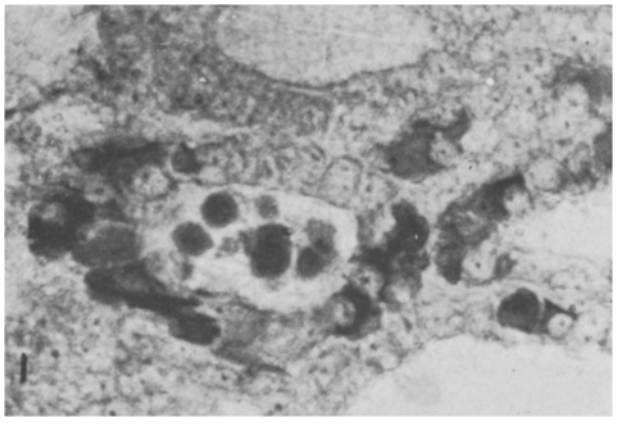

Fig. 1. Calcitonin cells in the monkey thyroid gland stained dark with lead haematoxylin. × 450.

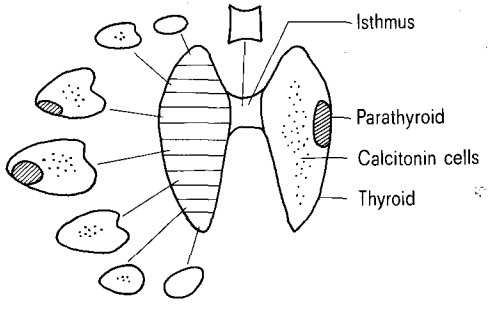

Fig. 2. Diagrammatic representation of the distribution of calcitonin cells in the thyroid of adult monkey.

(C cells range from 10.5 µm to 15.0 µm and their nuclei from 6.5 µm to 8.5 µm, whereas the follicular cells and their nuclei range from 6.5 µm to 8.5 µm and from 5.0 µm to 7.5 µm respectively). The C cells are intraepithelial, parafollicular and interfollicular in position (figure 1). They are located in the central region of the thyroid along the median axis. The anterior and posterior poles, the isthmus and peripherial regions of thyroid are completely devoid of C cells. The parathyroid also lacks C cells (figure 2). When stained with haematoxylin-eosin, the C cells take lighter stain. With lead haematoxylin and Davenport's silver impregnation[13], the C cells are selectively stained. In the former, the secretory granules of C cells take a deep blue-black stain (figure 1), whereas in latter the C cells demonstrated argyrophilic intracytoplasmic granules (figure 2).
Pilgrim[5] observed the C cells mainly in dorsomedial part of the thyroid of horse, monkey and man. Their total absence

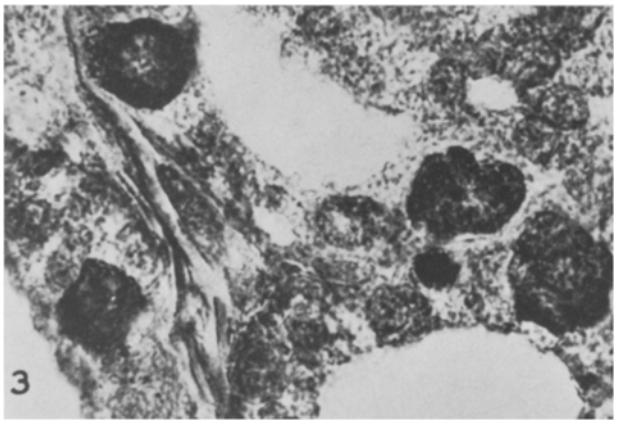

Fig. 3. Calcitonin cells in the monkey thyroid gland stained by silver impregnation. × 1000.

from the regions mentioned above (in monkey) has been reported from human thyroid[7,8]. Wolfe et al.[9] have, however, reported a meagre percentage of C cells from polar and isthmus regions of adult human thyroid, whereas in human neonates[10] the C cells were concentrated in a zone in the upper $^2/_3$ of the lateral lobes bilaterally, and were present in small groups in both intrafollicular and parafollicular positions. The interfollicular, intrafollicular and the parafollicular positions of C cells in monkey are similar to those of human adult[9].
On the basis of studies reported here, we conclude that C cells in Indian rhesus monkey show more or less a similar pattern of distribution as in human thyroid.

1 G.V. Foster, I. Mac Intyre and A.G.E. Pearse, Nature *203*, 1029 (1964).
2 A. G. E. Pearse, Proc. Roy. Soc. B *164*, 478 (1966).
3 Y. Kameda, Arch. histol. jap. *32*, 179 (1970).
4 K. Swarup and V. K. Das, Arch. histol. jap. *37*, 121 (1974).
5 C. Pilgrim, Dt. med. Wschr. *95*, 1074 (1970).
6 S. L. Teitelbaum, K. E. Moore and W. Shieber, Nature *230*, 334 (1971).
7 W. E. W. Roediger, J. Anat. *115*, 255 (1973).
8 P. J. McMillan, W. M. Hooker and L. J. Deftos, Am. J. Anat. *140*, 73 (1974).
9 H. J. Wolfe, E. F. Voelkel and A. H. Tashjian, Jr, J. clin. Endocr. Metab. *38*, 688 (1974).
10 H. J. Wolfe, R. A. Delellis, E. F. Voelkel and A. H. Tashjian, Jr, J. clin. Endocr. Metab. *41*, 1076 (1975).
11 R. S. Harris, J. R. Moor and R. L. Walker, J. clin. Invest. *40*, 1766 (1961).
12 E. Solcia, G. Vassallo and C. Capella, Histochemie *20*, 116 (1969).
13 Y. Kameda, Arch. histol. jap. *30*, 83 (1968).

Action potentials in non-tumor cells from the anterior pituitary gland

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Summary. Non-tumor cells of rat anterior pituitary gland are able, upon electrical stimulation, to generate action potentials which are based on an increase of the membrane permeability to both Na^+ and Ca^{2+}.

Action potentials have recently been recorded in several endocrine cells and their neoplastic derivatives[2-6]. Concerning the anterior pituitary, Kidokoro[7] has demonstrated that the clonal cell line GH_3 of a rat anterior pituitary tumor generates Ca-dependent action potentials. Biales et al.[8] have also shown that cultured cells of anterior pituitary tumors, including the GH_3 line, generate action potentials. The action potentials produced in the GH_3 cells were in this case dependent upon both Na^+ and Ca^{2+}. 2 main questions arise from these reports on neoplastic tumor cells: are non-

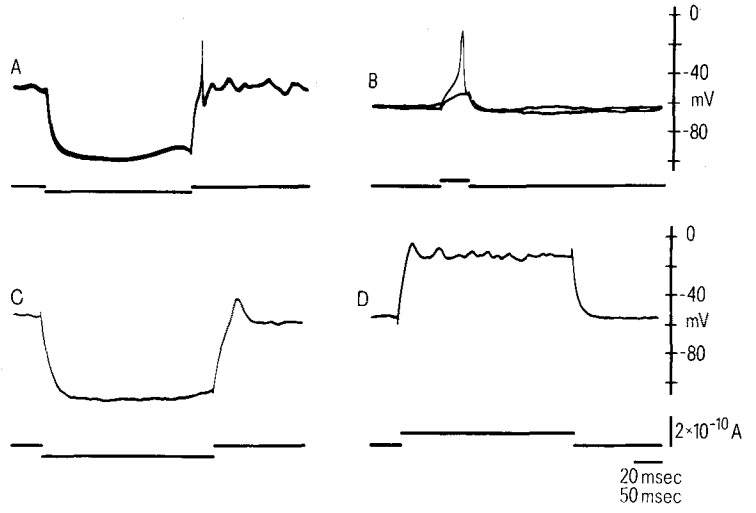

Fig. 1. Membrane responses of anterior pituitary cells to current pulses in normal saline (*A, B*) and Na-free solution (*C, D*). Resting potentials were -50 mV and -62 mV in *A* and *B*, respectively. Records *C* and *D* were taken from the same cell of which the resting potential was -55 mV. The calibration of 20 msec is applicable only to record *B*.

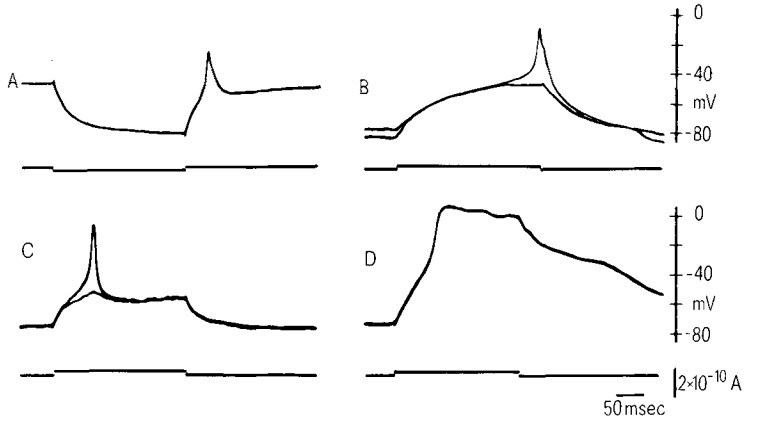

Fig. 2. Regenerative responses of anterior pituitary cells to current pulses in Na-free solution containing high concentration of alkaline earth cations. *A* and *B* responses in Na-free, 24 mM Ca^{2+} solution. Record *A* was taken immediately after penetration while the resting potential was -44 mV. In record *B* the membrane potential was shifted to -77 mV by background hyperpolarisation. *C* action potential in Na-free, 24 mM Sr^{2+} solution. The membrane potential was shifted to -75 mV before applying an outward current pulse. *D* prolonged action potential in Na-free, 24 mM Ba^{2+} solution recorded under similar condition as in *B* and *C*.

tumor anterior pituitary cells capable of generating action potentials? If so, upon which ions are these potentials dependent? No action potentials in normal anterior pituitary cells were found by the previous authors studying the electrophysiology of these cells[9-11], but Biales et al.[8] have indicated that such action potentials might occur.

Materials and methods. Slices of 300-500 µm thickness were prepared from the anterior pituitary of female rats. The preparation was superfused with oxygen-saturated saline at 37±1 °C. The solution had the following composition: NaCl 150 mM, KCl 5 mM, $CaCl_2$ 2.4 mM, $MgCl_2$ 1.3 mM, glucose 10 mM, buffered by Tris-HCl 5 mM to pH 7.4. Na-free solution was made by replacement of NaCl with choline-Cl. Na-free solutions of high Ca^{2+}, Sr^{2+} and Ba^{2+} concentration were prepared by replacing choline-Cl with $CaCl_2$, $SrCl_2$ and $BaCl_2$, respectively, on an isotonic basis. The electrode resistance of the glass micropipettes used for intracellular recordings ranged from 70 to 100 MΩ. The preamplifier was designed for injecting current through the recording electrode (Electric Biodyne, AM-1), and constant hyperpolarising current pulses of $2-5\times10^{-11}$ A intensity and 200-300 msec duration were continuously passed during advancement of the micropipette through the slice.

Results and discussion. Penetration of a cell was accompanied by sudden appearance of the resting potential and the hyperpolarising electrotonic potential induced by the current pulse. The resting potentials were in the range of -30 to -50 mV (39.6±13.2 mV, mean±SD n = 39). In 15 of the 39 penetrated cells action potentials could be elicited either after termination of an inward current pulse or during an outward current pulse (figure 1, A and B). The maximum rate of rise of the action potentials ranged from 11 to 35 V/sec (20.1±8.2 V/sec, n = 15). The steady-state current-voltage relationship in these cells was linear when the membrane potential was more negative than -50 mV. The slope decreased when the membrane potential was depolarised to values more positive than -35 mV. The cells hence have the property of outward rectification. The input resistance was calculated from the linear portion of the current-voltage relationship, and the values ranged form 130 to 960 MΩ (298±212 MΩ, N = 15). The values are similar to those reported for GH_3 cells[7,8].

All-or-none action potentials were not observed in the remaining 24 cells, but small regenerative depolarisations occurred at the termination of the hyperpolarising pulse. This observation has 2 possible explanations: the spike generating mechanism in these cells might be damaged by the penetration, or the difference in capability of generat-

ing action potentials might reflect 2 classes of cells. The 1st of these explanations was not supported by the resting potentials, which in fact was slightly lower in the cells generating action potentials than in the remaining cells.

All-or-none action potentials were not elicited after superfusion with Na-free solution for several min. The main component of the inward action current in normal saline is thus carried by Na^+. However, even in Na-free solution a significant regenerative depolarisation with a maximum rate of rise less than 3 V/sec occurred at the termination of a hyperpolarising current pulse (figure 1, C). Small graded responses were superimposed on the electrotonic potential when a depolarising current pulse was applied (figure 1, D). It is reasonable to consider that an increase of the membrane permeability to Ca^{2+} is responsible for the regenerative responses in Na-free solution. When the Ca^{2+} concentration was increased 10-fold to 24 mM, the maximum rate of rise of the anodal break response increased from 1.7 ± 0.6 V/sec ($n = 9$) to 5.7 ± 2.1 V/sec ($n = 5$). Figure 2, A shows the fast rising off-response in Na-free saline with 24 mM Ca^{2+}. When the membrane potential was hyperpolarised to a more negative level than -70 mV by DC current, an all-or-none action potential could be evoked by an outward current pulse (figure 2, B). The action potential was resistant to 2×10^{-6} g/ml tetrodotoxin.

In the cells which generate Ca spikes, Sr^{2+} and Ba^{2+} can replace Ca^{2+} as inward current carrier[12]. This is also the case in the anterior pituitary cells. When 24 mM Ca^{2+} in Na-free solution was exchanged with isomolar Sr^{2+}, action potentials occurred in response to a depolarising current pulse as seen in figure 2, C. The maximum rate of rise attained 11.1 V/sec in this cell. In Na-free solution with 24 mM Ba^{2+}, the action potential was markedly prolonged, and it was often seen to overshoot (figure 2, D). This prolonged action potential is probably due to the suppressing effect of Ba^{2+} on the delayed rectification mechanism.

The recorded cells were not identified in the present study. However, the number of parenchymal glandular cells greatly exceeds any other cells within the gland. It is therefore likely that the reported electrical activity was recorded mainly from glandular cells. The capacity of pituitary tumor cells to generate electrically induced Na- and Ca-dependent action potentials thus seems to be a physiological property retained from normal pituitary glandular cells. Our data will be presented in more detail elsewhere[14].

1 We thank Dr K. Yagi for critical reading of the manuscript.
2 P.M. Dean and E.K. Matthews, J. Physiol., Lond. *210*, 265 (1970).
3 B. Biales, M.A. Dichter and A. Tischler, J. Physiol., Lond. *262*, 743 (1976).
4 B.L. Brandt, S. Hagiwara, Y. Kidokoro and S. Miyazaki, J. Physiol., Lond. *263*, 417 (1976).
5 M.D. David and M.E. Hadley, Nature *261*, 422 (1976).
6 A. Tischler, M.A. Dichter, B. Biales, R. Delellis and H. Wolfe, Science *192*, 902 (1976).
7 Y. Kidokoro, Nature *258*, 741 (1975).
8 B. Biales, M.A. Dichter and A. Tischler, Nature *267*, 172 (1977).
9 J.V. Milligan and J. Kraicer, Science *167*, 182 (1970).
10 S. Martin, D.H. York and J. Kraicer, J. Endocr. *92*, 1084 (1973).
11 J.H. Poulsen and J.A. Williams, Nature *263*, 156 (1976).
12 P. Fatt and B.L. Ginsborg, J. Physiol., Lond. *142*, 516 (1958).
13 S. Hagiware and Y. Kidokoro, J. Physiol., Lond. *219*, 217 (1971).
14 S. Ozawa and O. Sand, Acta physiol. scand., in press (1978).

The effects of juvenile hormone analogues on the eggs of *Pieris brassicae* L.[1]

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Summary. Mortality was produced in the eggs of *P. brassicae* after treatment with 3 juvenile hormone analogues. The mortality was greatest in the early stages of embryonic development.

Embryonic development can be divided into a period of embryogenesis followed by a period of larval differentiation. These 2 periods are separated by the blastokinesis of the embryo. The effect of juvenile hormone analogues (JHAs) on post-oviposition egg development show that they are most effective in blocking embryonic development when they are applied during embryogenesis[2]. This disruption of embryonic development has been found in a wide variety of insects, e.g.: *Lygaeus kalmii* (Hemiptera)[3], *Lepismodes inquilunus* (Thysanura)[4], *Schistocerca gregaria* (Orthoptera)[5], *Hyalophora cecropia* (Lepidoptera)[6], and *Epilachna varivestis* and *Lasioderma serricorne* (Coleoptera)[7]. In the present experiment, the eggs of *P. brassicae* have been tested for mortality after applying JHAs to the eggs at different times between oviposition and hatching. The analogues used were Ro-84314[8], Ro-69550[9] and Law's Mimic[10].

Materials and methods. Cabbage plants with newly laid eggs on them were transferred from a greenhouse to a constant environment of 25 °C, 70% relative humidity and 16 h daylength. The eggs were treated topically with a JHA in acetone solution at the rate of 10 µg JHA/1 µl acetone/20 eggs. They were treated at the time of oviposition or at 1, 2 or 3 days later. Untreated eggs normally hatched at 4 days after oviposition. Control eggs were treated with acetone at the same time-intervals and with the same volume of acetone. The number of 1st instar larvae that hatched were counted and counts were continued daily until after ecdysis to the 4th instar.

Results. Although counts were made of the numbers of surviving larvae from hatching until the 4th instar, deaths were only found to occur at the time of hatching or at a larval ecdysis. The table shows the number of larvae surviving each of these critical periods. It can be seen that where the JHA produced a large mortality, most of this occurred before hatching to the 1st instar, but there was often another large mortality at the ecdysis to the 2nd instar. Most larvae that survived to the 2nd instar then survived to become adults. The most effective time for JHA treatment is immediately after oviposition, when there is nearly a 100% mortality for all analogues. 1 day after oviposition is also very effective in the analogues Ro-84314 and Law's Mimic but not Ro-69550. If the treatment is postponed until 2 or 3 days after oviposition, mortality is considerably reduced but this is still higher than the controls.

Discussion. The results show that the JHAs produce mortality in the eggs of *P. brassicae* throughout embryonic development, but mortality is greatest when the JHA is applied