Chungara, Revista de Antropología Chilena
ISSN: 0716-1182
calogero_santoro@yahoo.com
Universidad de Tarapacá
Chile

Appenzeller, Otto; Hoyle, Charles V.; Santoro, Calogero M.; Appenzeller, Martin
PALEONEUROBIOLOGY
Chungara, Revista de Antropología Chilena, vol. 32, núm. 1, enero, 2000, pp. 97-102
Universidad de Tarapacá
Arica, Chile

Chungara, Revista de Antropología Chilena
Volumen 32, N° 1, 2000. Páginas 97-102

PALEONEUROBIOLOGY

Otto Appenzeller[*], *Charles V. Hoyle*[**], *Calogero M. Santoro*[**]*, *Martin Appenzeller*[****]

Paleopathology has, with few exceptions, not identified diseases of the nervous system. We use the term Paleoneurobiology to encompass a branch of knowledge concerned with the study of the neurobiology of ancient humans and animals. To begin with we embarked upon an examination of sural nerves, ventral spinal roots, cutaneous perivascular nerves and glycosylated hemoglobin. Samples of the aforementioned were taken from mummified remains in Egypt (2.000-3.500 years BP) and the Atacama Desert (1000-3000 years BP), and the specific results for each will be presented. The preservation of neurochemicals for millennia in sural nerves opens the possibility to asses the presence of peripheral nerve disease in ancient civilizations. Although the significance of neurochemicals in ventral spinal roots remains to be determined the possibility that these might be involved in some neurologic disorders needs to be explored. Perivascular cutaneous nerve preservation suggest that intraepidermal nerve fibers may also be demonstrable in ancient skin. These fibers have been shown to be importantly affected in peripheral neuropathies. The possibility to establish the presence of diabetes mellitus in ancient civilizations by measuring glyocsylated hemoglobin in mummified blood promises to shed light on the genetics of the high incidence of this disease in contemporaneous Native American populations.

Key words: Sural nerves, perivascular cutaneous nerves, glycosylated, neurochemicals.

La Paleopatología, con pocas excepciones, no ha identificado las enfermedades del sistema nervioso. Usamos el término paleoneurobiología para abarcar el conocimiento relativo al estudio de la neurobiología de humanos y animales antiguos. Para comenzar, nos embarcamos en un examen de nervios surales, raíces espinales ventrales, nervios perivasculares cutáneos y hemoglobina glicosilato. Se tomaron muestras de lo anterior de restos momificados de Egipto (2.000-3.500 años AP) y el Desierto de Atacama (2.000-3.500 años AP), y se presentarán los resultados específicos. La preservación de neuroquímicos por miles de años en los nervios surales abre la posibilidad de evaluar la presencia de la enfermedad de los nervios periféricos en civilizaciones antiguas. Aunque no se haya determinado el significado de los neuroquímicos en las raíces espinales ventrales, hay que explorar la posibilidad de que tengan un rol en las enfermedades neurológicas. La preservación de los nervios perivasculares cutáneos sugiere que se puede identificar las fibras nerviosas intraepidermales en piel antigua. Se ha demostrado que estas fibras son afectadas por neurópatas periféricos. La posibilidad de establecer la presencia de diabetes mellitus en civilizaciones antiguas por la medición de hemoglobina glicosilato en sangre momificada revela la genética de la alta incidencia de esta enfermedad en poblaciones contemporáneas de los nativos de Norteamérica.

Palabras claves: *Nervios surales, nervios perivasculares cutáneos, hemoglobina glicosilata, neuroquímicos.*

Preservation of neurochemicals might give indication of disease of ancient human and contribute to social and biology history. We have demonstrated neurochemicals and nitric oxide synthase (NOS) in sural nerves from embalmed human Egyptian mummies ±3500 years old. Anterior roots were also dissected from 3 naturally mummified upper thoracic spinal cords ±3000 years old from Pisagua Bay (Chile). Single fibers of anterior roots were teased on gelatin coated slides. Forearm skin (n=3) from the Atacama desert (Chile) ca. 3000 years old was examined after blood vessels were teased from the superficial subcutaneum and stained with methylene blue. Lleum ca. 1300 year old Atacama desert (n=1) was also examined. Two intracardiac blood clots mixed with papillary muscle from the Atacama desert ca. 1100 years old were examined for glycosylated hemoglobin.

All tissues were examined after rehydration. The estimated dates of deaths were based on diagnostic artifacts, body position, snuff objects and carbon dating. Optimal dilution of rabbit polyclonal primary antibodies were made with phosphate buffered saline containing 0.1% sodium azide after pilot experiments. In sural nerves inmunoreactivity in nervi nervorum was found for type 1 nitric oxide synthase (NOS) and also, for the first time, NOS was demonstrated in two contemporaneous sural nerve controls. Inmumoreactivity for galanin, PGP 9.5 (a non-specifc axonal marker) and CGRP was found in 3, 2 and 1 sample respectively. Varicose NOS-containing fibers were found in teased anterior root fibers. Met-enkephaline (M-ENK) positive fibers were also present. Protein gene- product 9.5 (PGP 9.5) was extensively demonstrated in anterior roots. Perivascular nerve fibers were well preserved in the subcutaneum. The ileum contained nerve fibers and neurons showing 5-HT, PYY and NOS immunoreactivity. The fibers containing neurochemicals in anterior roots are likely to be in gray rami communicants. The tissues from heart chambers showed 5% hemoglobin A1C (contemporaneous normal 4.2-6.4%) of total hemoglobin.

One branch of paleobiology focuses on the neurobiology of ancient humans and animals; the term paleoneurobiology (Hoyle et al. 1997) has been proposed to encompass it. Because of the embalming practices in ancient Egypt, some peripheral nerves and the autonomic nervous system are the only tissues left in Egyptian mummies to yield paleoneurobiologic clues. On the other hand, naturally mummified tissues commonly found in arid climates such as the Atacama desert (Aufderheide and Rodríguez-Martin 1998) or unusual locations such as bogs (Aufderheide and Rodríguez-Martin 1998) occasionally have preserved central nervous tissue, and such mummies could yield information about nervous system function and diseases.

Complex bio-molecules have been found preserved to variable extent in Egyptian mummies. These include nucleic acids and proteins but large molecules seem better able to withstand the ravages of time than smaller molecules (Barraco 1978). Enzymes with substantial preservation of their catalytic activity have been reports in embalmed mummies from the Graeco-Roman period approximately 2000 years old (Wick et al. 1980; Kaup et al. 1994; Weser et al. 1995). Immunohistochemical studies have also shown preservation of some antigen in ancient tissues but this varies with the embalming method and climatic conditions to which the mummy was exposed (Fulcheri 1995). Immunofluorescence techniques have revealed macromolecules, e.g. collagen type I and III in naturally mummified skin from a Peruvian mummy. These findings suggest the possibility of not only recognizing extraskeletal diseases but also, to some

extent, of assessing function of ancient humans from their mummified remains.

The sural nerve has seemed to be a promising side to gain insights into the autonomic health and habits of long dead individuals. This nerve is frequently biopsied for diagnostic purposes in patients with a variety of peripheral neuropathies because the nerve is composed of sensory fibers and its partial surgical removal, usually under local anesthesia, does not cause paralysis. Because the nerve supplies primarily the skin it is rich in primary afferent fibers (Gundersen et al. 1985; Barajon et al. 1996). Neuropeptides are differentially affected in the sural nerve in some sensory neuropathies (Lincoln et al. 1993) such as diabetic and alcoholic neuropathy. The neuropeptides are present in the nervi vasorum and nervi nervorum and play an important role in the vasoregulation and blood flow through the vasa nervorum of the nerve (Lincoln et al. 1993). A comparison between the neurochemistry of ancient and modern people would be possible if neurochemicals could be shown to remain demonstrable after millennia in either embalmed or naturally mummified sural nerves.

Immunoreactivity for type I nitric oxide synthase (NOS), protein gene-product 9.5 (a non-specifics neuronal marker), galanin, and calcitonin gene-related peptide was found in sural nerves [from 7 embalmed from upper Egypt, Luxor and the Oasis of Kharga, 2000 (Graeco-Roman period) to 3500 (New Kingdom/Third intermediate period) years old] and 1 naturally mummified sural nerve (from Peru, 1000 years old) (Hoyle et al. 1997). It was not known if NOS- immunoreactivity was present in contemporaneous sural nerves until a study of modern control nerves confirmed that it is (Figure 1). Thus millenia old tissue led to the demonstration of a neurochemical that might have important pathogenetic implication in contemporaneous human sensory neuropathies.

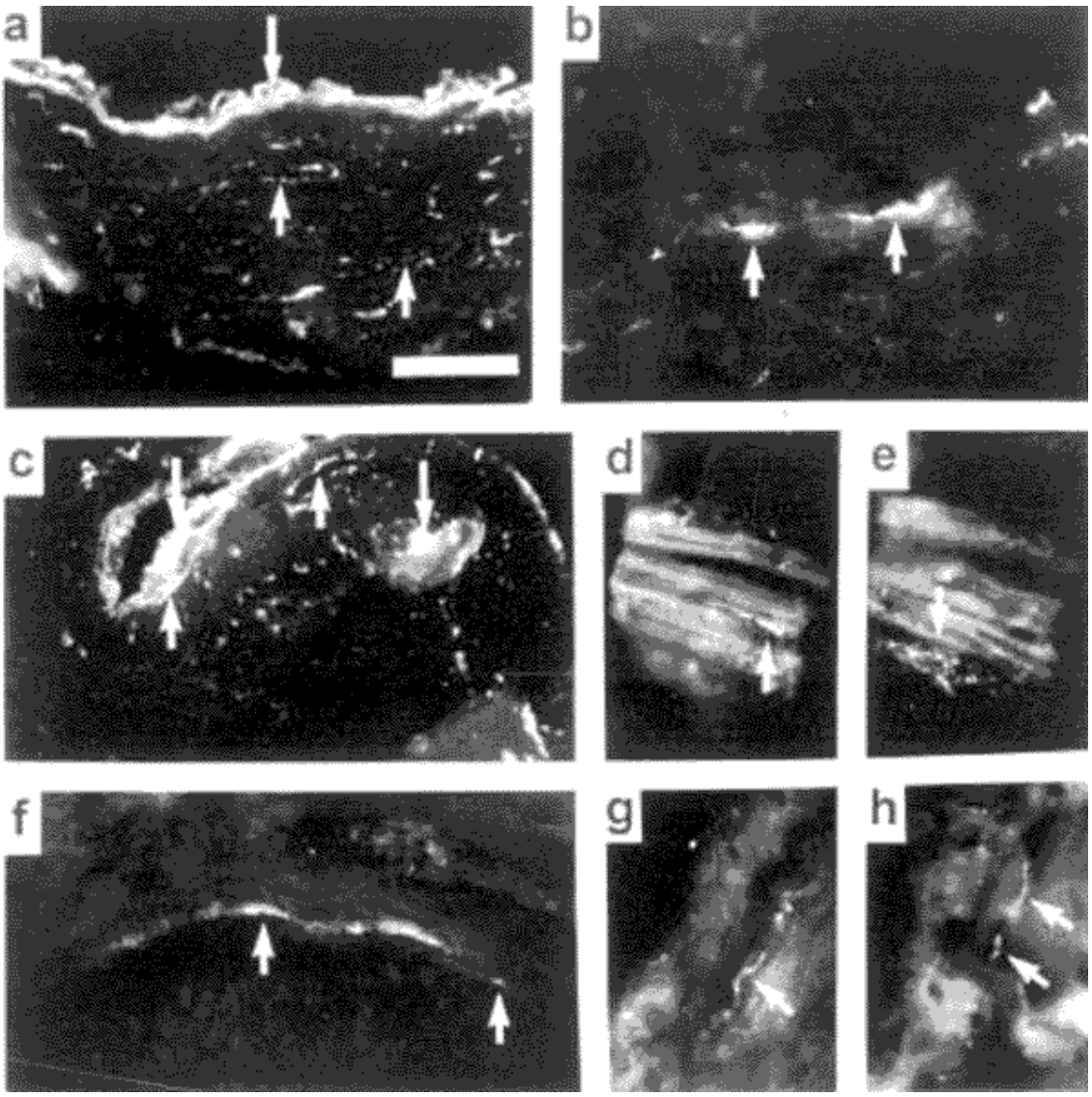

Figure 1. Immunoreactivity for PGP 9.5, calcitonin gene-related peptide (CGRP), galanin and nitric oxide synthase (NOS) in mummified human sural nerves 2000-3500 years old, and NOS in modern human sural nerves. (a) Immunoreactivity for PGP 9.5 in a sural nerve bundle from an Egyptian Mummy. Many varicose intrafascicular fibers were stained (small arrows). The perineurium (large arrow) and the connective tissue at the left of the micrograph were autofluorescent. Scale bar 100 mm. (b) PGP 9.5 immunoreactive varicose fibers (arrows) in epineurium from the sural nerve of an Egyptian mummy. Scale bar in (a) represents 50 mm. In (b). (c) CGRP- immunoreactivity in sural nerve bundle from another Egyptian mummy. Some varicose intrafascicular fibers were stained (small arrows) and there was a high level of autofluorescence from perineurial connective tissue (large arrows). Scale bar in (a) represents 100 mm. In (c) (d,e) Galanin-immunoreactivity in varicose fibers (arrows) in epi- or perineurium from the sural nerve of an Egyptian mummy. Scale bar in (a) represents 50 mm. in (d) and (e) (d,e) are nearly consecutive sections. (f) Immunoreactivity for NOS in varicose nerve fibers (arrows) in a modern sural nerve specimen obtained in 1995. Scale bar in (a) represents 50 mm. In (f) (g) NOS- immunorectivity in epineurial varicose nerve fibers (arrow) of a sample of sural nerve also dating from 1995. Scale bar in (a) represents 50 mm. In (g) (h) NOS-immunoreactive fibers (arrows) in epineurium from an Egyptian mummy. Scale bar in (a) represents 50 mm. In (h) From Hoyle et al. 1997.

In modern alcoholic and diabetic peripheral neuropathies there is a quantitative reduction in substance-P (SP) nerve/ epineural ratios in the sural nerves as determined by ELISA. This ratio is also reduced in the optic nerves of the same subjects with peripheral neuropathy even though the optic nerves are, unlike the sural nerves, histologically normal (Lincoln et al. 1993). In sural nerves from nineteen 1000-2000 years-old mummies from the Atacama desert quantitation of nerve/epineural SP ratios by ELISA showed a very low average of 1.3±0.3 (contemporaneous normal average ratio is 5.0). However, in one 2000 year old nerve the SP nerve/epineural ratio was 4.9 approximating normal contemporaneous values (Milner et al. 1999). Thus with better techniques it might become feasible to assess the presence of peripheral neuropathy by disturbed peptide ratios in ancient human remains. The ventral spinal roots of the upper thoracic cord were examined by immonohistochemistry in naturally mummified individuals who died approximately 3000 years ago in the Atacama desert. Conventional techniques readily revealed a leash of NOS and met-enkephalin containing varicose fibers and fibers that stained positively for protein gene-product 9.5 (Figure 2). Because of the varicose appearance of the fibers it is likely that these were preganglionic fibers, part of the gray rami communicants. (Appenzeller et al. 1997) It is not known, however, if these neurochemicals are also present in contemporaneous ventral roots. The appropiate controls are still to be examined. Moreover the role of these fibers and their neurotransmitters in diseases of the nervous system remains to be established.

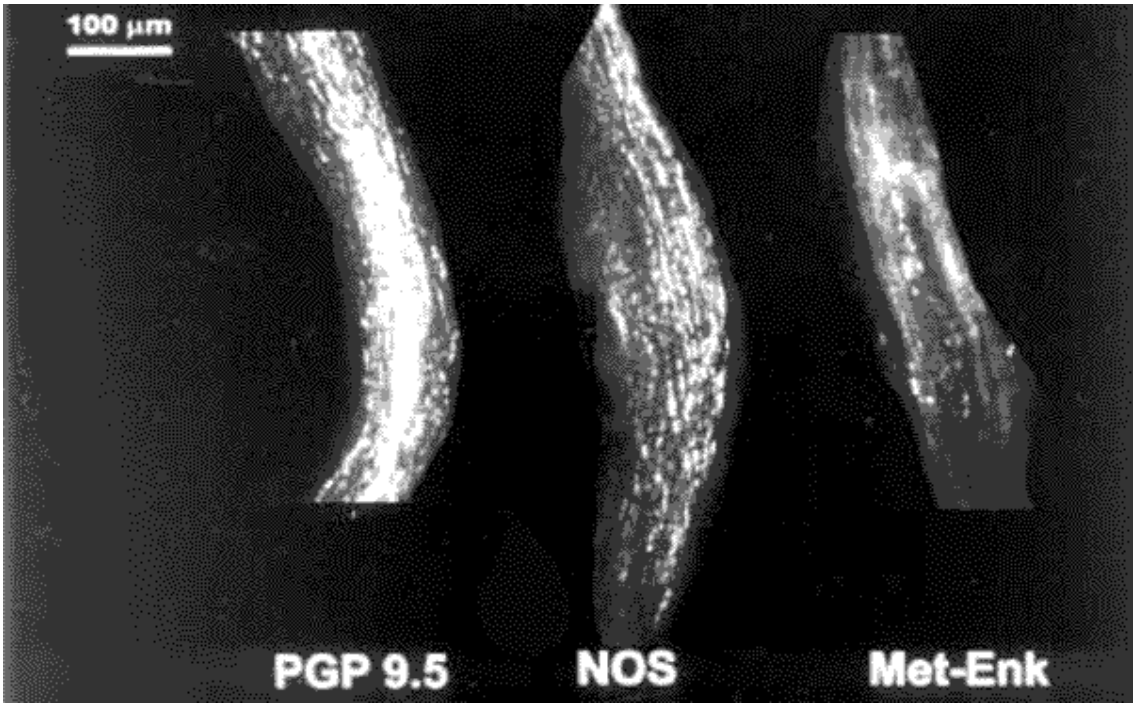

Figure 2. Immunoreactivity for PGP 9.5, nitric oxide synthase (NOS) and met-enkephalin (Met-Enk) in 3000 year old, naturally mummified, ventral spinal root fascicles from the upper thoracic spinal cord. Note varicose appearance suggesting pre-ganglionic fibers. Courtesy C.H.V. Hoyle.

The enteric nervous system contains more neurons than the spinal cord (Jessen et al. 1987). Thus it is not surprising that attempts have been made to demonstrate these autonomic neural networks in mummified tissue. Egyptian mummies were eviscerated during embalming but ileum from a naturally mummified body approximately 1300 years old from the Atacama desert was examined by standard immunohistochemistry. The enteric neuronsand fibers showed immunoreactivity for 5-HT, peptide YY (PYY); a mediator of inhibitory actions on gastrointestinal motility, secretion and blood flow (Jackerott and Larsson 1997 and NOS (Figures 3, 4, 5). The enteric nervous system plays an important role in the pathogenesis of some diseases of the gastrointestinal tract and in Chagas' disease. The demonstration of these neurochemicals in ancient tissue might give hints to the prevalence of diseases affecting the gut in some prehistoric civilizations.

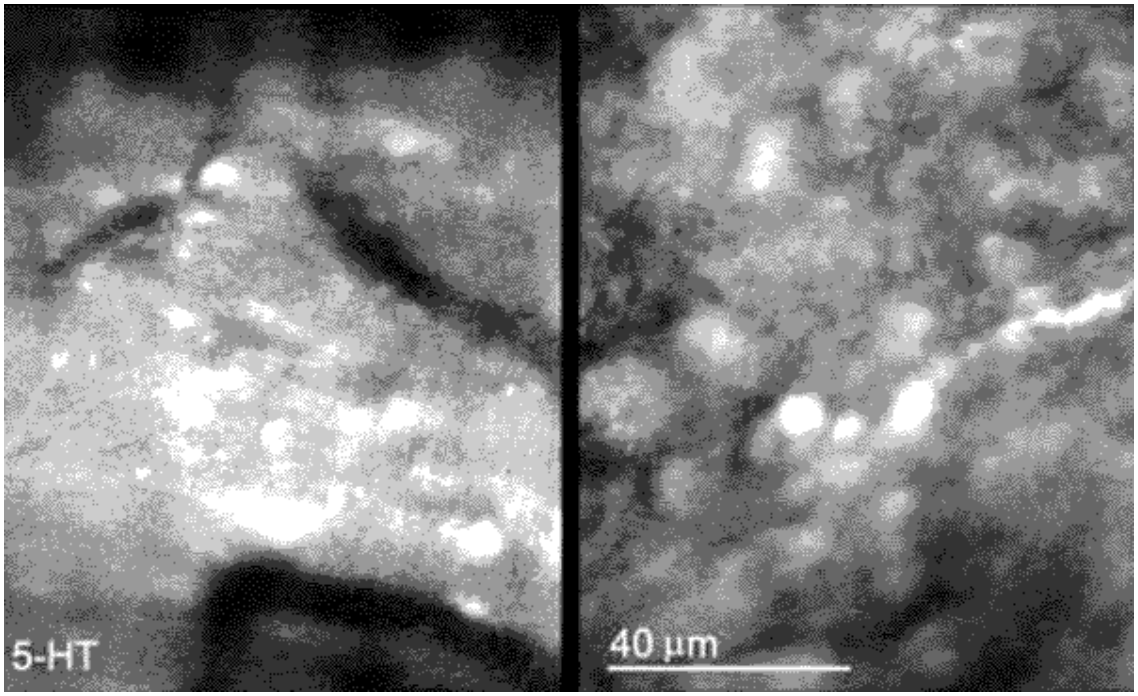

Figure 3. Immunoreactivity for serotonin (5-HT) in nerve fibers and neurons in a naturally mummified ileum from the Atacama desert about 1300 years old. Courtesy A. Belai.

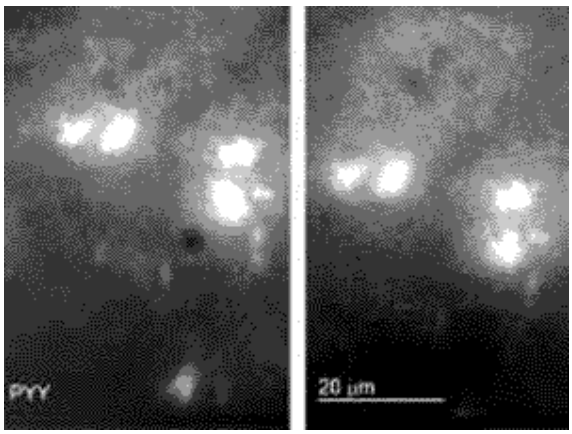

Figure 4. Immunoreactivity for peptide YY (PYY) in neurons of the same specimen as in Figure 3. Courtesy A. Belai.

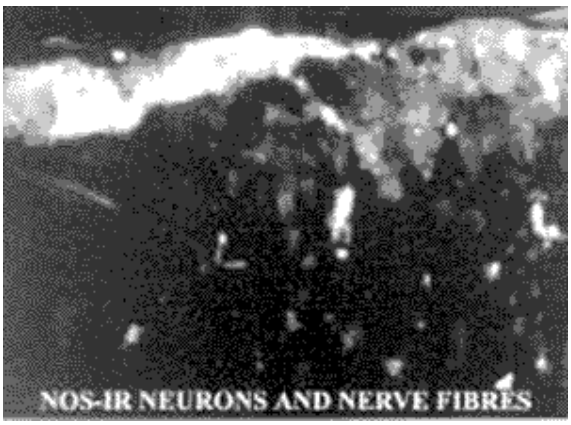

Figure 5. Immunorectivity for nitric oxide synthase (NOS-IR) of the same specimen as in Figure 3. Courtesy A. Belai.

Cutaneous nerves are affected in some peripheral neuropathies. The possibility that the nerve fibers in ancient skin might reveal peripheral nerve disease led to the examination of perivascular nerve fibers in the superficial subcutaneum. Forearm skin of 3 naturally mummified bodies from the Atacama desert, approximately 3000 years old, were shown to contain methylene-blue stained perivascular nerves (Figure 6).

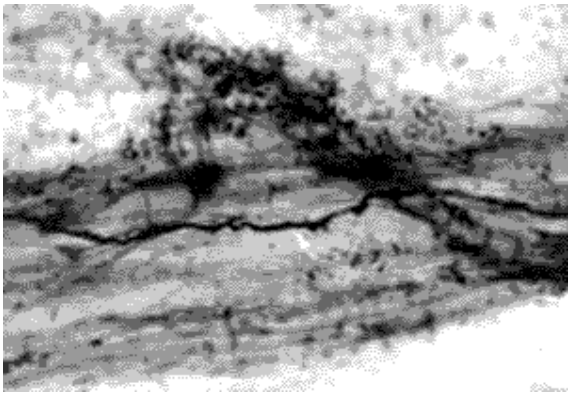

Figure 6. Perivascular nerve fiber in the superficial subcutaneum of 3000 year old naturally mummified skin from the Atacam desert stained with methylene blue (arrow). Courtesy M. Appenzeller.

Intraepidermal nerve fibers are known to decrease in number in a length-dependent fashion in peripheral neuropathies and also in the neuropathy of AIDS (McCarthy et al. 1995) but no intraepidermal nerve fibers have so far been demonstrable in mummified skin.

Diabetes mellitus is known to be a genetic disorder, but the individuals' life style, superimposed on genetic inheritance, determines whether he/she will actually become diabetic during life.

Present day American Native populations are afflicted by a high prevalence of diabetes (Campos-Outcald et al. 1995). The reasons for this prevalence are a subject of debate (Rousseau 1995). Some contend that the high prevalence of the disease is the result of a change in traditional life style and diet brought about by acculturation. This is superimposed upon a genetic predisposition to diabetes resulting from an admixture of Hispanic genes with the Native American gene pool during the conquest of the New World. Others opine that the genetic predisposition to diabetes was present

before the Spanish conquest of the New World and this together with acculturation contributed to the high prevalence of the disease. The examination of tissues from south American pre-Hispanic mummies might help resolve this debate since the finding of evidence for diabetes or its complications in these ancient remains could strengthen the latter hypothesis. Both diabetic and alcoholic profiles of peptides, or other evidence of these diseases in pre-Hispanic mummified tissues, might give meaning to the "improved understanding of the evolution of diseases and their role in human biologic and social history" (Zimmerman and Kelley 1982) to which paleoneurobiology can make significant contributions.

Acknowledgments. Work described here was supported by: New Mexico Health Enhancement and Marathon Clinics (NMHEMC) Research Foundation and the National Institutes of Health, Bethesda MD. Grant # IHSEP-02.

This is part of a Chapter entitled Paleoneurobiology and the Autonomic Nervous System by: Otto Appenzeller and A.C. Aufderheide to be published by Elsevier Science Publishers in Part I, the Autonomic Nervous System in the Handbook of Clinical Neurology, Amsterdam, The Netherlands, 1999.

We are grateful for tissue donations to Drs. David Hunt and Greta Hansen, Smithsonian Institution, Washington DC, USA. Dr. Sonia Guillén, Centro Mallqui, Ilo, Peru. Dr. Calogero Santoro, Department of Anthropology, University of Tarapacá, Arica, Chile and Dr. Arthur C. Aufderheide, Department of Pathology and Laboratory Medicine, University of Minnesota, Duluth, MN, USA.

References Cited

Appenzeller, O., C.H. Hoyle, C.M. Santoro and G. Burnstock 1997 Paleo-autonomic Biology of Human Anterior Spinal Roots. *Clin. Autonom. Res.* 7: 242. [Links]

Aufderheide, A.C., and C. Rodríguez-Martin 1998 *The Cambridge Encyclopedia of Human Paleopathology*. Cambridge U.K., Cambridge University Press [Links]

Barajon, I., M. Bersani, M. Quartu, M. Del Fiacco, G. Cavaletti, J.J. Holst and G. Tredici 1996 Neuropeptides and Morphological Changes in Cis-Platin Induced Dorsal Root Ganglion Neuropathy. *Exp. Neurol.* 138: 93-104. [Links]

Barraco, R.A., 1978 Preservation of Proteins in Mummified Tissues. *American Journal Phys. Anthropol*. 48: 487-491. [Links]

Campos-Outcald, D., J. Ellis and M. Aikin 1995 Prevalence of Cardiovascular Disease Risk Factors in a Southwestern Native American Tribe. *Public Health Rep*. 110: 742-748 [Links]

Fulcheri, I. 1995 Immunohistochemistry: a New Outlook in Histopaleopathology. *Bulletin Soc. Ital. Biol. Sper.* 71: 105-110. [Links]

Gundersen, K., O. Oktedalen and F. Fonnum 1985 Substance P in Subdivisions of the Sciatic Nerve, and in Red and White Skeletal Muscle. *Brain Res.* 329: 97-103. [Links]

Hoyle, C. H., P.K. Thomas, G. Burnstock and O. Appenzeller 1997 Immunohistochemical Localization of Neuropeptides and Nitric Oxide Synthase in Sural Nerves from Egyptian Mummies. *Journal Autonom. Nerv. System* 67: 105-108 [Links]

Jackerott, M. and L.I. Larson 1997 Immunocytochemical localization of the NPY/PYY Y1 Receptor in Enteric Neurons, Endothetial cells and Endocrine-like Cella of the Rat Intestinal Tract. *Journal Histochem Cytochem* 45: 1643-1650. [Links]

Jessen, K. R., R. Mirsky and J.M. Hills 1987 GABA as an Autonomic Neurotransmitter: Studies on Intrinsic GABA ergic Neurons in the Myenteric Plexus of the Gut. *TINS* 10: 255-261. [Links]

Kaup, Y., U. Baumer, J. Koller, R.E. Hedges, H. Werner, H.J. Hartman, H. Etspuler and U. Weser 1994 Zn2Mg Alkaline Phosphatase in an Early Ptolemeic Mummy. *Z. Naturforsch* 49: 97-103. [Links]

Lincoln, J., P. Milner, O. Appenzeller, G. Burnstock and C. Qualls 1993 Innervation of Normal Human Sural and Optic Nerves by Noradrenalin and Peptide Containing Nervi Vasorum and Nervorum: Effect of Diabetes and Alcoholism. *Brain Res.* 632: 48-56. [Links]

McCarty, B.G., S.T. Hsieh, A. Stocks, P. Hauer, C. Macko, D.R. Cornblath, J.W. Griffin and J.C. McArthur 1995 Cutaneous Innervation in Sensory Neuropathies: Evaluation by Skin Biopsy. *Neurology* 45: 1848-1855. [Links]

Milner, P., A. Belai, C.V. Hoyle, M. Appenzeller and O. Appenzeller 1999 Paleoneurobiology. *Clin. Autonom. Res.* (in press). [Links]

Rousseau, P. 1995 Native-American Elders. Health Care Status. *Clin. Geriatr. Med.* 11: 83-95. [Links]

Weser, U., H. Etspuler and Y. Kaup 1995 Enzymatic and Immunological Activity of 4000 Years Aged Bone Alkaline Phosphatase. *FEBS Lett.* 375: 280-282. [Links]

Wick, G., M. Haller, R. Timple, H. Cleve and G. Zziegelmayer 1980 Mummies from Peru. Demonstration of Antigenic Determinants of Collagen in the Skin. Int. Arch. *Allergy Appl. Immunol.* 62: 76-80. [Links]

Zimmerman, M.R. and M.A. Kelley (Editors) 1982 *Atlas of Human Paleopathology*. New York, Praeger Publishers. [Links]

* 1559 Fagle Ridge RD NE, Albuquerque NM 87122, U.S.A. E-mail: OttoArun12@aol.com.

** Department of Anatomy and Developmental Biology, University College, London, UK.

*** Departamento de Arqueología y Museología, Universidad de Tarapacá, Casilla 6-D, Arica, Chile. E-mail: csantoro@uta.cl.

**** Department of Pathology School of Medicine, University of California, San Francisco, CA, USA.

Recibido: marzo 1999. Aceptado: diciembre 2000.