Body weight, liver weight and area of sebaceous glands in rats with and without a portacaval shunt

N	Before PCS 5	After PCS 5	Before sham PCS 5	After sham PCS 5
Body weight (g)	307 ± 13[a]	241 ± 41[b]	291 ± 12	304 ± 18[c]
$\frac{\text{Liver weight}}{\text{Body weight}} \times 100$		2.15 ± 0.21		4.06 ± 0.35
$\frac{\text{Testes weight}}{\text{Body weight}} \times 100$		0.93 ± 0.07		0.99 ± 0.08
Number of sebaceous glands per micrograph	12.4 ± 2.7	6.2 ± 1.3[b]	10.8 ± 1.1	10.2 ± 2.1[c]
Area of sebaceous glands (mm^2)	598 ± 194	275 ± 88[b]	559 ± 170	567 ± 160[c]

[a] Mean ± ISD; [b] $p < 0.05$ vs the same group before PCS; [c] $p < 0.05$ vs the other group after PCS.

significance. The hyperoestrogenic state observed after PCS has several origins: among them, the porto-systemic shunt[17], the decreased hepatic blood flow[18], the increased conversion of androgen to oestrogens[19,10]. The atrophy of sebaceous glands after PCS is one more example of the importance of the skin as a hormonal receptor.

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Retrograde axonal transport and transneuronal transference of horseradish peroxidase in the rat ciliary ganglion[1]

P. Gomez-Ramos and E.L. Rodriguez-Echandia

Dept. Morfologia, Facultad de Medicina, Universidad Autonoma de Madrid, Arzobispo Morcillo 4, Madrid 34 (Spain), 10 April 1981

Summary. Horseradish peroxidase was injected into the ciliary body of the eye, and 24 h later it was found in the perikaryon and dendritic processes of the ciliary ganglion neurons, as well as in nerve terminals presynaptic to those neurons. These results indicate that horseradish peroxidase was retrogradely transported and subsequently transneuronally transferred.

Substantial evidence exists for a bidirectional transfer of substances from one neuron to another. Transneuronal migration of radioactive material following anterograde axonal transport was first shown in the visual system[2] and later in several other systems[3-5]. Radioactive tetanus toxin has been shown to travel transneuronally following retrograde axonal transport[6,7]. The present report presents some evidence of transneuronal transfer of horseradish peroxidase (HRP) a protein transported intraneuronally mainly in the retrograde direction[8-10] but also anterogradely[11,12]. Preliminary results have been presented elsewhere[13].

10 male albino rats (200–250 g) of the Sprague-Dawley strain were used. In 8 animals, 1 mg of HRP (Sigma type VI) in 3 µl of distillated water was injected into the ciliary body of one eye under light ether anesthesia. 2 control animals were injected with distilled water. The animals were sacrificed 24 h after the injection, and perfused through the heart with 250 ml of 1% paraformaldehyde and 1% glutaraldehyde in 0.12 M phosphate buffer (pH 7.2). The brains and the ciliary ganglia homolateral to the injected eye were dissected out and immersed in the same double aldehyde fixative at 4 °C for 4–6 additional h. The material was soaked overnight in 0.12 M phosphate buffer containing 15% sucrose at 4 °C and the peroxidase activity was determined by the method of Graham and Karnovsky[14]. The ciliary ganglia were post-fixed in 1%

OsO_4, dehydrated in ethanol and embedded in Epon 812. Ultrathin sections for electron microscopy were observed unstained or stained with lead citrate.

No endogenous peroxidase activity was detected in the control animals. 24 h after the HRP injection, the neurons of the ciliary ganglion homolateral to the injected eye showed HRP-containing vesicles throughout the cytoplasm. The nuclei and the Golgi complex were free of label, but many lysosomes exhibit HRP-reaction product. No diffuse HRP was found in the cytoplasm. HRP-reaction product was found at the presynaptic nerve terminals synapsing the ciliary neurons (fig. 1) and also in the extracellular space between neurons and Schwann cells but not in the connective tissue among the nerve fibers. Figures 2 and 3 (arrows) showed 2 different images which suggest endocytosis of HRP from the extracellular space by presynaptic nerve terminals.

No HRP-positive neurons were identified in the Edinger-Westphal nucleus, nor in any other mesencephalic structure in the 40-µm-thick sections of the brain of the HRP injected animals.

Diffusion of HRP out of the eye is limited, thus the HRP is very probably retrogradely transported by the axons of the short ciliary nerves, whose nerve endings are located in the ciliary body and whose cell bodies are in the ciliary ganglion[15]. No recurrent axon collaterals have been described for those neurons. When the HRP arrives at the neuronal soma, some of the HRP-containing vesicles might fuse with primary lysosomes as has been suggested previously[16,17]. In our material we have found lyosomes containing HRP-reaction product which confirm this idea. A number of the HRP-containing vesicles move to the periphery of the neurons and dendritic processes since there was a large proportion of HRP in dendritic processes at this time interval. The HRP might be released from the ganglion neurons into the extracellular space. The extracellular HRP present in our preparation was always located in the vicinity of the ciliary ganglion neurons. For these reasons we suggest that exocytosis of HRP may account for the extracellular HRP found in our preparation. The observed images of endocytosis at the presynaptic nerve terminals support the suggestion that some of the HRP released to the extracellular space is taken up by the nerve terminals of the preganglionic neurons synapsing with the ciliary neurons.

Our results confirm the incidental observation of Itaya et al.[18] that after anterograde transport HRP may cross synaptic junctions. Their results taken together with ours support the idea that HRP can be transferred from one neuron to another in both directions.

The use of retrograde transport of HRP as a neuroanatomical tool for identifying the origin of neuronal connections within the central nervous system is based on the fact that HRP after retrograde axonal transport is accumulated in

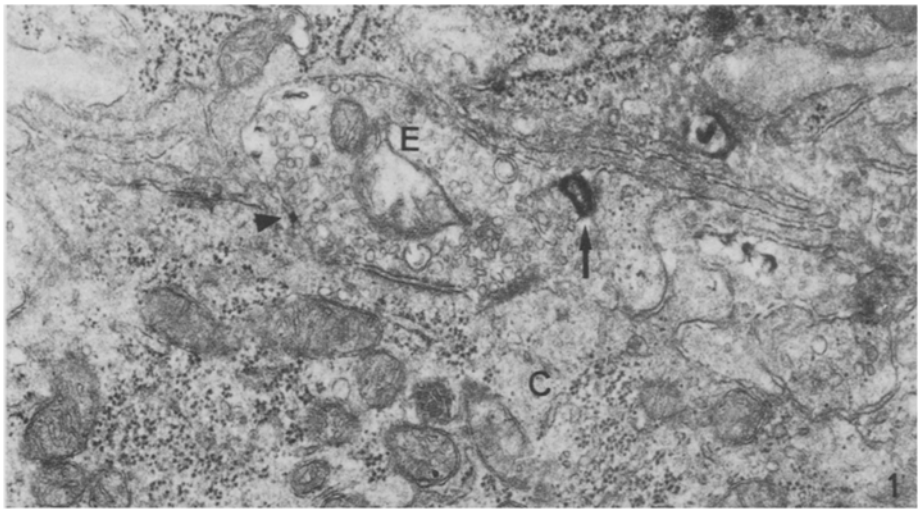

Figure 1. Electron micrograph of the ciliary ganglion of a rat 24 h after the injection of HRP in the ciliary ganglion. Arrow: HRP reaction-product in a nerve ending (E) which is making synaptic contact with the neuronal soma (C). Arrow-head: HRP reaction-product in the extracellular space between the nerve ending and the neuronal soma. × 28,800.

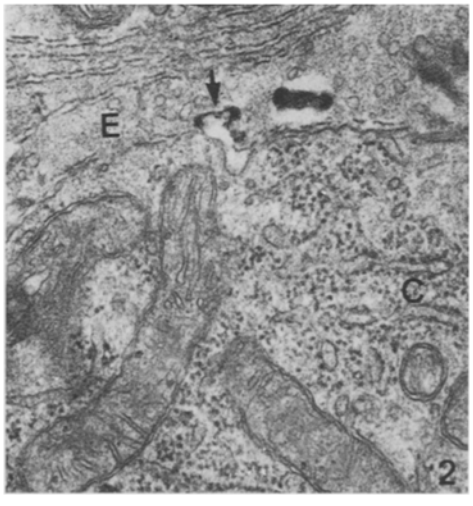

Figure 2. Same material as in figure 1. E: nerve ending. C: portion of the neuronal cytoplasm. The arrow points to a small HRP vesicle that is been endocyted by the nerve ending. × 36,000.

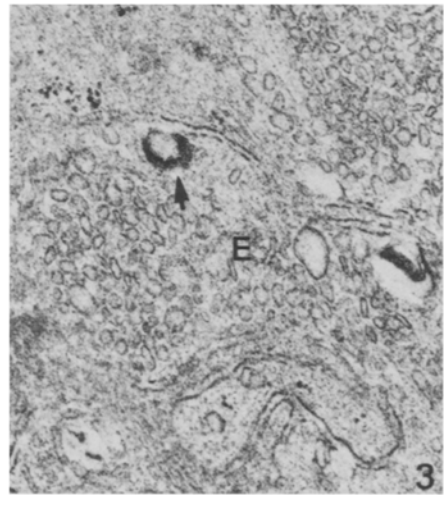

Figure 3. Same material as in figure 1. Another HRP vesicle that is forming in a nerve ending (E). × 44,000.

the neuronal soma, without labeling of the 2nd and 3rd neuron. Our suggestion that there is a transneuronal transfer of HRP does not invalidate this neuroanatomical method since the amount of HRP transferred is very small, but it should be a factor to consider.

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T-cell leukemia induced by 1-propyl-1-nitrosourea in Fischer rats[1]

N. Imamura, O. Saito, H. Dohy and T. Ogiu

Department of Internal Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Kasumi 1-2-3, Hiroshima (Japan), and Division of Pathology, Biological Safety Research Center, National Institute of Hygienic Sciences, Tokyo (Japan), 10 March 1981

Summary. T-cell leukemia was induced at a high rate in female Fischer rats by continuous oral administration of 1-propyl-1-nitrosourea. No association of oncorna viruses were found in the leukemogenesis.

We have reported on the high incidence of thymic lymphoma induction (41/48, 85%) in female Fischer rats following the continuous oral administration of 1-propyl-1-nitrosourea (PNU)[2]. Among those thymic lymphomas induced, 25 cases were successfully transplanted into syngeneic Fischer rats i.p. Here, we have reported on the preliminary characterization of 6 cases of thymic lymphoma transplanted in Fischer rats.

Female Fischer 344 rats purchased from Charles River Japan Co. (Atsugi, Kanazawa) were used through the experiment. Hematologically, all of the transplanted tumors were considered to be lymphoblastic leukemia. It was noted that the presence of leukemic cells with convoluted nuclei, which is usually found in human adult T-cell leukemia[3,4], was always observed in all of the transplanted cases. Cytochemical staining of leukemic cells revealed the focal distribution of acid phosphatase and β-glucuronidase activity which is typical for T-cells[5]. Peroxidase and alkaline phosphatase reactions were negative. Periodic acid-Schiff staining was also negative.

The results of the cell-surface and intracellular marker test are shown in the table. Antisera used in this experiment were mouse anti-Thy 1.1 alloantiserum, Searle Lab., London, fluorescein isothiocyanate (FITC)-conjugated rabbit anti-rat IgM serum, FITC-conjugated rabbit anti-rat IgG serum, FITC-conjugated rabbit anti-mouse IgG serum, Medical & Biological Lab. Ltd, Nagoya, and rabbit anti-calf terminal deoxynucleotidyl transferase (TdT) serum, monoclonal mouse anti-calf TdT serum, Bethesda Research Lab., Bethesda. The specificities of FITC-conjugated antisera were tested by the double immunodiffusion test described previously[6]. E-rosette formation with sheep erythrocytes, EA and EAC rosettes with bovine erythrocytes sensitized with IgG antibody (EA) or IgM antibody and mouse or human complement (EAC) were tested using an assay kit purchased from the Japan Immuno-Research Lab., Takasaki. E-rosette formation with guinea-pig erythrocytes (Hartley strain) was carried out as described previously[7].

Most of the leukemic cells from the 6 cases tested possessed Thy 1.1 antigen were detected as a result of the indirect

Cell-surface and intracellular marker studies on thymic lymphoma induced by 1-propyl-1-nitrosourea in Fischer rats*

Case No.	Cell-surface markers								Intracellular markers		
		SmIg		Receptors					CIg		
	Thy 1.1	M	G	E:sheep	Guinea-pig	EA(G)	EAC:human	Mouse	M	G	TdT
1	87	1	4	22.5	26	43.5	4.0	2.5	0	0	10
2	85	1	0	26.0	8	15.0	2.0	1.5	0	0	10
3	89	1	0	39.5	18	73.5	3.0	11.0	0	0	9
4	92	1	0	41.5	39	62.5	7.0	9.5	0	0	11
5	95	0	0	9.5	15	2.5	1.5	2.0	0	0	32
6	92	0	0	8.0	18	5.5	1.5	2.5	0	0	11

* Percent of positive cells.