a prophase gives rise to a metaphase, and so on. Thus, the duration of each phase can be calculated by means of the appropriate index once the duration of mitosis is known.

By the combined use of the partial mitotic index and the phase indices, we are enabled to calculate the natural or induced modifications in the cell-division cycle with less probability of error.

Résumé. Cet article propose l'emploi de l'index mitotique partiel et des index mitotiques des phases pour l'analyse cytologique quantitative des modifications apparaissant au cours du cycle de division cellulaire, et suggère une méthode rapide pour calculer tous les indices mentionnés.

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Sección de Citología, Instituto de Biología Celular, Madrid (Spain), February 5, 1965.

A Radioassay for Dopamine-β-Hydroxylase Activity

Dopamine-β-hydroxylase is a mixed function oxidase that catalyzes the terminal step in norepinephrine biosynthesis, namely, the conversion of dopamine to norepinephrine[1]. In addition to dopamine, other phenylethylamines such as tyramine, epinine, amphetamine, α-methyl dopamine, etc.[2–4] can also serve as substrates for the enzyme. The enzymatic activity of dopamine-β-hydroxylase was determined by measuring fluorometrically[5], the amount of norepinephrine formed from dopamine. While this is a relatively simple method for the determination of the enzymatic dopamine-β-hydroxylation, there are no simple methods available for the determination of the β-hydroxylation of other substrates. The specificity of the fluorometric method is also limited since the presence of other fluorescent compounds in the assay mixture may interfere with the determination.

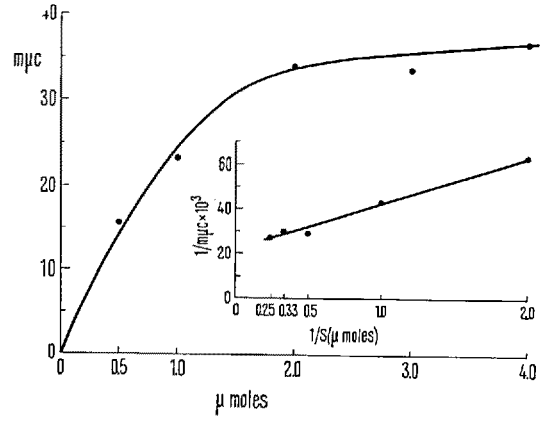
It has been shown that one of the two benzylic tritiums is lost during the enzymatic β-hydroxylation of dopamine-β,β-T[6]. Therefore it is conceivable that the rate of tritium released as water, according to the following reaction sequence:

$$\text{dopamine-}\beta\text{-T} + \text{ascorbate} + O_2 \longrightarrow \text{norepinephrine} + \text{TOH} + \text{dehydroascorbate}$$

is directly proportional to the rate of β-hydroxylation. The recent availability of dopamine-β-T made it possible to measure the release of TOH during the enzymatic β-hydroxylation. The tritiated water which is formed during the enzymatic β-hydroxylation can be separated by ion exchange chromatography from the substrate and product and subjected to radioassay. A similar procedure for the determination of tyrosinase[7] and tyrosine hydroxylase[8] was recently described.

The enzyme was prepared from adrenal medulla[1] and further purified by a previously described procedure[9]. Standard reaction mixtures contained, per tube, dopamine-β-T, 100–200 mμc/μmole (dopamine-β-T, with a specific activity of 137 mc/m*M* was obtained from New England Nuclear Corp., Boston, Mass.). The reaction was started by addition of enzyme, and the tubes were incubated at 37°C for 15 to 45 min. The incubation was stopped by addition of 0.5 ml glacial acetic acid, and the proteins were separated by centrifugation. With highly purified enzyme preparations, the amount of protein was so small that it did not require removal before transfer to the column. To count tritiated water, the reaction mixture was passed through a small Dowex 50 × Na^+ (0.3 × 4 cm) column. The column was washed with water to make an effluent volume of 10 ml. An aliquot (0.5 ml) was dissolved in a liquid scintillation solution which is suitable for counting aqueous samples[10]. A reagent blank, in which all additions were made except for the enzyme, was used to correct the results.

Rates were obtained by assaying TOH at different time intervals. There was a proportional increase of enzymatic activity with time. Reaction rates were also investigated as a function of enzyme concentration. Over the range tested, the reaction was a linear function of enzyme concentration. A typical plot of the reaction velocity vs. substrate concentration, and a double reciprocal plot of the reaction velocity vs. substrate concentration are shown in the Figure. Within a certain range, the velocity of the



Reaction rate (expressed in mμc of TOH formed) vs. substrate concentration (expressed in μmoles) and the double reciprocal plot of the reaction rate vs. substrate concentration.

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reaction was directly proportional to the substrate concentration.

It is evident from the above results, that the detritiation rate of dopamine-β-T is directly proportional to the dopamine-β-hydroxylase activity. It should be pointed out that the enzymatic removal of the benzylic tritium is stereospecific and, providing there is no isotope effect, only one mole of TOH is formed from 2 moles of dopamine-β-T. The presently described assay is even more sensitive for the determination of dopamine-β-hydroxylase than the previously applied fluorometric procedure. The sensitivity can be increased by the use of higher specific labeled dopamine. This procedure can also be used to determine the rate of β-hydroxylation of other dopamine-β-hydroxylase substrates, provided that these substrates are tritium labeled in the β-position of the side chain. This radioassay was also applied for the determination of dopamine-β-hydroxylase activity in neuroblastoma and pheochromocytoma tumors. In two pheochromocytoma tumors, and in three out of five neuroblastoma tumors, dopamine-β-hydroxylase activity was found to be high. All tested neuroblastoma and pheochromocytoma tumors also showed high tyrosine hydroxylase activity. Thus it was demonstrated that the enzymes which catalyze the catecholamine production are present in these tumors, and their activity must be responsible for the overproduction of the catecholamines in these diseases. A detailed study on the determination of dopamine-β-hydroxylase and tyrosine hydroxylase activities in these tumors is in progress and will be reported elsewhere[11].

Zusammenfassung. Es wurde eine Isotopenmethode zur Bestimmung der Dopamine-β-hydroxylase-Aktivität beschrieben, die auch zur Bestimmung der enzymatischen β-Hydroxylierung sämtlicher Substrate der Dopamine-β-hydroxylase verwendet werden kann. Die Methode bewährte sich für Aktivitätsbestimmungen in Neuroblastoma- und Pheochromocytoma-Tumoren.

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Isolation and Partial Characterization of the Moult-Inhibiting Hormone of the Crustacean Eyestalk

The earliest observations of accelerated moulting and precocious growth in crustaceans from which both eyestalks had been ablated were made by Zeleny[1] and Megušar[2]. This has been repeatedly observed in several crustaceans[3]. It has been demonstrated that the moult-inhibiting hormone is secreted by the neurosecretory cells of the medulla terminalis X-organ and transported to the sinus gland where it is released into the blood[4]. The moult-inhibiting hormone of the X-organ sinus gland complex appears to inhibit the Y-organ and prevent the release of the moulting hormone[5,6]. Although attempts have been made to isolate the moulting hormone[7], the isolation of moult-inhibiting hormone in crustaceans has not been achieved.

Bilateral ablation of eyestalks in *Ocypode macrocera* results in precocious growth and moulting[8]. In the present study specimens of *Ocypode macrocera* (23 to 25 mm carapace width) collected from the Visakhapatnam beach were used. Eyestalks of the crab, *Ocypode macrocera*, were dissected, the eyestalk ganglia were isolated and dehydrated in acetone. Eyestalk ganglia from 1000 eyestalks were dried in the above manner and this material (10.35 g) was used for the extraction.

The acetone dried eyestalk ganglia were homogenized successively with acetone (5.0 ml/g) and twice with chloroform. The material was washed in acetone and dried at room temperature (28°C) in air. The crude eyestalk ganglia powder was suspended in 90% phenol and stirred for 2 h. Five volumes of a solution of acetic acid-acetone (1:4) and 0.005 *M* NaCl were added and the mixture was cooled at 4°C for 2 h. The mixture was filtered and an equal volume of ether was added to the filtrate. After allowing the mixture to stand overnight in the cold, the precipitate was collected on a filter paper, washed in acetone and dried in a vacuum desiccator. The dried extract was dissolved in 5.0 ml of 80% acetic acid, 15.0 ml of water was added and the inert matter was precipitated by adding 6% NaCl. To the filtrate, TCA was added to the concentration of 2.5% and the mixture was allowed to cool at 4°C for 2 h. The precipitate collected by centrifugation was washed with 2.5% TCA and dried in a vacuum desiccator. The dried material was extracted with absolute ethanol and centrifuged. The supernatant was isolated and allowed to evaporate and the residue after evaporation was collected and weighed. The resulting substance was a white crystalline material weighing 2.15 mg.

The isolated substance was tested for moult-inhibiting effect in the following manner. Bilateral ablation of eyestalks was performed in 32 specimens of *Ocypode macrocera* that were in C_4 stage of the intermoult cycle and the crabs were divided into three groups. Group A, consisting of 10 crabs, was left untreated. Group B, consisting of 12 crabs, was injected with a dose of 2 μg/0.05 ml of the isolated substance in sea water on the 6th day after eyestalk

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