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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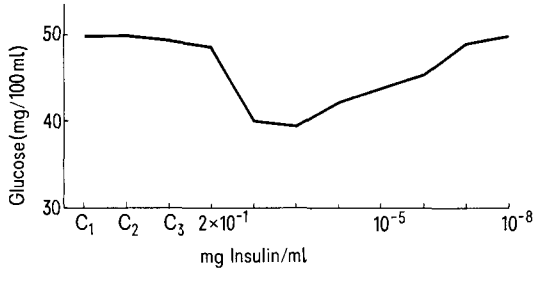
Effect of Insulin on the Glucose Uptake of Protozoa

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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min, and glucose concentration was determined in the supernatant by the *o*-toluidine photometric technique (Boehringer-Mannheim GmbH, Blutzucker Farbtest). Under the given conditions of experiment, addition of 50 mg/100 ml final glucose concentration at 10 min proved to be optimal. 10 replica experiments under such conditions had unequivocal results.

The results are shown in the Figure. It can be seen from the curve that the glucose intake of the unicellular organisms was markedly increased by the presence of insulin. The decrease of glucose content in the medium reached 20% at a concentration of 2×10^{-2} mg/ml and almost as much at 2×10^{-3} mg/ml. At the subsequent lower concentrations, the curve began to ascend slowly, until reaching the control level at 2×10^{-7}. The glucose consumption of the unicellular organisms differed significantly ($0.1 > p > 0.05$) between the insulin concentrations 2×10^{-2} and 2×10^{-4}.

The effect of insulin on *Tetrahymena* was formerly studied by HILL[6] and WAITHE[7] with negative results, to which HILL[6] referred in his book as unpublished data, so that no explanation of the failure is possible. WAITHE[7] used too high concentrations of insulin for a too long reaction time; we did not find any effect ourselves under such conditions. The present results however unequivocally show the influence of insulin on *Tetrahymena*, which accords well with our previous observation[3,4] that Protozoa are responsive to certain hormones of higher animals. The phenomenon cannot be explained unless it is postulated that insulin receptors are present in *Tetrahymena*. This does not seem possible unless the receptor corresponds with a given pattern of cell membrane which is already present at the lowest levels of phylogenesis, viz. also in cells not normally related to hormonal activity[8]. The structure and function of the membrane pattern are naturally not regarded as, or referred to as, a receptor until an interaction with a given hormone takes place. This seemed to be true ab ovo for animo acid type hormones, with the reservation that such hormones, being generally present in living biengs, could as well occur in unicellular organisms or their surroundings. The response of *Tetrahymena* to insulin, a polypeptide hormone occurring exclusively in higher animals, is unequivocally in favour of the above hypothesis.

Summary. Insulin stimulates the glucose uptake of *Tetrahymena pyriformis*. This shows the presence of insulin receptors in *Tetrahymena*, consequently receptors may be present in a level of phylogenesis, where the natural contact between the given hormone and the cell is unnecessary and impossible.

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Effects of Hypophysectomy, Bilateral Adrenalectomy and Hormone Replacement Therapy upon Organ Monoamine Oxidase Activity

The in vivo activity of monoamine oxidase (MAO) results from a delicately balanced input of a variety of physiological stimuli, including hormonal secretions[1-4].

Rat heart MAO has been reported to decrease after hypophysectomy (HX)[5], but no change was found by LANDSBERG and AXELROD[6]. Adrenal enzyme activity has been found to decline[7,8]. The in situ removal of pituitary function in the rabbit fetus by decapitation increased MAO activity in several tissues, including the adrenal[9]. The authors suggested that these increases might be related to the ensuing lack of glucocorticoids, since bilateral adrenalectomy (AX) enhances MAO activity[9-11]. However, further studies seem pertinent, since ontogenetic development affects MAO[12] and decapitation is a traumatic procedure.

In the present study, the effects of HX and AX on organ MAO are compared, and the effects of replacement doses of adrenocorticotropic hormone (ACTH) in HX rats and dexamethasone in AX rats were determined.

Materials and methods. Male albino Sprague-Dawley rats, weighing between 160 and 200 g were used. AX, bilateral adrenal demedullation[13] and sham-operations were carried out under pentobarbital anesthesia. The animals were given 0.9% W/V NaCl to drink and were sacrificed 10 days after surgery. Rats were checked visually for remaining adrenal or medullary tissue. Some animals received dexamethasone sodium phosphate (Decadron, Merck Sharp and Dohme, 2×30 µg/rat/day, i.p.) for 10 days.

HX and sham-operated rats were obtained from Zivic Miller Laboratories, Inc., Pittsburgh, Pennsylvania. The animals were kept at 26–27 °C on a normal laboratory diet plus pears and were given water containing 0.9% W/V NaCl and 5% W/V sucrose. The rats were sacrificed 10 days after surgery. Since HX retards growth, the sham-operated rats consisted of 2 groups. Group I was of equivalent age to the HX rats, whereas the group II was younger, so as to be of an equivalent mean weight to the HX rats at sacrifice time. In some experiments, purified ACTH in gelatin (Cortrophin Gel, Organon) was administered (4 units/rat/day, s.c.) for 10 days. The adequacy

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