in blocking the motor effects of the muscarinic agent oxotremorine, 1-(2-oxopyrrolidino)-4-pyrrolidino-2-butyne, while the effects on peripheral cholinergic symptoms, such as mydriasis and acetylcholine-induced spasms of guinea-pig ileal strips, are of lower magnitude[1–6]. Consequently, these compounds can be regarded as anticholinergic agents with selectivity for the central nervous system.