APUD Characteristics and Immunocytochemistry of Avian Pituitary Corticotrophs

In the human and murine adenohypophysis both corticotrophs and somatotrophs (probably also the prolactin cells) have been shown by TAKOR TAKOR and PEARSE[1] to possess the classical amine precursor uptake and decarboxylation (APUD) characteristics. Indeed, mammalian corticotrophs have for some years been accepted as members of the APUD cell series. Our studies were designed to investigate the situation in the avian hypophysis, taking advantage of the fact that it is possible to demonstrate ACTH, by immunocytochemical procedures, in formaldehyde-fixed tissues.

Material and methods. Adenohypophyses were taken from quail and chick embryos at the end of the incubation period (14 days for quails and 15 to 17 days for chicks) and 1 week after hatching in both species. In the embryos, 100 mg/kg of L-3, 4 dihydroxyphenylalanine, (Sigma) (L-DOPA) were injected, 1 h before sacrifice, into a blood vessel of the chorio-allantoic membrane. After birth, L-DOPA was administered i.p. The pituitary glands were immediately dissected out and quenched in liquid propane cooled with liquid nitrogen. After freeze-drying in a thermoelectric tissue dryer for 16 h at −40 °C they were exposed to formaldehyde vapour at 60 °C for 4 h and then vacuum embedded in paraffin wax.

Control tissues were 1. freeze-dried unfixed material and 2. pituitary glands from uninjected animals.

Fluorescence microscopy. Sections from all the blocks were cut a 5 μm, placed on dry slides and mounted in buffered glycerine. They were then examined by fluorescence microscopy, and photographed, using a) a Zeiss (Oberkochen) Standard Universal Microscope fitted with an HBO 200 mercury arc lamp. Filters used were UG (1/2 mm) and BG 38/4 for excitation at 365 nm, with a K430 barrier filter, and Schott IL406 and BG 1 (2/3 mm) for excitation at 406 nm, with a K 470 barrier filter; b) a Leitz Orthoplan Microscope fitted with HBO 100 and XBO 75 arc lamps and a Ploem illuminator. For formaldehyde-induced fluorescence (FIF) excitation at 406 nm employed a K 470 barrier filter and a TK 510 dichroic mirror. For excitation of fluorescein isothiocyanate (FITC) labelled antibody at 490 nm two KP 490 interference filters were used with a TK 510 dichroic mirror. The barrier filter was K 515. Photomicrographs were taken on Ilford FP4 film.

Microspectrofluorometry. Where necessary sections prepared as above were dewaxed in warm paraffin oil and mounted in the same fluid (Light liquid paraffin, B.D.H.). They were then examined with a Leitz Microspectrograph[2] modified for microspectrofluorometry by the provision of monochromatic epi-illumination, a reference channel and a measuring system using photon counting with automatic recording of data in digital form[3]. Paraffin oil was used as the immersion medium for a Zeiss Ultrafluor objective and no coverglass was employed.

For the measurement of emission spectra only, some sections were mounted in DPX medium (R.A. Lamb) without removal of wax, covered with a coverslip and examined with a Leitz X 95/1.30 oil immersion objective. Epi-illumination was used in all cases, and control readings were taken from adjacent tissue areas lacking specific fluorescence. Catecholamines were differentiated from 5-hydroxytryptamine by their emission spectra which were expressed as relative quanta per quantum energy (frequency) interval.

Immunocytochemistry. Sections subjected to the FIF procedure were subsequently dewaxed with petroleum ether (light petroleum) and an indirect immunofluores-

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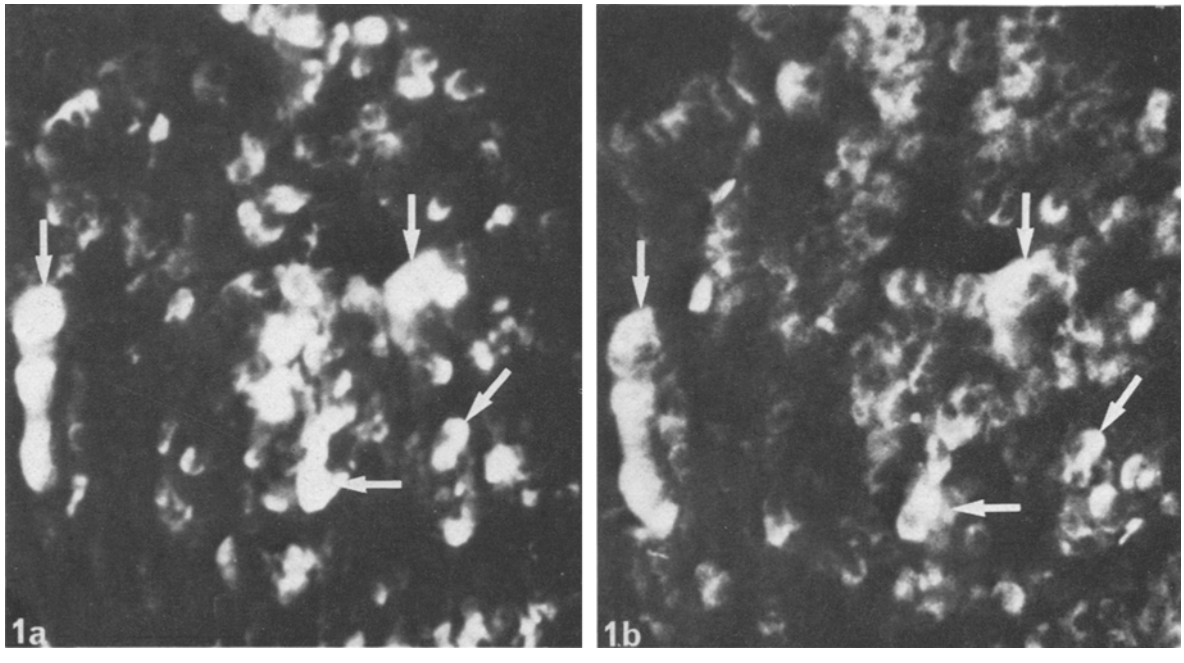

a) Part of a cephalic lobe of a chick embryo pituitary gland. FIF preparation after uptake and decarboxylation of L-DOPA shows fluorescent cells. Arrows point out groups of these cells for comparison with Figure b. ×670.

b) The same section as Figure a, subjected to an indirect immunofluorescent procedure using anti-ACTH serum, shows positive cells. Arrows point out groups of cells corresponding to those in Figure a. ×670.

cence technique[4] was applied, using as the first layer an antiserum against purified porcine ACTH (whole molecule) and as the second layer fluorescein-labelled goat anti-rabbit IgG globulin.

Results. The pituitary glands from normal, uninjected birds, freeze-dried but untreated with formaldehyde vapour, showed no fluorescence, Control glands freeze-dried and treated with the vapour showed minimal fluorescence in the cephalic lobe and none in the caudal lobe. The spectral characteristics of this fluorescence could not be determined.

In glands from the injected series of birds, treated by the FIF procedure, a greenish-yellow fluorescence was observed in a variable proportion, estimated as about one third, of the cells in the cephalic lobe (Figure a). This fluorescence exhibited the correct spectral characteristics for dopamine (excitation maximum 410–420 nm, emission maximum 480–485 nm).

Slides treated for the second time, with anti-ACTH serum, showed a characteristic fluorescence in cells located in the cephalic lobe and it was possible to find correspondence between cells showing FIF (Figure a) and those with specific peptide immunofluorescence (Figure b).

Discussion. In their studies on mouse pituitary gland TAKOR TAKOR and PEARSE[1] found direct correspondence between cells responsible for L-DOPA uptake and cells with immunofluorescence for ACTH and growth hormone. In this report we demonstrate that in chick and quail embryonic pituitary cells reacting positively with an immune serum prepared against the whole ACTH molecule are responsible for L-DOPA uptake and decarboxylation. Thus the corticotrophs of avian adenohypophysis are to be regarded as APUD cells. On the other hand, as shown by FERRAND, MIEGEVILLE and LE DOUARIN[5] all the cells of chick and quail pituitary, with formol induced fluorescence after L-DOPA injection, are also PAS-positive. Thus, as observed in various species of mammal, ACTH cells of embryonic or young quail and chick are characterized by PAS positive reaction. The same observation was previously made by DUBOIS[6] in the hen pituitary gland.

Summary. L-DOPA is taken up and decarboxylated by cells in the avian adenohypophysis, which are situated largely in the cephalic lobe. These APUD cells have been shown by sequential formaldehyde-induced and immunofluorescence to be corticotrophs and the relationship between the two procedures has been found to have a reciprocal quality.

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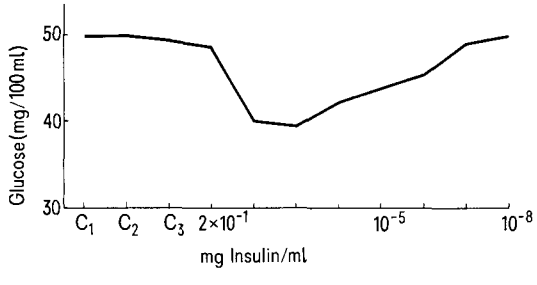
Effect of Insulin on the Glucose Uptake of Protozoa

Protozoa do not normally possess a hormonal regulating mechanism, but they have been shown to contain certain hormone-like substances, e.g. *Tetrahymena pyriformis* was shown to contain adrenaline and serotonin[1]. It was also shown experimentally that unicellular organisms are able to react to hormones of higher animals. BLUM[2] reported that triiodothyronine has an influence on *Tetrahymena* and others[3] found that its receptors respond selectively to the various iodine-containing hormones. Histamine and serotonin enhance the phagocytic activity of *Tetrahymena*[4] in the same manner as in cells of higher animals. In addition *Tetrahymena* is able to distinguish between serotonin and the chemically related plant hormone, indole acetic acid[4]. All hormones tested up to now for a possible influence on *Tetrahymena* were simple amino acid derivatives, and although the presence of receptors was shown in certain cells not normally related to hormonal activity, it seemed worthwhile to investigate the effect of certain hormones which occur exclusively in higher animals.



Effect of insulin on glucose intake by *Tetrahymena pyriformis*. C_1, glucose control; C_2, glucose + insulin control; C_3, glucose + *Tetrahymena* control.

Two-day cultures of *Tetrahymena pyriformis* GL were used in stationary phase at 25 °C. The medium was 1% Bactotrypton (Difco) containing 0.05% yeast extract. 24 h before the start of the experiments, the *Tetrahymenae* were centrifuged at 500 rpm, then separated from the medium and subsequently kept in LOSINA-LOSINSKY's solution[5]. After 24-h starvation, glucose (concentration range: 12.5–100 mg/100 ml) and bovine insulin (concentration range: 2×10^{-1}–2×10^{-8} mg/ml; 1 mg = 17 IU) were added to the culture tubes at times ranging from 10 min to 24 h at 25 °C, to find the optimal time and concentration. Each culture tube contained 3,000 cells per ml. Control series were set up with glucose alone, glucose + insulin and glucose + *Tetrahymena*. 10 min later the protozoa were precipitated with 0.3 *N* trichloroacetic acid, the cultures were centrifuged at 3000 rpm for 10

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