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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[11] Acknowledgment: This work was supported by USPHS Research Grant No. 5 R01 MH-02717-07 and by the Institutional Grant No. IN-14G from the American Cancer Society.

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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ablation. Group C animals (10 crabs) were each injected with 0.05 ml of sea water. During the period of experimentation the crabs were fed twice weekly on pieces of fish. The experiment was continued until all the crabs had moulted, and the duration of proecdysis in each crab was noted. The experiment was repeated once, and the results are shown in the Table.

From the Table, it is evident that the isolated fraction of the eyestalk ganglia has a pronounced moult-inhibiting effect. The proecdysal duration of the eyestalkless *Ocypode* that were injected with a dose of 2 μg of the isolated fraction is significantly higher ($P = < 0.001$ in both the experiments) than the values of untreated animals and those injected with sea water. The differences in proecdysial duration between the untreated eyestalkless crabs and those injected with sea water are not statistically significant.

Proecdysial duration in the Crab *Ocypode macrocera*

Experiment no.	Experimental group	No. of crabs	Treatment	Duration of proecdysis (in days mean ± S.D.)
I	A	10	Untreated eyestalkless crabs	20.8 ± 1.5
	B	12	Eyestalkless crabs injected with the isolated fraction of the eyestalk	36.8 ± 3.7
	C	10	Eyestalkless crabs injected with sea water	21.3 ± 2.2
II	A	8	[a]	19.5 ± 0.7
	B	9	[a]	38.8 ± 3.3
	C	8	[a]	20.1 ± 1.2

[a] Treatment same as in experiment I.

The isolated fraction showed no chromatophorotropic activity when tested on chromatophores of *Ocypode*, *Palaemon* and *Metapenaeus*. It has no effect on premoult water uptake in the crab, *Ocypode macrocera*. In the light of these observations, the isolated fraction was identified as the moult-inhibiting hormone, with the implicit reserve that further experiments as to its effect on other metabolic changes in relation to the moult cycle are necessary to confirm this statement.

Tests on solubility properties revealed that the hormone is soluble in ethanol, methanol and phenol. It is insoluble in acetone, ether and chloroform. Ascending paper chromatography, using a water saturated mixture of *n*-butanol and amyl alcohol as the solvent, revealed that the hormone has an Rf value of 0.54. The hormone is inactivated by 1:1000 trypsin. It is concluded that the moult-inhibiting hormone is most likely a peptide[9].

Résumé. Un fragment soluble dans le phénol de pédoncule oculaire du Crabe *Ocypode macrocera* a, sur la mue, un effet inhibitif prononcé. Cette inhibition est sans effet sur les chromatophores et sur l'absorption de l'eau pendant la pré-mue du crabe. L'hormone est soluble dans l'éthanol, le méthanol et le phénol et inactivée par la trypsine. L'hormone d'inhibition de la mue paraît être une peptide.

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[9] Acknowledgments: The author is grateful to Dr. NAGABHUSHANAM for guidance, to Prof. P. N. GANAPATI for facilities and keen interest in the work, and to the Government of India for the award of a scholarship.

Studies of Selective Mating Using the Sex-Linked Mutants *White* and *Bar* of *Drosophila melanogaster*

A previous paper[1] was devoted to the measurement of sexual isolation between the melanistic mutants *ebony* (e^{11}) and *black* of *Drosophila melanogaster*, and their wild-type allele. This one concerns the two sex-linked mutant genes *white* and *Bar*. Exactly the same methods were used. The degree of sexual isolation was determined according to the 'multiple choice method' by direct observation of 100 flies (25 pairs of both strains), 4 or 5 days old, introduced together in a wooden box covered by a thick glass, the copulating pairs being recorded at intervals of 5 min on a paper reproducing the same grid as the chequered canvas extended beneath the box. The locomotor activity of the fly in a group was determined according to EWING[2], 25 flies of the same sex and strain being introduced together in the first of a series of 6 tubes and the number of flies in each tube being recorded at 3 min intervals.

The results of the observations concerning selective mating are given in Table I as cumulated percentage of females fertilized in each mating type. The graphic expression of such data would be exponential curves approaching a plateau, but it is possible to turn it into straight lines by logarithmic transformation, using the WATTIAUX formula

$$dx_A / (n_A - x_A)\, dt = b\, K\, e^{-K t},$$

where n_A is the total number of individuals of one sex and genotype, dx_A the number of flies mating at the intervals of time dt, and K and b constants which can be calculated from the regression line fitting to the transformed data[3]. The values of measurement becoming independent of time, any bias due to the few copulations taking place eventually after the $2^1/_2$ h of observation is avoided. Figure 1 (time in abscissa and values $dx_A/n_A - x_A$ in ordinate) gives the regression lines permitting comparison

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