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.

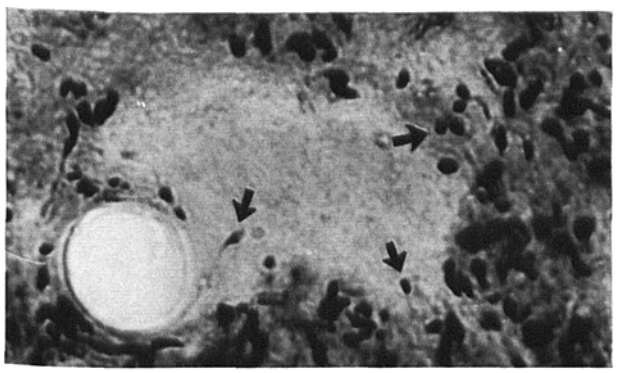

Fig. 1. Nerve cell in the lateral cervical nucleus on the operated side surrounded by degenerating structures, many of which have been interpreted as boutons (some indicated by arrows). The lumen to the left belongs to a capillary. NAUTA-LAIDLAW preparation. × 2000.

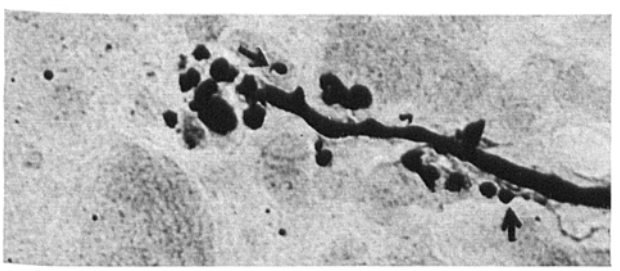

Fig. 2. Degenerating mossy fibre in the anterior cerebellar lobe on the operated side surrounded by apparently degenerating boutons (arrows). Note the thin fibres in connection with some of them. NAUTA-LAIDLAW preparation. × 1000.

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.

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[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).

light microscope and the thicker ones were post-fixed in osmiumtetroxide and prepared for electron microscopy[2].

Numerous droplets indicating AP activity appeared in the cells after 40 min incubation (Figure 1). The controls contained few granules (Figure 2). The multivesicular bodies (Figure 3) and dense bodies were loaded with lead precipitate to different degrees (Figure 4), whereas the small bodies (Figure 5) were as a rule negative (Figure 4).

The stained bodies obviously belong to the lysosomal system. Since the intestinal epithelium shows a strong endocytotic activity[4] most of its lysosomes are presumably of the heterophagic[7] type. This indicates that be-

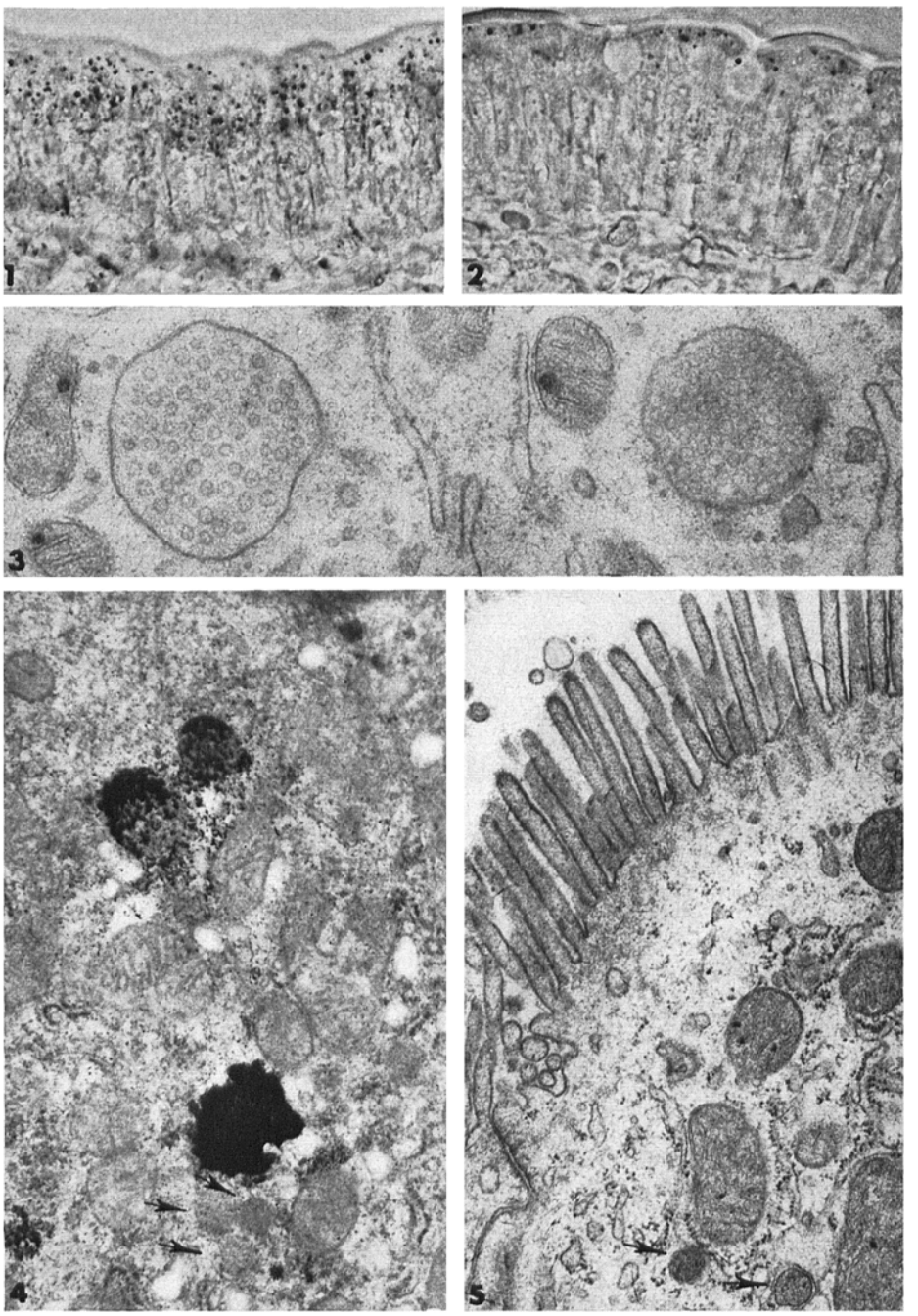

Fig. 1. Intestinal epithelium after 40 min incubation. Numerous AP-positive granules indicate a high frequency of lysosomes. Photomicrograph. × 640.

Fig. 2. Control specimen after 40 min incubation with NaF added to the substrate. The few positive granules possibly represent alkaline phosphatase. Photomicrograph. × 640.

Fig. 3. Multivesicular bodies of different degrees of development. Electronmicrograph. × 36,000.

Fig. 4. Intestinal cell after 40 min incubation showing cytoplasmic bodies with different degrees of activity. The small round structures presumably representing microbodies are negative (arrows). Electronmicrograph. × 20,000.

Fig. 5. Apical portion of intestinal cell with 2 small bodies (arrows). × 20,000.

sides the extracellular digestion in the intestine there is also an intracellular digestion. However, since the life span of the absorbing cells is only 3 days[8] autophagic lysosomes may also be present.

The small bodies are generally negative and structurally they conform to microbodies. Such bodies are frequently found in renal and hepatic cells. Recently, structures probably representing microbodies have been demonstrated also in other organs (epididymis, Sertoli cells) by AFZELIUS and NICANDER[9]. This points to a greater ubiquity of these bodies than had been assumed earlier, and their possible presence in the absorbing intestinal epithelium is, therefore, not surprising.

Zusammenfassung. Zytoplasmatische Körperchen absorbierender Zylinderzellen (Saugferkeldarm) wurden licht- und elektronenmikroskopisch auf saure Phosphatase (SP) untersucht: Zytoplasma enthält mehrere SP-positive Körnchen (offenbar lysosomales System) sowie kleinere, SP-negative Körperchen, als «Microbodies» gedeutet. Den heterophagischen Lysosomen wird eine intrazelluläre Verdauungsfähigkeit zugesprochen.

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[7] CH. DE DUVE and R. WATTIAUX, A. Rev. Physiol. *28*, 435 (1966).
[8] H. A. PADYKULA, Fedn. Proc. Fedn Am. Socs exp. Biol. *21*, 873 (1962).
[9] B. AFZELIUS and L. NICANDER, J. Ultrastruct. Res., in press.

Early Detection of Infection of Sugarcane by Ratoon-Stunting Disease (RSD)

RSD which depresses the growth of the ratoon crop of sugarcane is believed to be a virus disease and is difficult to detect before it exerts its growth-retarding effect[1]. VAN KAMMEN and BROUWER[2] described an increase in levels of polyphenoloxidase in tobacco leaves inoculated with tobacco mosaic virus; a similar observation was recorded for maize infected with maize rough dwarf virus by HARPAZ and KLEIN[3]. We attempted to use this procedure for the detection of RSD in sugarcane.

Healthy leaves of sugarcane about 130 cm long were cut into sections 33 cm long and split along the midrib. One half of the split section was inoculated by dusting it lightly with carborundum and rubbing it on either side with each of two fingers wetted with inoculant. The inoculant for the test half was juice squeezed from RSD-infected cane stalks, that for the control half juice from healthy stalks of the same variety. Inoculum was rinsed off with running water, the bottom 1 cm of the half-leaves was cut off under water and the split halves were put into tubes (33 cm by 28 mm) containing 30 ml distilled water. The tops were closed with 'parafilm' and the tubes, in racks, were incubated at 24 °C for various periods up to 7 days under fluorescent lights (13.5 h day). At the end of the incubation the half-leaves were removed from the tubes, blotted dry, cut up with scissors and representative samples of 1–1.5 g were ground[4] with 15 ml McIlvaine buffer[2] for 10 sec; a second grinding with 7 ml for 5 sec served to rinse the machine and disperse any fibres on the head. The dispersion was made up to 25 ml with buffer, stirred in the presence of a few drops of *iso*-octanol and centrifuged for 20 min at 8000 *g* and 4 °C. Polyphenoloxidase was determined with chlorogenic acid as substrate by a spectrophotometric method similar to that described in [2], except that the reaction was carried out in a Perkin-Elmer 4000 A recording spectrophotometer and that the extinction values from the recordings were plotted against time on semilogarithmic paper. A straight-line relationship between log extinction and time held for 2-3 min after the reaction was started by addition of chlorogenic acid to the test sample and the slope of this line was taken as a measure of anzyme activity. The ratio of activity/g wet weight for the test extract to the activity/g wet weight for the control extract, called the R value, was found to vary considerably from one test to the next. For 151 tests the mean R value was 1.23 and this was significantly greater than unity ($P < 0.001$). Although many modifications of each step of the method were tried we were unable to obtain more reproducible results. Thus the minimum number of replicate samples which would have to be analysed to give an accuracy in diagnosis of 9/10 and 99/100, respectively, were 29 (23) and 70 (56) for 2 groups of experimental results. In view of the large number of replicate analyses required, the method in its present form is not suitable for routine detection of RSD.

Zusammenfassung. Im Einklang mit Erfahrungen bei Tabak- und Maispflanzen konnte in Blättern des Zuckerrohres nach Impfung mit Saft von an «Ratoon Stunting Disease» erkranktem Zuckerrohr eine Erhöhung des Polyphenoloxydasen-Spiegels festgestellt werden. Eine praktische Anwendung dieser Methode zur frühen Diagnose dieser Viruskrankheit ist noch nicht möglich.

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Colonial Sugar Refining Company, Research Laboratories, Roseville (N.S.W., Australia), 14th November 1966.

[1] D. R. L. STEINDL, Proc. int. Soc. Sug. Cane Technol, 7th Congr. 457 (1950).
[2] A. VAN KAMMEN and D. BROUWER, Virology *22*, 9 (1964).
[3] I. HARPAZ and M. KLEIN, Experientia *20*, 274 (1964).
[4] The grinding was carried out in 50 ml polypropylene tubes (Nalge Co., Rochester, N.Y., USA) using an Ultraturrax TP 18/2 (Janke & Kunkel KG, Staufen I. Br., West Germany).