From these results we conclude that tannic acid at low concentrations forms a soluble complex with protein modifying its spectrum with subsequent disappearance of the peak at 520 nm for the cytochrome C and at 500 nm for the metamyoglobin[5,6]; addition of Tween 80 to the solution splits the linkage of cytochrome C and metamyoglobin with the tannic acid and causes the reappearance of the respective peaks. If tannic acid is added to the protein solution in large quantities, a precipitate containing the tannic acid-protein complex is formed. With cytochrome C, this precipitate if suspended in the presence of Tween 80, gives cytochrome C with the characteristic spectrum of the reduced form[7].

Riassunto. L'acido tannico a basse concentrazioni forma complessi solubili con il citocromo C e la metamioglobina, modificandone i relativi spettri di assorbimento nel visibile. Il Tween 80 aggiunto alle soluzioni contenenti i suddetti complessi determina la rottura del legame proteina-acido tannico e la ricomparsa dello spettro caratteristico delle proteine in esame.

A. ZANOBINI, P. VANNI
and A. M. FIRENZUOLI

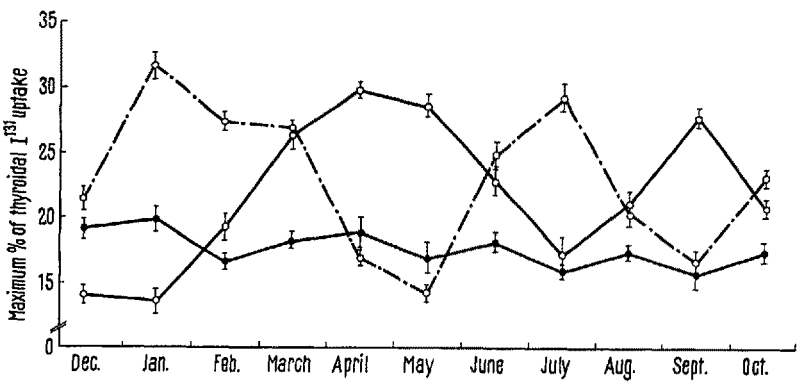
Istituto di Chimica biologica dell'Università di Firenze (Italy), 22 June 1967.

[5] S. PALEUS and K. G. PAUL in *The Enzymes* (Ed. P. D. BOYER H. LARDY and K. MYRBACH, Academic Press, New York, London 1963, vol. 8, p. 97.
[6] H. M. RAUEN, *Biochemisches Taschenbuch* (Springer Verlag 1956), p. 393.
[7] M. DIXON and E. C. WEBB, in *Enzymes* (Longmans, London 1964), p. 386.

Influence of Photoperiods on the Seasonal Fluctuations of TSH Content of the Pituitary in a Freshwater Catfish, *Mystus vittatus* (Bloch)

In spite of the availability of a vast amount of literature on seasonal, morphological and physiological changes of teleostean pituitary[1-15], very little is known about variations in their hormone content. Using the thyroidal I^{131} uptake of rainbow trout, WOODHEAD and FONTAINE[16] have assessed the thyrotropic potency of cod. SWIFT and PICKFORD[17] have evaluated seasonal fluctuations in thyrotropic activity of the pituitary in *Perca fluviatilis* by stimulating the thyroid of hypophysectomized *Fundulus heteroclitus*. However, they have used only a histometric technique for the evaluation of the activity. In the present investigation an attempt has been made to ascertain the effects of photoperiods on seasonal variations in the TSH level of the pituitary of a freshwater catfish *Mystus vittatus*. To avoid species specificity[18] effects, the same species has also been used for the assay of thyrotropic potency. Thyroidal I^{131} uptake in response to homogenized pituitary was taken as criterion for the evaluation of TSH potency. To suppress endogeneous TSH secretion in the receptor fish, they were pretreated with L-thyroxin.

Three groups of adult specimens of *M. vittatus* of both sexes with an average weight of 11.0 g and total length of 10.0 cm were subjected to different photoperiods for 12 months (November–October). Group 1 was kept under normal photoperiods, group 2 was subjected to continuous illumination and group 3 to total darkness. For the assessment of thyrotropic content, pituitary glands from each group in every month were collected for 11 months (December–October). The pituitaries were acetone dried and stored separately in sealed vials. Mature specimens of *M. vittatus* pretreated with 150 µg of L-thyroxin in 0.25 ml physiological saline solution/fish twice a week for 3 weeks, were utilized for assay. They were divided into 34 batches of 12 specimens each. Each batch from 1–33 received pituitary extracts of an experimental group pooled during a month. Thus batches 1–11 were injected with pituitary homogenates of group 1 collected from December–October respectively. Batches 12–22 were given pituitary homogenates collected from group 2 in the same monthly sequence as that of group 1 and the batches 23–33 received pituitary extracts drawn from group 3 in the above sequence. In all batches each specimen received extracts of 2 pituitaries divided into 6 doses, that is 2 doses/week for 3 weeks. The pituitary extracts were kept in sealed vials and stored in deep-freeze, but before injection extracts were brought to room temperature. The pituitary homogenates were prepared in physiological saline solution and the quantity of each injection fluid was 0.2 ml.



Seasonal variations in the TSH level of the pituitary under varied photoperiods in *M. vittatus*. O——O, pattern of cyclic variations in TSH level of group 1 (normal photoperiods); O---O, cyclic variations in TSH level of group 2 (continuous illumination); ●——●, cyclic variations in TSH level of group 3 (total darkness).

All injections were given i.p. The 34th batch was treated as control and given physiological saline injections. The last injection in all batches was followed by a tracer dose of 5.0 μc of I^{131} which was administered i.p. for the evaluation of thyroidal iodine uptake. The region of the lower jaw of the specimens containing thyroid follicles was cut out, blotted dry and its thyroidal iodine uptake was measured in well type scintillation counter.

Using the induced I^{131} uptake as an indicator of TSH activity, I found that this species exhibited seasonal cyclical variations in its thyrotropic potency. With normal photoperiodic conditions there were 2 peaks in TSH content alternating with phases of very low activity. From the Figure it can be seen that April and September gave peak values, January and July low values. The pattern of cyclic fluctuations was the same in group 2, exposed to continuous illumination, except that the peaks were encountered 2 months earlier than with group 1. In contrast to this, TSH level in the samples of group 3, which was kept under total darkness, was very low throughout the year and may be compared with the minimal potencies of the other 2 groups (Figure). The 34th batch which was treated as control showed very low uptake of I^{131}, due to the blocked indigenous secretion of TSH.

Under normal photoperiods the pattern of cyclic variations in TSH level resembles to some extent that of *P. fluviatilis*[17], where June and September samples showed an increase in TSH content but August samples were found to be inactive. Continuous illumination appears to be one of the important external factors responsible for the early activation of the pituitary without disturbing the basic plan of cyclic activity and thereby raising the peaks of thyrotropic content by 2 months in advance as compared to that kept under normal photoperiods. Total darkness seems to be an other external regulator which tends to abolish the inherent capacity of pituitary for cyclical activity[19].

Zusammenfassung. *Mystus vittatus* zeigt unter natürlichen Bedingungen jahreszeitliche Variationen des TSH Gehaltes der Hypophyse. Veränderung der Lichtverhältnisse kann diesen TSH Zyklus beeinflussen: Dauerlicht verschiebt die Phasen, Dauerdunkel unterdrückt die zyklischen Veränderungen auf einen geringen Aktivitätsgrad.

T. P. Singh

Department of Zoology, University of Udaipur, Udaipur (Rajasthan, India), 10 May 1967.

[1] M. Olivereau, Ann. Inst. Oceanogr. *29*, 96 (1954).
[2] B. Verhoeven and G. E. Van Oordt, Proc. Sect. Sci. K. ned. Akad. Wet. C *58*, 628 (1955).
[3] H. W. Sokol, Anat. Rec. *122*, 451 (1955).
[4] J. M. Dodd, Mem. Soc. Endocr. *4*, 166 (1955).
[5] In *Techniques in Endocrine Research* (Ed. P. Eckstein and F. Knowles; Academic Press Inc., London 1963), p. 161.
[6] J. M. Dodd and T. Kerr, Symp. zool. Soc. Lond. *9*, 5 (1963).
[7] G. E. Pickford and J. W. Atz, *The Physiology of the Pituitary gland of Fishes* (New York Zoological Society, New York 1957).
[8] G. E. Pickford, in *Comparative Endocrinology* (Ed. A. Gorbman; John Wiley and Sons, New York 1959), p. 404.
[9] A. G. Sathyanesan, Cellule. *63*, 283 (1963).
[10] A. G. Sathyanesan and T. P. Singh, J. zool. Soc. India, *14*, 165 (1963).
[11] O. H. Robertson and B. C. Wexler, J. Morph. *110*, 157 (1962).
[12] M. P. Schreibman, Zoologica, *49*, 217 (1964).
[13] M. D. Lagios, Gen. comp. Endocr. *5*, 207 (1965).
[14] Y. Honma, Bull. Jap. Soc. scient. Fish. *32*, (1966).
[15] Y. Honma and E. Tamura, Bull. Jap. Soc. scient. Fish. *31*, 863 (1965).
[16] A. D. Woodhead and Y. A. Fontaine, Bull. Inst. océanogr. Monaco *56*, 1137 (1959).
[17] D. R. Swift and G. E. Pickford, Gen. comp. Endocr. *5*, 354 (1965).
[18] M. Fontaine and Y. A. Fontaine, Gen. comp. Endocr. Supplement *1*, 63 (1962).
[19] I am grateful to Dr. A. G. Sathyanesan, Department of Zoology, Banaras Hindu University for suggestions and encouragements. My thanks are also due to Prof. P. Starr, Pasadena, California, Dr. Th. Garrett, Illinois and Baxtor Laboratories Inc., Morton Grove, Illinois, for generous supply of crystalline L-thyroxin, to Prof. S. P. Raychaudhuri for providing facilities and encouragements.

Time Course of the Distribution of in vivo Administered $^{89}Sr^{++}$ in Rat Liver Subcellular Fractions

A recent study of the subcellular distribution of injected $^{89}Sr^{++}$ in rat liver has shown that, a few min after the injection, mitochondria contained a very large proportion of the total radiostrontium of the cell[1]. Experiments with uncouplers of oxidative phosphorylation have led to the conclusion that energy-linked mechanisms for Sr^{++} uptake are operating in the liver cell. Smaller amounts of $^{89}Sr^{++}$ were associated with the microsomes, and with the fraction that contains the nuclei of the cells. The affinity of the cellular organelles for Sr^{++} is evidently very high, since only negligible amounts of radioactivity were recovered in the cell sap. Preliminary experiments were also carried out on the possible variations in the subcellular distribution of radioactivity as a function of time after the injection of $^{89}Sr^{++}$: shifts in the pattern of distribution were indeed detected, indicating that the subcellular pools of Sr^{++} were not irreversibly sequestered in the organelles, but were to a certain extent interchangeable, and communicating with each other. A sequential transfer of Sr^{++} from one subcellular structure to another could then be a part of the process by which the liver cell assumes, maintains, and discharges the injected $^{89}Sr^{++}$.

A detailed study of the problem has therefore been carried out, with special emphasis on the times immediately following the injection of $^{89}Sr^{++}$. The results obtained have shown that definite changes in its intracellular distribution do indeed occur early after the injection, and that they involve all subcellular fractions studied; it is thus possible to suggest a sequence of events for the fate of Sr^{++} in the liver cell.

Methods. Commercial rats were used, weighing 250–300 g, and fasted 16 h before the sacrifice. $^{89}SrCl_2$ (20 μc/100 g b.w., in 0.5 ml distilled water) was injected i.p. Fractionation and analytical methods have been described

[1] E. Carafoli, J. gen. Physiol *50*, 1849 (1967).