Laboratory of Histology and Embryology, Faculty of Science, University of Rome, June 8, 1954.

Sommario

È comparato lo sviluppo degli occhi e del tetto ottico di un pesce cieco delle caverne (*Anophthyctys jordani*) con quello di forme affini viventi all'aperto e con occhi normalmente sviluppati. Pur essendovi una notevole ipoplasia del tetto i neuroni specifici a neurite ricorrente conservano le loro peculiari caratteristiche strutturali. Ciò dimostra che l'adattamento ecologico non ha ridotto i fattori intrinseci del differenziamento di questi neuroni.

PRO EXPERIMENTIS

An Experimental Method of Tissue Surface Measurements of Beta Activity *in vivo*

Several workers have considered the problem of *in vivo* external measurements of radioactivity in tissues. Studies have been carried out with β emitters and particularly with radiophosphorus by LOW-BEER[1], FRIEDELL *et al.*[2], GEFFEN *et al.*[3] on human subjects and by SODARO and SHEPPARD[4], SCHÖNENBERG and MENZEL[5] on rabbits. The physical aspect of these measurements has been investigated by STRAJMAN[6] with theoretical and experimental analysis.

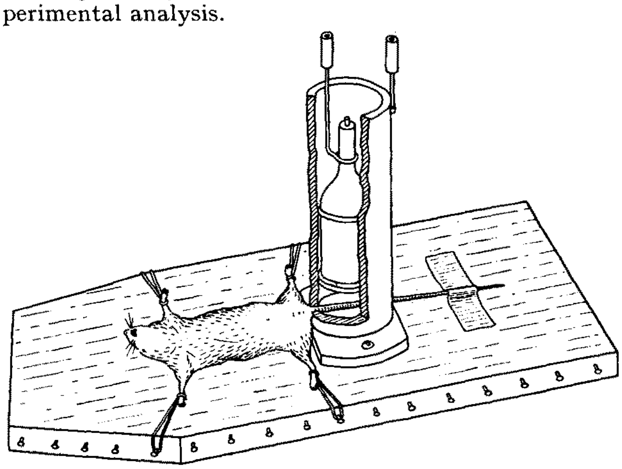

Fig. 1

As the interpretation of the results of external β activity measurements was not thorough, it seemed useful to study the behaviour of radiophosphorus in a normal animal by means of varying the route of administration only. We have therefore developed a simple and convenient experimental procedure, which enables us to obtain reliable surface β measurements in mice, over a lapse of some hours. Such procedure ensures strictly constant counting efficiency as well as rather comfortable conditions for the animal.

The mouse is laid on its back upon a small table with the four limbs held by means of rubber elastics. The tail is passed through two diametrically opposite small holes at the base of an aluminium cylinder firmly fixed on the table: the free extremity of the tail is then kept in place by adhesive tape (Fig. 1). Cotton wool, properly placed, prevents any spreading of radioactive excreta. The aluminium cylinder houses a β G.M. tube of the bell jar type with thin mica window. The thickness of the cylinder wall is sufficient to stop all β particles except those emitted from the counting area.

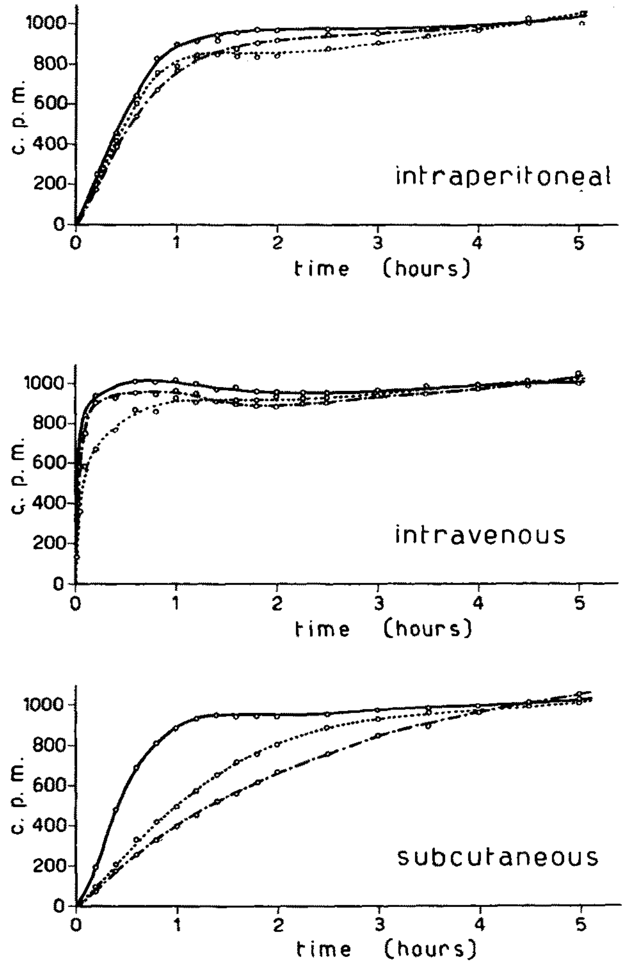

Fig. 2

We have employed white male mice of a body weight ranging from 23 to 27 g. The room temperature was constant (about 20°C). A solution of P^{32} in the form of Na_2HPO_4 with $\sim 85\,\gamma$ of stable phosphorus per ml of isotonic NaCl solution at pH = 7·2 was injected intraperitoneally (0·5 ml of solution), intravenously (0·1 ml into the vena jugularis) and subcutaneously (0·5 ml) respectively. We have made a series of brief integral preset-time countings for each animal starting before the injection and extended over 4 to 6 h.

Under these conditions of experiment, the counting rate depends on the following variables: (1) geometrical conditions of the counting (e.g. size of tail, portion of the tail in counting area, etc.); (2) route of administration; (3) quantity of injected P^{32}; (4) individual biological variability.

The counts-per-minute (corrected for dead time, background and radioactive decay) make "build up" curves when drawn in cartesian co-ordinates. A comparison between the "intravenous", "intraperitoneal" and "subcutaneous" curves indicates that their trend, different in the initial portions, tends gradually to become similar at the beginning of the third hour; thereafter the curves show a very slow and continuous slope of a few units per cent per hour. Furthermore the counting rate at the

[1] B. V. A. LOW-BEER, *The Clinical Use of Radioactive Isotopes* (C. C. Thomas, Springfield, Ill., 1950).

[2] M. T. FRIEDELL *et al.*, Arch. Int. Med. *83*, 608, 620 (1949); *85*, 667 (1950).

[3] A. GEFFEN, R. LOEVINGER, and B. S. WOLF, Radiology *46*, 856 (1951).

[4] R. M. SODARO and C. W. SHEPPARD, Nucleonics *8*, No. 6, 40 (1951).

[5] H. SCHÖNENBERG and K. MENZEL, Z. Kinderheilk. *73*, 17 (1953).

[6] E. STRAJMAN, Univ. Calif. Publ. Physiology *8*, 333 (1951).

fifth hour after the injection is independent of the administration route. We registered 450 counts-per-minute per μc of injected P^{32} with a standard deviation of $\pm$ 33%. The "build up" curves can therefore be normalized by equalizing to 1000 the mean value of the countingrate at the fifth hour. Figure 2 shows examples of the normalized curves.

The observation of these curves permits us to affirm that the geometrical and biological variabilities do not prevent the study of other variables introduced at will: one may study particularly the administration route (as we have done), the room temperature, the effects of the pharmacological substances administrated.

The discussion of the significance of our measurements is out of the scope of the present note. However, from the analysis (to be published) of these curves, and considering the results of our other researches, we can say that what is measured is the activity of the extracellular and cellular spaces, while the blood activity is not significantly measured.

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Riassunto

Gli autori descrivono una tecnica che consente di attuare determinazioni esterne di radioattività β su tessuti superficiali nel topo, mantenendo costante il rendimento di misura per una durata di alcune ore. Vengono presentati e brevemente commentati alcuni esempi di curve ottenute con questa tecnica in seguito ad iniezioni di P^{32} per via endovenosa, endoperitoneale e sottocutanea.

Informations - Informationen - Informazioni - Notes

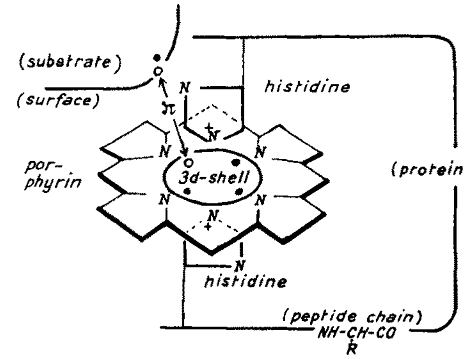
THEORIA

An Electronic Theory of Transition Metal Enzymes and Catalysts

It is well-known that the transition metal is a good catalyst for oxidation and reduction. We have as industrial catalysts: platinum black, Raney's nickel, cobalt for hydrocarbon synthesis, and iron for ammonia synthesis; and as biocatalysts: heme-protein (cytochrome, cytochrome-oxidase, peroxidase, catalase, hemoglobin), and copper-protein (phenol- and ascorbic-acid-oxidase, hemocyanin).

In the heme-protein, an iron porphyrin and two histidines of protein sometimes form the d^2sp^3 octahedral six covalent iron complex salt[1]. As the 3d-orbitals of iron atom in the complex are saturated by the electrons of ligands, the 3d-electrons can be assumed to form a closed shell similar to the 3d-electrons of metal copper. It has been found that a bond radius of iron in the complex is about 1·23 Å[2], and an atomic radius of metal copper is about 1·27 Å[3]. The π-electrons of conjugated double bond in the iron porphyrin will be able to move freely among atoms just as do the 4s-electrons of metal copper. By taking it for granted that each of six nitrogen atoms of porphyrin and two histidines has the same relation to the central iron atoms and forms a symmetrical structure to the latter, an analogy to the metallic model that is, a polyhedron of metal copper calculated quantum-mechanically by Wigner-Seitz's method may exist as an isolated form in the prosthetic group of heme-protein. The copper-protein acts in the same way as does the heme-protein. Since the 3d-orbitals of copper atom are almost filled, the copper-protein will have no need to make a complex salt with the aid of porphyrin. The state containing 9 electrons in d-orbitals will be called hereafter "platinum-like state", since Pt is the most powerful catalyst for oxidation-reduction. One electron coming in and out of the platinum-like state may play a decisive role in the oxidation-reduction process for the metal enzymes, as shown in the Figure[4].

Transition metal atoms are ordinarily arranged according to the well-known order of the periodic table by their physical and chemical properties. However, it has been found to be convenient for a survey of catalytic activities to rearrange them according to the number



A Model of Metal Enzymes.

of their d-electrons[1]. One of its representations is shown in Table where the number of d-electrons are taken from the Slater's treatise[2]. Hydrogenation by hydrogen molecule has taken place through the presence of 2nd class metals which include the 3rd class metals. Both Pt and Pd are the best catalysts for hydrogenation. Cu and Ni usually require some treatments in order to make these metals as much effective as catalysts as Pt, e.g. mixing CuO with Cr_2O_3 as in the case of Adkins' catalyst, or dissolving out Al by alkali from Al-Ni alloy to leave porous Ni as in the Raney's catalyst. In the d-electron deficient state, the metals will accept electrons from substrates and form at their surface some intermediate complex compounds with substrates which will later be decomposed and reduced, like Co in the Fischer's synthesis and Fe in the Haber's synthesis.

[1] C. D. Coryell and L. Pauling, J. Biol. Chem. *132*, 769 (1940). - H. Theorell and Å. Åkesson, J. Amer. Chem. Soc. *63*, 1812 (1941).

[2] L. Pauling, *The Nature of the Chemical Bond* (Cornell Press, 1939) p. 182; J. Amer. Chem. Soc. *69*, 542 (1947).

[3] F. E. Fowler, Smithsonian Physical Tables *1933*, 491.

[4] K. Ohmori and K. Ohki, Kagaku (Science) *18*, 36 (1948); Chem. Abstr. *45*, 1174 (1951). - K. Ohki, J. chem. Soc. Japan *70*, 68 (1949); C. A. *45*, 4135 (1951); Kagaku *19*, 152, 203 (1949); C. A. *45*, 2992 (1951); Kagaku *19*, 476 (1949); C. A. *46*, 3578 (1952); Kagaku *20*, 156 (1950); C. A. *44*, 6690 (1950). - H. Taketa and K. Ohki, Kagaku *20*, 186 (1950); C. A. *46*, 3398 (1952).- K. Ohki, *Quantum Chemistry in Biology* (Japanese edition, Kyoritsu Publ. Co., 1950).

[1] K. Ohki, Kagaku *22*, 42 (1952); C. A. *46*, 2408 (1952); Kagaku *22*, 420 (1952); C. A. *46*, 9903 (1952); *Quantum Chemistry of Organic Compounds* (Japanese edition, Kyoritsu Publ. Co., 1952).

[2] J. C. Slater, *Introdution to Chemical Physics* (McGraw-Hill, p. 346, 347, 1939).