from Struthioniformes to Passerines; others 1-6 have also described similar distribution in their studies in avian pituitary gland, except HERRING7 who has observed deep staining granular cells in the anterior two-thirds of the pars glandularis and clear cells in the posterior third part, and TILNEY 8 who found central acidophils and peripheral basophils in chicken. RAHN<sup>3</sup> has divided the pars glandularis into two lobes on the basis of change of celltypes and their distribution and designated them as the 'cephalic' and the 'cadual' lobes in the chick. The cephalic lobe usually contains light staining acidophils with their concentration toward periphery of the lobe, whereas the caudal lobe lying nearer the infundibular process has dark staining acidophils and thus provides demarkation of the boundary line of the two lobes. Almost all workers who described the bipartitate nature of the avian pituitary gland have, however, referred to a similar type of separation. While studying cytomorphology of the pituitary gland in a few Indian birds, in A. tristis, Linn. a connective tissue strand is observed traversing the pars glandularis through its periphery (Figure 1) thereby dividing it into two separate lobes named the cephalic and the caudal in accordance with previous literature. It is evident from the review of the literature that such a type of separation of the two lobes in avian pituitary gland is hitherto not described.

Colloidal secretions surrounded by degranulated cells were numerably observed in the pars glandularis and the zona tuberalis. The zona tuberalis connects the pars glandularis with pars tuberalis—a character resembling

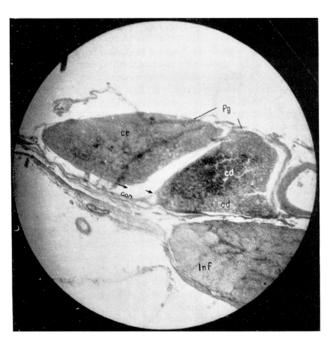


Fig. 1. Pars glandularis (pg) and Infundibular (inf) process. Note separation of the 'Cephalic' (cc) and 'Cadual' (cd) lobes by a connective tissue (con).

## Flamingoes Raise their Young on a Liquid Containing Blood

Since 1958 seventeen chicks have been raised in the Basle Zoo flamingo colony, which is composed of all three races of *Phoenicopterus ruber*. In 1962 the juice with

more the mammalian pars anterior (Figure 2). The colloids are basic in nature and thus stain blue with Mallory's and Cleveland-Wolfe stains and give positive colour with PAS methyl blue Orange G.



Fig. 2. A part of pars glandularis. Note zona tuberalis (zt), acidophils (ac), basophil (bs) and colloidal secretions (col).

Résumé. Les oiseaux femelles possèdent une glande pituitaire plus grande que celle des mâles. La glande est couverte entièrement par les couvertures meningées. Deux types d'aciophiles, un basophile violet et PAS positif, et puis une cellule chromophobe ont été observés. Les lobes céphaliques et la caudale sont separés par un toron de tissu connectif, d'un caractère inconnu jusqu'ici dans la biparitate de la glande pituitaire des oiseaux. Les secretions colloidales de caractère basique sont nombreux dans la pars glangularis et dans la zona tuberalis.

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- <sup>2</sup> F. PAYNE, Biol. Bull. 82, 79 (1942.
- <sup>3</sup> F. PAYNE, Anat. Rec. 96, 77 (1946).
- <sup>4</sup> H. Rahn, J. Morphol. 64, 483 (1939).
- <sup>5</sup> H. RAHN and B. T. PAINTER, Anat. Rec. 79, 297 (1941).
- <sup>6</sup> J. P. Schooley, Cold Spring Harbor Symposium on Quantitative Biology, vol. 5 (1937).
- 7 P. T. HERRING, Quart. J. exp. Physiol. 6, 73 (1913).
- <sup>8</sup> F. TILNEY, Amer. Anat. Mcm. 2, 1 (1911).
- My thanks are due to Prof. L. S. Ramaswami for suggesting the problem and for painstaking guidance during the course of work at Department of Zoology, University of Rajasthan, Jodhpur (India).

which both parents feed their single chick was analysed for the first time. Three analyses were performed (I–III) on juice taken from the crops of freshly fed 9–17 day-old chicks, and drops of juice obtained direct from an adult bird during the feeding act were submitted to histological examination (IV). The juice is of a watery consistency

and, for the first few weeks after the chicks have hatched, is bright red. Thereafter, however, its colour gradually fades. It was examined with particular reference to carotenoids (which are also responsible for the red colouring of the plumage) and to blood. The results were as follows:

I. H. THOMMEN (F. Hoffmann-La Roche & Co. Ltd., Basle): Canthaxanthin 5.4 µg/ml. F. STUDER and H. P. LOREZ (of the same firm): pH approximately 8. 22,000 erythrocytes pro mm³, corresponding to a dilution of blood in the juice of 1:100. Numerous bacteria, single epithelial cells and leucocytes.

II. H. THOMMEN: Canthaxanthin 4.9 µg/ml, Xanthophyll 1.8 μg/ml, β-Carotene traces. E. Undritz (Sandoz Ltd., Basle): Benzidine reaction: positive. Haematocrit (packed cell volume): 1/2% (normal haematocrit value of flamingoes = 50%) corresponding to a blood dilution in the juice of 1:100; erythrocytes, many leucocytes and thrombocytes well preserved which argues against a mixture with gastric juice. K. Zehnder (Sandoz Ltd.): (a) Membrane electrophoresis revealed a similarity to normal human serum, save that the main band migrates as far as human  $\alpha_1$ -globulin. The extinction curve is also very similar to that of normal human serum. (b) Density 0.973, pH (potentiometric) 8.48, total protein 8.7 g/100 ml, Albumin 3.0 g/100 ml, Chloride ions 350 mg/100 ml, Glucose (enzymatic estimation) 190 mg/100 ml, Calcium 14.3 mg/100 ml, Phosphorus 6.0 mg/ml, total cholesterol 975 mg/100 ml, ether-soluble fraction 17.5% (w/w). Flamingo juice is therefore very rich in fat and contains an appreciable amount of glucose.

III. H. Thommen: Canthaxanthin 1.6 µg/ml, Xanthophyll 1.0 µg/ml,  $\beta$ -Carotene traces. H. P. Lorez: pH 7.6, Erythrocytes 14,000/mm³, single epithelial cells and leucocytes, few bacteria.

IV. Smears on microscope slides were made with a few drops of juice collected direct from a parent bird. According to Undritz many erythrocytes were present, also a blood clot. The benzidine reaction was strongly positive.

From these reports it can be concluded that the juice owes its red coloration to blood as well as carotenoids. Furthermore, the juice is very nourishing. This is apparent not only from the analyses but also from the fact that a chick weighing less than 100 g when hatched attains a body weight (according to race and sex) of 2–4 kg in two months without taking any appreciable amount of additional nourishment (at 8–10 weeks the filter apparatus in the beak is not yet fully developed).

It is estimated that this nutrient juice fed to the young by both parents amounts to at least 200 ml per day.

There can be no question of predigested food, for, if this were the case, distinct food particles would be present in the juice. Moreover, neither before nor during feeding, when the juice flows continuously, can regurgitating motions be observed. Histological investigations were carried out by S. Lindt (Veterinary Pathological Institute, Universität Bern, Switzerland) on two birds. They give indications as to the source of the secretion in question:

The whole of the upper digestive tract (pharynx to glandular stomach) is thickly lined by acinose glands with merocrine hypersecretion connected to the surface by a narrow excretary duct passing through a desquamative multilayer of pavement epithelium.

Hyperaemic capillaries form a fine network around the glands and also underlie the basal membrane of the stratified pavement epithelium. From this plexus the capillaries also penetrate the stratum basale, and enter and often pass through the stratum spinosum to the surface.

It is possible to observe diapedesis of individual erythrocytes both in the glands and at the surface. Blood constituents mixed with gland mucus form a layer on the surface of the epithelium.

It appears, therefore, that the whole upper digestive tract is responsible for the secretion described.

This kind of nourishment of the flamingoes seems comparable only to the crop milk of pigeons, a sort of crop milk secreted by emperor penguins, an oily secretion in some species of the procellariiformes and mammals' milk<sup>1</sup>.

Zusammenfassung. In einer brütenden Flamingokolonie des Zoologischen Gartens Basel konnte der von den Altvögeln den Jungen verabreichte Nahrungssaft gewonnen und untersucht werden. Nach diesen Untersuchungen handelt es sich nicht um aufgewürgte vorverdaute Nahrung, sondern um ein Sekret, das im Bereich des Vorderdarmes produziert wird. Es ist rot gefärbt, enthält Blut, reichlich Carotinoide und kann nach seinem Nährwert mit der Milch der Säugetiere verglichen werden.

E. M. LANG

Zoologischer Garten, Basel (Switzerland), July 19, 1963.

Besides the scientists already mentioned, A. Thiersch and H. Wackernagel took part in the observations.

## The Changing Pattern of Neutral-Red Staining with Morphogenesis in Certain Polychaete Embryos

Vital stains have been used by a number of workers for many years as a biochemical index for the localization of different substances in mosaic and regulation eggs. Most of these earlier attempts, as summarized by Needham¹, were not based on any sound chemical footing. As late as 1956, Reverberl² opened a new chapter in this field which was later thoroughly explored by himself and his school and summarized³.⁴. This new trend in the science of vital staining, being based on the sure knowledge that janus green specifically stains mitochondria, is remarkably more exact than the work¹ of earlier investigators who

mostly detected changes in the pH only. Recently, following Reverberi's idea, Brahmachary and Bhattacharya studied *Limnaea* embryos with neutral red. As it may be interesting to extend this project with other material, polychaete embryos have now been studied by the same procedure.

<sup>&</sup>lt;sup>1</sup> J. NEEDHAM, Biochemistry and Morphogenesis (Cambridge 1950), p. 131.

<sup>&</sup>lt;sup>2</sup> G. Reverberi, Exper. 12, 55 (1956).

<sup>&</sup>lt;sup>3</sup> G. REVERBERI, in Advances in Morphogenesis (Academic Press 1961), p. 55.

<sup>&</sup>lt;sup>4</sup> G. REVERBERI, Ricerca Scientifica 31, 263 (1961).

<sup>&</sup>lt;sup>5</sup> R. L. Brahmachary and A. Bhattacharya, Exper. 19, 317 (1963).