

chopped grass had pH values of 4.03, 4.00 and 4.13, while another sample of the same crop ensiled at the same time in an unchopped condition had a final pH of 4.46. The carbohydrates liberated by the chopping can replace molasses, and it is conceivable that, with the accompanying liberation of plant amino-acids, a further stimulus is given to the growth of lactobacilli—a possibility suggested by the findings of Dolby and Waters<sup>2</sup> and Harry<sup>3</sup> in connexion with studies on *Lactobacillus casei*. Further, the findings of Brouwer<sup>4</sup>, on the inter-relationship of dry matter content and pH in silage, mentioned by de Man, have been confirmed by Brown<sup>5</sup> and extended by myself<sup>6</sup> to include the nitrogen content as well.

A. J. BARNETT

Division of Agricultural Biochemistry,  
Department of Biological Chemistry,  
Marischal College,  
Aberdeen.

<sup>1</sup> de Man, J. C., *Nature*, **169**, 246 (1952).

<sup>2</sup> Dolby, D. E., and Waters, J. W., *Nature*, **153**, 139 (1944).

<sup>3</sup> Harry, E. G., *Biochem. J.*, **49**, 5 (1951).

<sup>4</sup> Brouwer, E., *Vers. Land. Onderz.*, **43**, 55 (1937).

<sup>5</sup> Brown, W. O., *J. Brit. Grassland Soc.*, **5**, 225 (1950).

<sup>6</sup> Barnett, A. J. G., *J. Brit. Grassland Soc.*, **5**, 93 (1950).

### $\beta$ -Glucuronidase Activity of Peripheral Nerve during Wallerian Degeneration

WHEN a peripheral nerve is cut, the portion of the nerve distal to the point of section undergoes a series of changes characteristic of Wallerian degeneration. The axon and, later, the myelin sheath are destroyed and there is an increase in the number of both Schwann cells and endoneurial cells (chiefly fibrocytes and macrophages) found along the course of the nerve<sup>1,2</sup>. We have now shown that in such a degenerating peripheral nerve there is a marked increase in the activity of the enzyme  $\beta$ -glucuronidase.

The  $\beta$ -glucuronidase activity of the sciatic nerve of the cat increased by a factor of 30-40 sixteen days after nerve section (see table). The enzyme activity of whole homogenates of nerve was determined in acetate buffer pH 4.5 by the method of Fishman, Springer and Brunetti<sup>3</sup>, using biosynthetic phenolphthalein mono- $\beta$ -glucuronide as substrate.

In addition to the increase in  $\beta$ -glucuronidase activity, there was also an increase in the concentration of both deoxypentose nucleic acid and pentose nucleic acid. Since considerable difficulty was experienced in applying the usual methods for the

quantitative determination of nucleic acids to tissue from the nervous system, resort was made to an ultra-violet absorption method<sup>4</sup>. The increase in the concentration of deoxypentose nucleic acid (by a factor of 3) and of pentose nucleic acid (by a factor of 4-5) is a reflexion of the well-known increase in the cellularity of the nerve. If, as has been suggested, the mean amount of deoxypentose nucleic acid per diploid cell is constant for any given species, the concentration of deoxypentose nucleic acid gives an estimate of the total number of cells in the nerve. Although cell nuclei contain small quantities of pentose nucleic acid, most of the pentose nucleic acid is in the cytoplasm. The concentration of pentose nucleic acid thus gives an indication of the total cytoplasmic mass of all the cells in the nerve, and the ratio of the concentration of pentose- to deoxypentose-nucleic acid gives an indication of the mean quantity of cytoplasm per cell. In the degenerating nerve the concentration of pentose nucleic acid increased more than the concentration of deoxypentose-nucleic acid. There was thus a significant increase in the ratio of pentose- to deoxypentose-nucleic acid, suggesting an increase in the mass of cytoplasm per cell, an increase that has been observed microscopically for Schwann cells<sup>4</sup>.

The great increase in  $\beta$ -glucuronidase activity is of interest, since Levvy, Kerr and Campbell<sup>5</sup> have suggested a possible relation between  $\beta$ -glucuronidase activity and cellular proliferation. The last line of the table indicates that in the degenerating nerve the mean  $\beta$ -glucuronidase activity per  $\mu$ gm. deoxypentose nucleic acid phosphorus, that is, the mean activity per cell, was some twelve times greater than that of the control nerve. However, in other experiments the mean  $\beta$ -glucuronidase activity per unit deoxypentose nucleic acid was very much less four days after nerve section, a time when cellular proliferation is at its height. Also, the  $\beta$ -glucuronidase activity per unit deoxypentose nucleic acid was still at the high 16-day level thirty-two days after nerve section. At this time the cellularity of the nerve, as judged by deoxypentose nucleic acid determinations, was decreasing. This would suggest, as found by Mills, Smith, Stary and Leslie<sup>6</sup> for rat liver regenerating after subtotal hepatectomy, that the peak in the  $\beta$ -glucuronidase activity per unit deoxypentose nucleic acid occurs after the phase of rapid cellular proliferation.

Similar changes were observed in the concentration of nucleic acids and  $\beta$ -glucuronidase activity when the nerve was crushed rather than sectioned.

This work was aided by grants from the National Research Council of Canada and the National Mental Health Grants.

D. M. HOLLINGER  
J. E. LOGAN  
W. A. MANNELL  
R. J. ROSSITER

Department of Biochemistry,  
University of Western Ontario,  
London, Canada.  
Nov. 12.

<sup>1</sup> Young, J. Z., *Physiol. Rev.*, **22**, 318 (1942).

<sup>2</sup> Abercrombie, M., and Johnson, M. L., *J. Anat., Lond.*, **80**, 37 (1946).

<sup>3</sup> Fishman, W. H., Springer, B., and Brunetti, R., *J. Biol. Chem.*, **173**, 449 (1945).

<sup>4</sup> Logan, J. E., Mannell, W. A., and Rossiter, R. J. (in the press).

<sup>5</sup> Levvy, G. A., Kerr, L. M. H., and Campbell, J. G., *Biochem. J.*, **42**, 462 (1948).

<sup>6</sup> Mills, G. T., Smith, E. E. B., Stary, B., and Leslie, I., *Biochem. J.*, **47**, xlviii (1950).

$\beta$ -GLUCURONIDASE ACTIVITY AND NUCLEIC ACIDS OF CAT SCIATIC NERVE  
(Figures in brackets represent the number of animals in each group).  
Mean  $\pm$  Standard Error of Mean

	Normal	Degenerating 16 days	Percentage increase
$\beta$ -Glucuronidase (Fishman units (see ref. 3) per 100 mgm.)	34.8 $\pm$ 1.3 (56)	1,280 $\pm$ 160 (6)	3,580
Deoxypentose nucleic acid (mgm. P per 100 gm.)	4.8 $\pm$ 0.1 (52)	14.4 $\pm$ 0.9 (6)	200
Pentose nucleic acid (mgm. P per 100 gm.)	3.9 $\pm$ 0.1 (52)	18.5 $\pm$ 0.8 (6)	370
Ratio of pentose- to deoxypentose-nucleic acid	0.81	1.29	59
$\beta$ -Glucuronidase (Fishman units (see ref. 3) per $\mu$ gm. deoxypentose nucleic acid P)	7.3	88.9	1,120