With respect to these experiments, the transport in yeast cells is to be accepted as the rate-determining step. Having accepted the carrier mechanism as working hypothesis for further studies, it can easily be deduced that it is not necessary for the carrier to have the chelating activity desirable for an appreciable equilibrium-shift in an isolated system cell-metal-carrier. Evidently, the desorbing solution outside the cell will steadily disturb this inner equilibrium, tending to establish the equilibrium distribution of metal between the cell and the solution. The chelating activity is rather only one of more rate-determining factors. Hence, some chelating agents of medium strength with convenient selectivity and acidity can be expected to be effective too, if substituted with an alkyl chain, in altering the metal permeability of cells in a desired manner and sufficient degree.

Zusammenfassung. Es wird ein neues lipophiles Derivat der Nitrilotriessigsäure (NTA) dargestellt, eine Decylnitrilotriessigsäure (DNTA). Aus mit Ce^{144} kontaminierten Hefezellen wird durch Äthylendiamintriessigsäure (EDTA) bedeutend mehr Ce ausgewaschen, wenn die Zellen mit DNTA vorbehandelt worden sind, während Vorbehandlung mit NTA keine Steigerung bewirkt. Wahrscheinlich wirkt DNTA dank seiner Lipophilie als «Carrier» des Metalls durch die Zellmembran. Es wird auf die Möglichkeit hingewiesen, durch Alkylierung die Membrandurchlässigkeit auch anderer Chelatbildner zu verändern.

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Effect of Tween 80 on Protein-Tannic Acid Complex[1]

In previous experiments we demonstrated that Tween 80 could be used for the preparation of enzyme active extracts from the needles of adult conifers[2]. The same product has also been used by GOLDSTEIN et al.[3] to prevent tannic acid inhibition of a commercial preparation of β-glycosidase and by us of an extract from the bark of *Pinus pinea* on three purified dehydrogenases[4]. The hypothesis was advanced that tannic acid and polyphenols in general, at low concentrations, formed soluble complexes with enzymes, blocking their activities and Tween 80 reactivates these activities by separating the polyphenols from the protein.

In the present work we intended to confirm this hypothesis by studying the formation and breaking of the tannic acid-protein complex by means of Tween 80. We have measured the absorption spectra respectively of the protein, the tannic acid, and of the complex protein-tannic acid and, at last, of the same in the presence of Tween 80. Cytochrome C and metamyoglobin were chosen because the spectrum of these proteins shows a very characteristic behaviour in the visible light, whereas tannic acid and Tween 80 instead have slight and undifferentiated absorption. Cytochrome C was purchased from Boehringer, Mannheim (Germany), metamyoglobin from Kock-Light Laboratories Ltd., Colnbrook Buckinghamshire (England), tannic acid from Manetti and Roberts, Florence (Italy), Tween 80 from Fluka AG, Buchs (Switzerland).

Absorption spectra were done with a Beckman DB spectrophotometer equipped with a Sargent mod. SR recorder. The results are reported in Figures 1 and 2.

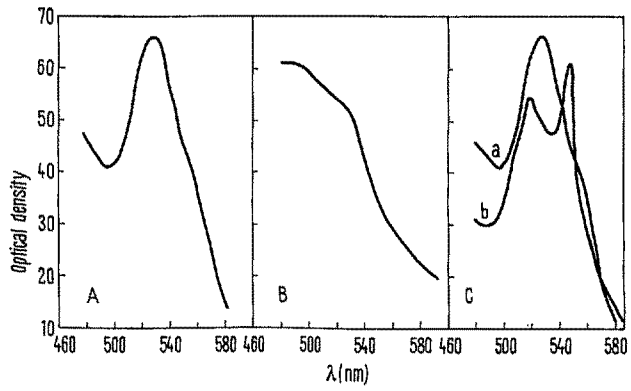

Fig. 1. (A) Spectrum of 0.1 mM oxidized cytochrome C, pH 6; final volume 2 ml. (B) Spectrum of 0.1 mM oxidized cytochrome C – 1.5 mM tannic acid complex, pH 6; final volume 2 ml. (C) (a) Spectrum of 0.1 mM cytochrome C – 1.5 mM tannic acid complex after the addition of 50 μl of Tween 80, pH 6; final volume 2 ml. (b) Spectrum of 0.1 mM cytochrome C reduced by its precipitation with 10 mM tannic acid and resuspension with 50 μl of Tween 80, pH 6; final volume 2 ml.

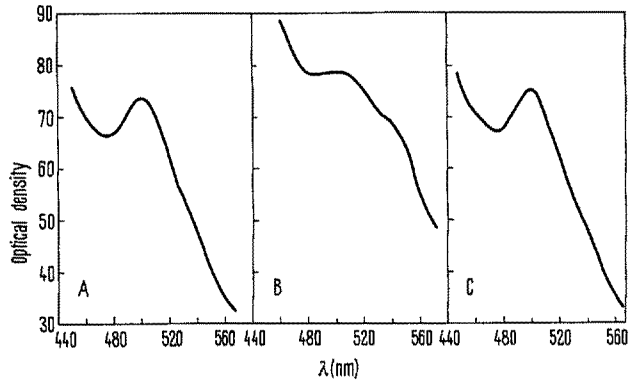

Fig. 2. (A) Spectrum of 0.1 mM metamyoglobin, pH 6; final volume 2 ml. (B) Spectrum of 0.1 mM metamyoglobin – 1.5 mM tannic acid complex, pH 6; final volume 2 ml. (C) Spectrum of 0.1 mM metamyoglobin – 1.5 mM tannic acid complex, after addition of 50 μl of Tween 80, pH 6; final volume 2 ml.

[1] This work was supported by a grant from the «Impresa Enzimologia» of the Italian Consiglio Nazionale delle Ricerche and by Grant No. FG-It-128 from the U.S. Department of Agriculture.

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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.

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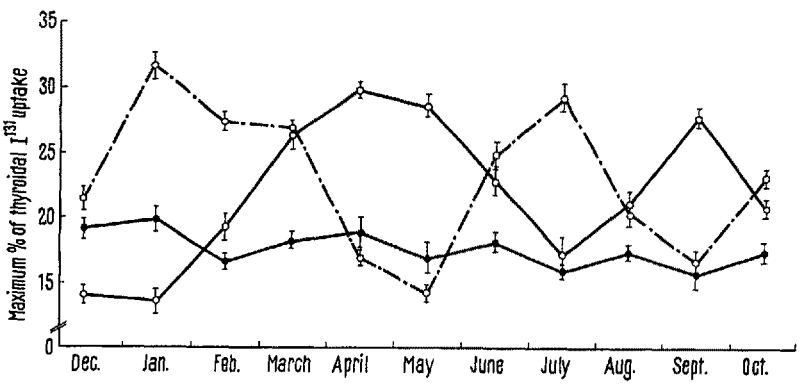
[5] S. PALEUS and K. G. PAUL in *The Enzymes* (Ed. P. D. BOYER H. LARDY and K. MYRBACH, Academic Press, New York, London 1963, vol. 8, p. 97.
[6] H. M. RAUEN, *Biochemisches Taschenbuch* (Springer Verlag 1956), p. 393.
[7] M. DIXON and E. C. WEBB, in *Enzymes* (Longmans, London 1964), p. 386.

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).