$MgCl_2 \cdot H_2O$ 1.2 mM/l, glucose 16.7 mM/l, and polyethylene glycol (PEG) 2 g/l.

The effect of i.v. secretin was determined by comparing the net electrolyte fluxes in the period following secretin (Boots Pure Drug Company, London) with those in the alternate period following i.v. saline. In Group I ($n = 5$), saline was injected 5 min after the start of the first perfusion period and secretin was injected 5 min after the start of the second. In Group II ($n = 5$), injections were given in the reverse order.

The net fluxes of sodium, potassium, chloride and bicarbonate were calculated as follows:

$$\text{ion flux} = \text{initial volume} \left(\text{final ion conc.} \frac{\text{initial PEG conc.}}{\text{final PEG conc.}} - \text{initial ion conc.} \right)$$

The sign of the flux denotes movement into (+) or out of (−) the perfusion fluid.

Statistical significance was assessed with a t-test for paired samples.

Results and discussion. The Table summarizes the mean net fluxes of bicarbonate, chloride, sodium and potassium that were measured after administration of saline and secretin. Large single doses of secretin failed to cause a significant change in the flux of any of the ions. Because a dose of 5 U elicits a near-maximal response in secretory rate from the rat pancreas[2], it was considered a stimulus sufficient to determine whether secretin affected intestinal bicarbonate secretion. The biologic effects of a single submaximal dose of secretin in the rat, as determined by its pancreatic exocrine activity, are maximal within 20 min and minimal after 30 min[2], so the period of observation used in this study should have detected any measurable change. There is little reason to doubt the activity of the preparation since vials from the same batch used in clinical studies elicited normal responses in humans.

Ion	Mean net flux (μEq)		S.E.	P
	Saline	Secretin		
HCO_3^-	+ 60.7	+ 55.9	3.44	> 0.10
Cl^-	− 190	− 173	15.3	> 0.10
Na^+	− 115	− 117	20.6	> 0.90
K^+	− 1.45	− 1.93	0.614	> 0.40

The Table compares the net fluxes of HCO_3^-, Cl^-, Na^+ and K^+ into (+) and out of (−) the fluid perfusing the ileum in 10 rats. The standard error (S.E.) and P-value are shown. (Student t-test.)

Conclusion. Single large i.v. doses of secretin failed to influence the net fluxes of bicarbonate, chloride, sodium or potassium in fluid perfusing the ileum of the rat. The results suggest that bicarbonate secretion in the ileum is controlled by a mechanism which differs from that of the pancreas and biliary tree.

Zusammenfassung. Es wird gefunden, dass einmalige, grosse i.v. Dosen von Sekretin keinen Einfluss auf den Netto-Flux von Bikarbonat, Chlorid, Natrium und Kalium in der Perfusionsflüssigkeit des Ileums der Ratte ausüben: offenbar ist der Kontrollmechanismus der Bikarbonatsekretion im Ileum von dem in Pankreas und Leber verschieden.

K. A. HUBEL
with the technical assistance of
BARBARA COLBERT

Gastroenterology Research Laboratory, Department of Medicine, University of Iowa, Iowa City (Iowa 52240, USA), 10th November 1966.

[2] J. RAMIREZ, K. A. HUBEL and J. A. CLIFTON, Am. J. Physiol. *211*, R260 (1966).

Improved Impregnation of Degenerating Boutons in NAUTA-LAIDLAW Preparations

It is a well-known fact that the NAUTA method is not well suited for the demonstration of terminal boutons. It may well impregnate such structures[1], but the occurrence of impregnated particles, which for various reasons can be classified as degenerating boutons, seems to be rare.

In a preparatory NAUTA study on the spinal cord in the cat, in which we made use of that modification of the NAUTA method which incorporates the Laidlaw solution[2], we replaced the perfusion method of KOENIG, GROAT and WINDLE[3] with the method of HOLT and HICKS[4] which we have used for an electron microscopic study[5]. However we used 5% sucrose instead of 7.5%. We were struck by the presence of a great number of structures interpreted as degenerating boutons. Therefore we undertook a series of experiments with the methods of KOENIG, GROAT and WINDLE and HOLT and HICKS, respectively. In 1 of the 2 solutions, according to HOLT and HICKS, the pH of the solution was buffered to 5.5. The pH of the solutions of KOENIG, GROAT and WINDLE was 4.2. The dorsal part of the lateral funiculus was cut on the left side in the first lumbar segment in 4 cats. This cut interrupted spinal afferents to the lateral cervical nucleus[6] and fibres in the dorsal spinocerebellar tract[7]. After 6 days' survival, the animals were perfused with one of the solutions. The central nervous system (including the upper cervical region and the cerebellum), and also the lesion of the 4 cats were immediately dissected and 3 of

[1] R. W. GUILLERY and H. J. RALSTON, Science *143*, 1331 (1964). – F. WALBERG, J. comp. Neurol. *122*, 113 (1964).
[2] W. J. NAUTA, in *New Research Techniques of Neuroanatomy* (Ed. W. F. WINDLE; Thomas, Springfield, Ill. 1957).
[3] H. KOENIG, R. A. GROAT and W. F. WINDLE, Stain Technol. *20*, 13 (1945).
[4] E. J. HOLT and R. M. HICKS, J. biophys. biochem. Cytol. *11*, 31 (1961).
[5] J. WESTMAN and G. GRANT, Acta Soc. Med. Upsal. *70*, 259 (1965).
[6] A. BRODAL and B. REXED, J. comp. Neurol. *98*, 179 (1953).
[7] G. GRANT, Acta physiol. scand. *56*, suppl. 193 (1962).

them were placed in the respective solutions mentioned above. The preparation of 1 of the 2 cases perfused according to KOENIG, GROAT and WINDLE was, however, placed in formol saline solution. They were kept in a refrigerator. After 2 weeks the first cervical segment and after 4 weeks the left paravermian part of the cerebellum were cut into sections on a freezing microtome to a thickness of 20 μ. Sections taken from the various cases were processed in parallel for impregnation according to the NAUTA[2] method. The potassium permanganate time in this method was varied from 3–15 min with intervals of 3 min. This was done for each separate staining, giving 5 groups of preparations. Other time factors were kept constant.

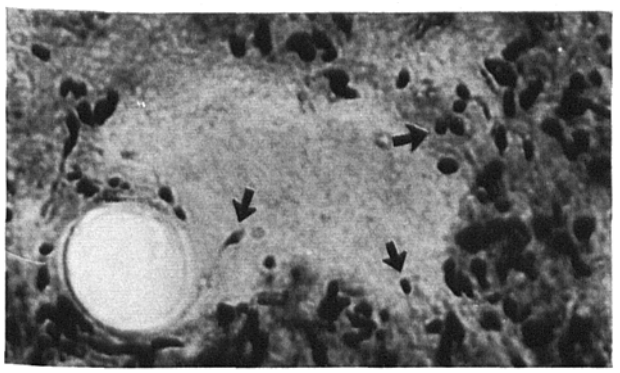

Fig. 1. Nerve cell in the lateral cervical nucleus on the operated side surrounded by degenerating structures, many of which have been interpreted as boutons (some indicated by arrows). The lumen to the left belongs to a capillary. NAUTA-LAIDLAW preparation. × 2000.

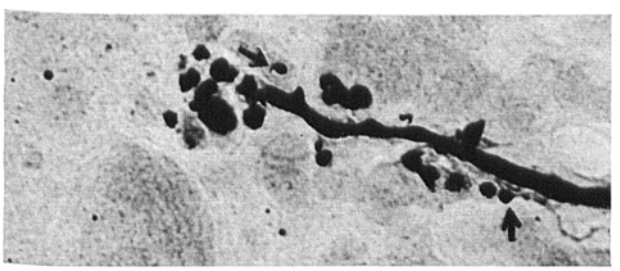

Fig. 2. Degenerating mossy fibre in the anterior cerebellar lobe on the operated side surrounded by apparently degenerating boutons (arrows). Note the thin fibres in connection with some of them. NAUTA-LAIDLAW preparation. × 1000.

The impression gained at the preparatory study was confirmed. In the material which had been perfused according to HOLT and HICKS (pH 7.2) we found on the operated side, in addition to degenerating fibres, numerous impregnated structures that must be interpreted as degenerating boutons. Figure 1 demonstrates the appearance of the degeneration in the lateral cervical nucleus. Note the close resemblance of the picture with the appearance of normal boutons as visualized with the RASMUSSEN[8] technique. Figure 2 illustrates a degenerating mossy fibre with boutons (for comparison see also Figure 3k in BRODAL and GRANT[9] and Figure 2a in GRANT[10]).

Provided degenerating boutons from other parts of the nervous system are as well impregnated as the ones investigated here, the NAUTA method as we have used it will become a valuable additional method for the tracing of degenerating fibre systems. It would have the great advantage over otherwise excellent methods for impregnation of boutons, e.g. GLEES' method[11], of being specific for degenerating systems.

Zusammenfassung. In NAUTA-LAIDLAW-Präparaten werden die degenerierenden Endfüsschen im Nucleus cervicalis lateralis sowie im Cerebellum der Katze viel vollständiger dargestellt, als nach andern Imprägnationstechniken; Voraussetzung dazu ist jedoch, dass die Perfusion gemäss KOENIG, GROAT und WINDLE[3] durch diejenige von HOLT und HICKS[4] ersetzt wurde.

G. GRANT and J. WESTMAN

Institute of Human Anatomy, University of Uppsala, Uppsala (Sweden), 18th September 1966.

[8] G. L. RASMUSSEN, in *New Research Techniques of Neuroanatomy* (Ed. W. F. WINDLE; Thomas, Springfield, Ill. 1957).
[9] A. BRODAL and G. GRANT, Expl Neurol. *5*, 67 (1962).
[10] G. GRANT, Expl Neurol. *5*, 179 (1962).
[11] P. GLEES, J. Neuropath. exp. Neurol. *5*, 54 (1946).

Acid Phosphatase Activity in Cytoplasmic Bodies of the Absorbing Intestinal Cells from Suckling Pigs

The columnar absorbing cells of the mouse intestine contain multivesicular bodies and dense bodies of different types[1]. They partly contain acid phosphatase (AP) activity and thus conform to the lysosome concept[2]. Such heterogeneous structures and also small cytoplasmic particles resembling microbodies as described for example by AFZELIUS[3] are also present in the intestinal epithelium of suckling pigs[4].

In order to study the occurrence of AP in the mentioned bodies we obtained material from 2 litters of suckling pigs[4]. Small tissue cubes were processed for electron microscopy with standard methods. Other samples were fixed in glutaraldehyde[5], freeze-sectioned (15 and 50 μ), and processed for AP[6]. The incubation time was 30–45 min. Controls were run with NaF added to the substrate (0.042%). The thinner sections were examined in the

[1] H. ZETTERQVIST, *The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum* (Godvil AB, Stockholm 1956).
[2] T. BARKA, J. Histochem. Cytochem. *12*, 229 (1964).
[3] B. AFZELIUS, J. biophys. biochem. Cytol. *26*, 835 (1965).
[4] M. SIBALIN and N. BJÖRKMAN, Expl. Cell Res. *44*, 166 (1966).
[5] D. D. SABATINI, K. BENSCH and R. J. BARRNETT, J. biophys. biochem. Cytol. *17*, 19 (1963).
[6] G. GOMORI, *Microscopic Histochemistry: Principles and Practice* (Univ. Chicago Press, Chicago 1952).