

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 $\mu\text{g/ml}$. F. STUDER and H. P. LOREZ (of the same firm): pH approximately 8. 22,000 erythrocytes pro mm^3 , 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 $\mu\text{g/ml}$, Xanthophyll 1.8 $\mu\text{g/ml}$, β -Carotene traces. E. UNDRITZ (Sandoz Ltd., Basle): Benzidine reaction: positive. Haematocrit (packed cell volume): $\frac{1}{2}\%$ (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 α_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 $\mu\text{g/ml}$, Xanthophyll 1.0 $\mu\text{g/ml}$, β -Carotene traces. H. P. LOREZ: pH 7.6, Erythrocytes 14,000/ mm^3 , 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 excretory 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' milk¹.

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.

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

¹ 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, REVERBERI² opened a new chapter in this field which was later thoroughly explored by himself and his school and summarized^{3,4}. 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 BHATTACHARYA⁵ 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.

¹ J. NEEDHAM, *Biochemistry and Morphogenesis* (Cambridge 1950), p. 131.

² G. REVERBERI, *Exper.* 12, 55 (1956).

³ G. REVERBERI, in *Advances in Morphogenesis* (Academic Press 1961), p. 55.

⁴ G. REVERBERI, *Ricerca Scientifica* 31, 263 (1961).

⁵ R. L. BRAHMACHARY and A. BHATTACHARYA, *Exper.* 19, 317 (1963).

Polychaete egg masses anchored to the bottom of the soil were collected from the brackish water of Negombo lagoon, Negombo (Ceylon). PILLAI⁶ had identified these polychaetes to be *Marphysa borradalei* (Negombo) and had carried out a detailed study of the sequential stages of their morphogenesis. The embryos, as recognized to be at the early metatrochophore stage by comparing with PILLAI's figures, have a brownish or yellowish colour. When viewed under the microscope in full light, they showed a deep yellow or green natural tint. In spite of this they could be stained very well with neutral red after keeping for some time in a solution of neutral red added to the petri dish with brackish water. The staining was practically uniform (see a) but a band of red granules was seen to stretch between the two eye spots, around the primitive mouth and at the posterior region. This staining pattern persisted up to the late metatrochophore stage (b) when a pair of lashing setae had appeared on both sides.

PILLAI⁶ noted that the cilia are completely lost by the time the second parapodium has three jointed setae and that new parapodia are added successively between the

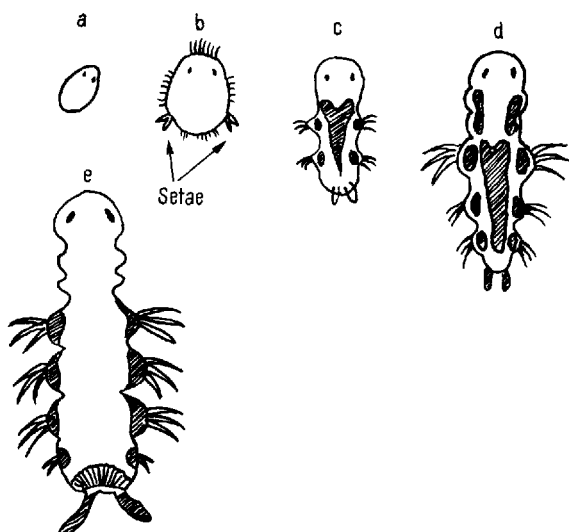
last one formed and the anal segment. Some of these later stages were studied. As soon as the specimen had acquired a visible stain they were thrown back to brackish water and observed after 3–4 h. The Figures sum up the results. Two salient features are immediately noticeable. (1) The gut region is intensely stained up to the three parapodia stage (d) but at the four parapodia stage (e) (with the fifth emerging) the stain in the gut region has vanished. This is comparable with the progressive localization⁵ of stain in the gut region of *Limnaea* embryos which was later verified and it was indubitably shown that at a later stage only the posterior part of the alimentary system retains the stain and the anterior part loses it⁷. (2) Secondly, there is intense localization of the stain at the bulges of the parapodia after they appear and just before they appear. The latter point is evident from (e) (fifth appearing).

The observations were carried out up to the eight parapodia stage and the above remarks were found to be valid till then⁸.

Résumé. L'auteur a étudié une espèce de polychète de Ceylan par des colorations au rouge neutre. La couleur s'accumule uniformément dans l'embryon au stade métatrochophore. Après l'apparition des parapodes, le conduit alimentaire est fortement teinté, mais la couleur s'évanouit dès le stade des « quatre-parapodes ». La couleur est toujours localisée dans les parapodes arrondis, et se montre déjà à leur point de germination immédiatement avant qu'ils fassent saillie au dehors.

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Different stages of *Marphysa borradalei*. a, b: whole region is stained. c, d, e: the stained region is shown by shading.

⁶ T. G. PILLAI, Ceylon J. Sci. 1, 94 (1958).

⁷ R. L. BRAHMACHARY, unpublished data.

⁸ I am deeply indebted to Dr. A. C. J. WEERAKOON and Mr. G. PILLAI of the Colombo Fisheries Research Station for kindly providing me with the laboratory facilities and the material, and to Dr. N. DE SILVA who carried out the observations during my illness and thereby made this work possible.

Effects of Presso- and Chemoceptive Components of the Cat's Aortic Nerve on Sham Rage Behaviour¹

It has recently been demonstrated that the sham rage behaviour of the acute thalamic cat is under the inhibitory control of the carotid sinus pressoreceptors², while it is excited by chemoceptive afferents from the carotid bodies³. In order to study whether similar actions are also characteristic of the presso- and chemoceptive afferents coursing in the aortic nerves, we have resorted to electrical stimulation of these nerves. As demonstrated by DOUGLAS and SCHAUMANN⁴, and later confirmed by us⁵, stimulation of an aortic nerve with pulses of increasing amplitude or duration or frequency successively activates low threshold depressor fibres, intermediate threshold pressor fibres and high threshold depressor fibres. As the excitation of the intermediate component is also associated with the appearance of hyperpnoea, this group of fibres has been in-

terpreted as composed of chemoceptive afferents, while the two depressor effects have been ascribed to pressoreceptive fibres.

Methods. The experiments were carried out in 14 acute thalamic cats, whose brain was transected just in front of the anterior pole of the thalamus. The left aortic nerve

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² C. BARTORELLI, E. BIZZI, A. LIBRETTI, and A. ZANCHETTI, Arch. ital. Biol. 98, 308 (1960).

³ E. BIZZI, A. LIBRETTI, A. MALLIANI, and A. ZANCHETTI, Amer. J. Physiol. 200, 923 (1961).

⁴ W. W. DOUGLAS and W. SCHAUMANN, J. Physiol. 132, 173 (1956).

⁵ G. BACCCELLI, M. GUAZZI, A. LIBRETTI, and A. ZANCHETTI, Nature, in press.