Table II. Blastogenic effect of bovine spinal cord antigen on human blood lymphocytes from a variety of hospital patients

Sex	Age	Reason for confinement	Percentage large cells + mitoses		
			No antigen	With antigen (μg/ml) 5	50
♂	34	Multiple sclerosis	2.63	5.59[a]	7.69[a]
♂	19	Multiple sclerosis	1.60	3.27[b]	3.91[a]
♂	46	Multiple sclerosis	0.81	1.00	3.33[a]
♂	55	Advanced multiple sclerosis	1.18	1.69	2.12
♀	53	Multiple sclerosis? Parkinsonism?	0.69	0.63	0.94
♀	36	Advanced multiple sclerosis	1.00	0.87	1.51
♂	50	Multiple sclerosis	1.62	3.37[b]	2.19
♂	59	Tuberculosis, diabetes	2.37	2.06	2.43
♂	63	Leukemia	1.19	1.67	1.50
♀	44	Pain, unknown etiology	2.31	2.81	1.24
♂	49	Open heart surgery	1.61	1.50	2.62
♀	50	Cerebrovascular accident	1.12	1.25	2.75[b]
♂	55	Cord lesion (cyst? syringomyelia?)	0.44	–	2.25[a]
♀	83	Fracture	1.06	3.13[a]	2.87[a]
♀	77	Osteoarthritis	1.31	1.81	5.24[a]
♂	81	Tumor of spine?	1.81	5.18[a]	4.62[a]

[a] Chi-square test compared to control, $P < 0.001$. [b] Chi-square test, $0.001 < P < 0.01$.

central nervous lesions and those in their eighth or ninth decade of life. The latter raises an interesting geriatric question which demands further investigation.

With such limited data one is unwilling to generalize broadly, but our results are fully consistent with those of HUGHES et al.[5] who reported that lymphocytes from patients with multiple sclerosis are transformed when cultured in the presence of an encephalitogenic protein, not dramatically but to a greater extent than those from patients with other neurological disease and those from normals[14].

Résumé. On a constaté de la blastogenèse dans une culture de protéine encéphalitogène de moelle épinière bovin faite avec des lymphocytes de malades atteints de sclérose en plaques ou présentant des lésions du système nerveaux central, ou encore agés de plus de 70 ans.

J. A. BROCKMAN and A. V. STIFFEY

Metabolic Chemotherapy Department,
Lederle Laboratories Division,
American Cyanamid Company,
Pearl River (New York 10965, USA), 20 November 1969.

[14] Acknowledgement. We thank Drs. T. CASSARA, S. ALEXANDER, and B. ELKIN for their cooperation, and their patients and our friends for their blood donations.

Comparative Study of the Ultrastructure and Hormonal Content of the Proximal and Distal Stumps of the Transected Neurosecretory Hypothalamo-Hypophysial System[1]

The hypothalamus is linked to the neural lobe of the hypophysis via the hypothalamo-neurohypophysial tract. Within the axons of this tract neurosecretory material – the polypeptide hormones oxytocin and vasopressin, and a carrier protein (neurophysin) – which can histologically be demonstrated by a positive Gomori reaction is carried by a proximo-distal axoplasmic flow from the synthesizing perikarya into the neural lobe of the hypophysis where it is stored and released[2,3].

After transection of the hypothalamo-neurohypophysial tract an increased amount of Gomori positive substance is not only observed in the proximal stump but also in the *distal stump*[4–8]. As it is very unlikely that neurosecretory material is produced within disconnected axons and as the light microscope alone could not give a satisfactory explanation for the observed phenomenon, the present studies were carried out.

For the ultrastructural studies 3 grass frogs (*Rana pipiens*) were sacrificed at each of the following time periods following transection of the proximal neurohypophysis[8]: 6, 12, 24, 36, 48 h, 6 and 9 days; 7 sham operated animals served as controls. After decapitation, fixation of the stumps was achieved by direct application and ventricular perfusion of a threefold aldehyde mixture[9] in which the dissected tissue remained for 2 h; this procedure was followed by 2 h postfixation in 1% osmium tetroxide (pH 7.2), en bloc staining with uranyl acetate[10], embedding into araldite 502, and staining of the sections with lead citrate[11].

The pharmacological extracts were obtained from 2 groups of 6 animals (controls and 6 h), 2 groups of 7 and 6 animals (1 day), 2 groups of 8 and 7 animals (2 days), 1 group of 6 animals (9 days), and 1 group of 3 animals (15 days) by pooling and homogenizing in 0.25% acetic acid at 0 °C the proximal (including the hypothalamus caudal to the optic chiasm) and the distal stump (including the median eminence) separately for each group. Further treatment of the homogenates included immersion in boiling water for 3 min and subsequent centrifugation at 3000 rpm for 10 min. The bioassays[12] [(2+2)] assay

[1] Supported by grants from the National Institute of Health and the Space Sciences Research Center of the University of Missouri, Columbia (Missouri, USA).

[2] W. BARGMANN, in *Neurohypophysial Hormones and Similar Polypeptides* (Springer, Berlin-Heidelberg-New York 1968), p. 1.

[3] M. GINSBURG, in *Neurohypophysial Hormones and Similar Polypeptides* (Springer, Berlin-Heidelberg-New York 1968), p. 286.

[4] W. HILD, Virchows Arch. path. Anat. *319*, 526 (1951).

[5] J. F. CHRIST, in *Neurosecretion* (Academic Press, New York 1962), p. 125.

[6] W. ETKIN, Gen. comp. Neurol. *2*, 161 (1962).

[7] F. C. ITURRIZA and M. A. RESTELLI, Z. Zellforsch. *81*, 297 (1967).

[8] H.-D. DELLMANN and P. A. OWSLEY, Z. Zellforsch. *87*, 1 (1968).

[9] E. M. RODRIGUEZ, Brain Res., *15*, 395 (1969).

[10] N. J. KARNOVSKY, J. Cell. Biol. *35*, 213 (1967).

[11] J. VENABLE and R. COGGESHALL, J. Cell. Biol. *25*, 401 (1965).

[12] J. DEKANSKI, Br. J. Pharmac. *7*, 567 (1952).

design were performed on Dibenzyline (300 μ g/100 g) treated and ethanol (12%) anesthetized rats with pitressin as standard preparation.

In Table I our ultrastructural findings are summarized. The first 3 phases are observed between the operation and 1–2 days in the proximal as well as in the distal stumps, the fourth phase begins at around 2 days in the proximal and 1–$1^1/_2$ days in the distal stump, the fifth phase's events are first observed at 9 days in the proximal stump and at 6 days in the distal stump; in both stumps overlappings between phases are observed which are due to a non-synchronized reaction of the severed axons and a varying reaction speed of the individual axons. Our interpretation of these postoperative changes is summarized in Table II.

A comparison of the light- and electron-microscopic findings in the proximal and distal stumps reveals that during the first 3 phases the observed increased amount of Gomori positive material is due to the presence of an increased number of neurosecretory granules and also very likely to neurohypophysial hormones contained within tubular formations (the neurohypophysial hormones have been shown to give a positive Gomori reaction[13]); during the last 2 phases the same phenomena are responsible for the positive Gomori reaction in the proximal stump; in the distal stump, however, dense lamellar bodies of varying sizes represent the Gomori positive material observed in the light microscope.

The pharmacological results are represented in the Figure. In comparing the ultrastructural and bioassay results the similarity of the morphological and pharmacological reactions in the proximal and the distal stump during an initial period of approximately 24 h becomes very striking; then, beyond approximately 24 h after the transection, with the diverging curves of the hormone content of the proximal and the distal stumps (increase in the proximal and decrease in the distal stump) the ultrastructural reactions in the 2 stumps are also dissimilar.

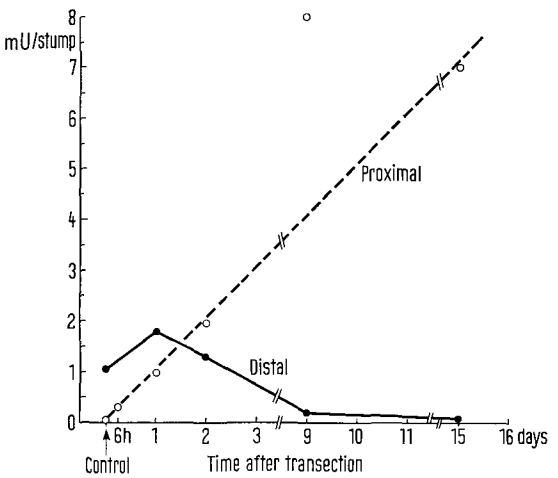
Table I. Postoperative ultrastructural changes

	In the proximal stump	In the distal stump
1st Phase	Increase of neurosecretory granules at the site of transection[a]	Increase of neurosecretory granules at the site of transection
2nd Phase	Appearance of vesicles and tubular formations	Appearance of tubular formations
3rd Phase	Formation of neurosecretory granules takes place within the enlarged tubular formations	
4th Phase	Accumulation of neurosecretory granules in more proximal portions of the axon[b]	Beginning degeneration of axons (appearance of numerous small and large dense lamellar bodies) and glial phagocytic activity, tubular formations decrease in density and width
5th Phase	Establishment of neurovascular contacts; beginning formation of a new neural lobe	Continuing and definite degeneration of axons (appearance of largest dense lamellar bodies), increased glial phagocytic activity

[a] This very early increase in thought to be due to a 'passive' accumulation of neurosecretory granules which were on their way to the neural lobe before the transection and which were stopped by the sealing of the axonal membranes at the site of transection. [b] This is thought to be an 'active' accumulation of neurosecretory granules due to local packaging and proximo-distal flow.

Table II. Possible origin of the various structures

	In the proximal stump	In the distal stump
neurosecretory granules	Packaging from tubular formations and from the more proximal parts of the neurosecretory neuron through a *proximo-distal* flow	Packaging from tubular formations and from the neural lobe through a *backflow*
Tubular formations	From tubular formations normally present in the axon	
Dense lamellar bodies	None	Small dense lamellar bodies from mitochondria Large dense lamellar bodies from localized disturbed lipid metabolism Largest dense lamellar bodies from degenerating axons



Hormonal content of the proximal and distal stumps.

The ultrastructural changes and the bioassay results very clearly indicate that the reaction to the injury in the proximal stump leads to the reorganization of a neural lobe – median eminence structure; in the distal stump an initial reaction similar to the initial phases of reorganization observed in the proximal stump occurs, however, in the absence of a perikaryon the disconnected axons very rapidly degenerate.

Zusammenfassung. Nach Durchtrennung des Hypophysenstiels wird eine Akkumulation von Neurosekretgranula sowohl im proximalen wie auch im distalen Stumpf des Stiels nachgewiesen. Elektronenmikroskopische und pharmakologische Untersuchungen machen wahrscheinlich, dass die Akkumulation im distalen Stumpf auf einen Reflux der Neurosekretgranula zurückzuführen ist.

H.-D. Dellmann and E. M. Rodriguez

Department of Veterinary Anatomy, School of Veterinary Medicine, University of Missouri, 125 Connaway Hall, Columbia (Missouri 65201, USA), 16 October 1969.

[13] N. Guttierrez and J. C. Sloper, Histochemie *17*, 73 (1969).