diseased muscle is envisaged before its mode of action is fully understood.

1 H. Zalkin, A.L. Tappel, K.A. Caldwell, S. Shibko, I.D. Desai and T. Holliday, J. biol. Chem. *237*, 2678 (1962).
2 I.M. Weinstock and M. Lucas, Enzyme Biol. Clin. *5*, 89 (1965).
3 C.M. Pearson and N.C. Kar, in: Clinical Studies in Myology, p. 89. Elsevier Publishing Co., Amsterdam 1971.
4 R.M. Stephen, R.F. Mayer and L. Vogelsang, Archs Biochem. Biophys. *146*, 227 (1971).
5 N.C. Kar and C.M. Pearson, Clin. chim. Acta *40*, 341 (1972).
6 N.C. Kar and C.M. Pearson, Proc. Soc. exp. Biol. Med. *141*, 4 (1972).
7 V. Dubowitz, in: Disorders of Voluntary Muscle, p. 310. Churchill Livingstone, London 1974.
8 R.J.T. Pennington, in: Disorders of Voluntary Muscle, p. 488. Churchill Livingstone, London 1974.
9 S. Dhingra, S.S. Katoch and R.K. Malhotra, Exp. Path. *15*, 97 (1978).
10 G. Gomori, Am. J. clin. Path. *16*, 347 (1946).
11 L. Weil and M.A. Russel, J. biol. Chem. *136*, 9 (1940).
12 C.H. Fiske and Y. Subbarow, J. biol. Chem. *66*, 375 (1925).
13 R.K. Malhotra, S. Sarin and A.K. Bindal, Experientia *32*, 513 (1976).
14 A.E.F.H. Meijer and R.P.M. Vossenberg, Histochemistry *52*, 45 (1977).
15 J. Kendrick-Jones and S.V. Perry, Biochem. J. *103*, 207 (1967).
16 G. Karpati and W.K. Engel, Nature *215*, 1509 (1967).
17 S. Schiaffino, S. Pierobon Bormioli and M. Aloisi, in: Exploratory Concepts in Muscular Dystrophy, p. 519. Elsevier Publishing Co., Amsterdam 1973.
18 T.L. Munsat, D. McNeal and R. Walters, Archs Neurol. *33*, 608 (1976).

Effect of amputation and limb regeneration on the pars distalis of the newt, *Notophthalmus viridescens*[1]

D. Louise Barber and S.R. Scadding

Department of Zoology, University of Guelph, Guelph (Ontario, Canada N1G 2W1), 6 March 1978

Summary. A study of the pituitary of the newt, *Notophthalmus viridescens*, during limb regeneration indicated no observable changes in histology or ultrastructure of any of the cells of the pars distalis when compared with the pars distalis of unamputated control newts.

That the pituitary gland is essential for limb regeneration in the adult newt, has been confirmed many times since the initial observation by Schotté[2]. Subsequently, workers have suggested that specific pituitary hormones are the necessary factors which are required for limb regeneration. ACTH[3], growth hormone[4,5], and prolactin[6], have all been proposed as the specific pituitary hormone that is necessary to support limb regeneration. Other investigators[7,8], have claimed that there is no specific requirement for any one hormone but that the pituitary is necessary to provide the appropriate hormonal milieu in which regeneration could occur. It has been demonstrated that in some cases where there is a large increase in requirements for a specific hormone, that there are immediate cytologically detectable changes in the pituitary. Shiino and Rennels[9] have demonstrated an increase in exocytosis of secretion granules in growth hormone cells of the rat pituitary following partial hepatectomy. DeVolcanes and Weatherhead[10] have shown degranulation of pars intermedia cells 8 h following transfer of *Xenopus laevis* from a white to a black background. If, then, there is a sudden release of a pituitary hormone during salamander limb regeneration, as some authors have suggested, then we might expect to see observable changes in pituitary cells. Consequently, this study was undertaken to see if there were any histologically or ultrastructurally observable changes in the pituitary of the newt following limb amputation.

Methods. Limbs of 37 newts, *Notophthalmus viridescens* were amputated and fixed at intervals from day 1 to day 28 following amputation of the limb and compared to control unamputated animals. This covers the period in which the pituitary is reported to be essential for limb regeneration[11]. For light microscopy, the brain with attached pituitary was fixed in 10% buffered formalin, embedded in methacrylate plastic, sectioned sagittally at 2 µm, and stained with Herlant's polychrome stains[12]. For electron microscopy, pituitaries were fixed in 3% glutaraldehyde followed by 1% osmium tetroxide, embedded in Epon, and stained with uranyl acetate and lead citrate.

Results and discussion. Methacrylate plastic embedding proved to be superior to paraffin embedding for observation of pituitary cells, since the thinner sections allowed better visualization of cytoplasmic granules. The 2 acidophils and 2 basophils previously reported for newt pituitary could be recognized, and had the same distribution as that previously reported[13–15]. The cell types, numbers, locations within the pars distalis, and ultrastructure were compared in amputated and unamputated control newts, and the observations were the same. No differences could be detected in the pituitary between regenerating and control newts. No evidence could be found to support hypersecretion of any particular hormone. Our results support the conclusion of Vethemany-Globus and Liversage[8], that the pituitary does not provide a specific factor for regeneration, but that the pituitary is necessary because it contributes to the environmental mileau that supports growth and differentiation of the blastema cells of the regenerating limb.

1 This investigation supported by a grant from the National Research Council of Canada to S.R. Scadding.

2 O.E. Schotté, C. r. Soc. Phys. Hist. nat. Genève *43*, 67 (1926).
3 O.E. Schotté and J.L. Chamberlain, Rev. Suisse Zool. *62*, 253 (1955).
4 J.A. Wilkerson, J. exp. Zool. *154*, 223 (1963).
5 S.C. Bromley and C.S. Thornton, J. exp. Zool. *190*, 143 (1974).
6 T.G. Connelly, R.A. Tassava, and C.S. Thornton, J. Morph. *126*, 365 (1968).
7 R.A. Liversage and S.R. Scadding, J. exp. Zool. *170*, 381 (1969).
8 S. Vethamany-Globus and R.A. Liversage, J. Embryol. exp. Morph. *30*, 397 (1973).
9 M. Shiino and E.G. Rennels, Cell Tissue Res. *163*, 343 (1975).
10 B. DeVolcanes and B. Weatherhead, Neuroendocrinology *22*, 127 (1976).
11 O.E. Schotté and A.B. Hall, J. exp. Zool. *121*, 521 (1952).
12 G.L. Humason, in: Animal Tissue Technique, p. 352. W.H. Freeman and Co., San Francisco 1972.
13 R.L. Holmes and J.N. Ball, in: The Pituitary Gland: A Comparative Account, p. 235. Cambridge University Press, Cambridge 1974.
14 P.G.W.J. van Oordt, in: Perspectives in Endocrinology, p. 405. Ed. E.J.W. Barrington and C.B. Jorgenson. Academic Press, New York 1968.
15 R.R. Cardell, J. Ultrastruct. Res. *10*, 317 (1964).