

of this insect are innervated by the branches of 2 nerves, an anterior nerve originating from the interganglionic connective between the suboesophageal and prothoracic ganglia, and the other posterior nerve being the transverse branch of the median nerve from the prothoracic ganglion (Figure 1). These innervating nerves divide into several branches before entering the gland and, on entry, ramify further to form a network of nerve fibres which occupy the intercellular spaces of the gland, each segment of the network surrounding closely a gland cell (Figure 2). On close examination, we could follow this pattern of innervation even with a routine stain like hematoxylin.

This network-like innervation pattern, hitherto unrecorded, seems significant in view of the fact that neurosecretory material has been demonstrated within the innervating nerves of the prothoracic glands in many other insects⁶⁻⁸ and our preliminary observations indicate a similar situation in *P. demoleus* also. It is, therefore, evident that these nerves serve to transport neurohormones to the gland and the innervation pattern observed here would ensure a direct hormone delivery to the individual gland cells⁹.

Zusammenfassung. Die Prothorakaldrüse der Schmetterlingsraupe *Papilio demoleus* L. ist mit Sicherheit innerviert, wobei jede einzelne Drüsenzelle von Nervenendigungen umspunnen ist. Der Befund spricht für eine Aktivierung der Häutungsdrüse durch Neurosekret, das durch Nerven zugeführt wird.

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⁷ B. SCHARRER, Z. Zellforsch. mikrosk. Anat. 64, 301 (1964).

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⁹ We wish to express our thanks to Prof. S. P. RAY-CHOUDHURI for providing working facilities and to the C.S.I.R., Government of India, for supporting the work with a grant. One of us (H. H. S.) held a junior research fellowship.

The Effect on the Onset of Puberty of Whole Body Irradiation of Infant Female Rats with and Without Hypothalamic Lesions

As far as we know there are no statistical data to show how whole body X-irradiation acts on the factors controlling the onset of puberty in laboratory animals, as measured by the opening of the vaginal orifice. MANDEL and GRIESEWOOD¹ have claimed that the vagina of rats open precociously after irradiation of the ovarian region, but BEAUMONT² has failed to confirm this finding. DONOVAN and VAN DER WERFF TEN BOSCH^{3,4} as well as other authors⁵⁻⁷ have reported that electrical injuries of the hypothalamus of infant rats may accelerate the opening of the vagina.

Our experiments have been designed to test whether whole body X-irradiation affects the onset of puberty in normal infant rats and in infant animals with hypothalamic lesions. Albino rats bred randomly for more than 30 years and maintained under the same conditions of temperature, lighting and diet have been used and the number of young per mother has been restricted to 6 in experimental and in control groups.

At 8 days of age animals have been exposed to doses of 200, 400 or 500 R of whole body X-rays from a Siemens set under the following conditions: 200 kV, 16 mA, added filter of 0.5 mm Cu, FSD 42 cms, dose rate 107.5 R/min. Bilateral lesions 1 mm apart have been produced by microcoagulation aimed at the basal region of the arcuate nucleus. The majority of the lesions have been placed close to the desired site. Some irradiated rats have had their microcoagulations on the 20th and others on the 25th day after birth and non-irradiated animals on the 17th or on the 25th day. The number of rats in the control, irradiated, operated and operated plus irradiated groups are given in the Table. Student's *t*-test has been used to determine the significance of differences in mean values.

The results are presented in the Table, in which mean values and standard deviations are given for the age and body weight of animals at vaginal opening.

The vaginal orifice becomes patent at about the same time in controls and animals exposed to 200 R. Doses of

400 R delay significantly the onset of puberty. Only 8 of 50 rats given 500 R have survived, but have been too weak for the microcoagulation of the hypothalamic region. The occurrence of the opening of the vagina of these animals is also significantly delayed and for a longer period than in rats given 400 R.

The hypothalamic lesion accelerates significantly the onset of puberty which occurs at the lowest body weight of all groups. A dose of 200 R and even more so one of 400 R delays significantly the opening of the vagina as compared with non-irradiated animals with a hypothalamic lesion. Puberty occurs earlier in irradiated and operated rats than in animals treated by irradiation only or in controls.

Daily vaginal smears have been performed after the onset of puberty and the first appearance of pure epithelial cells in the vaginal smear recorded. In a majority of irradiated rats with and without the hypothalamic lesion, the vaginal smears indicate a fairly regular oestrus cycle during the period of observation.

It was found that both types of injury widened the mean interval between the occurrence of opening of the vagina and the first appearance of pure epithelial cells in the smear, except when 500 R dose was applied. The

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² H. BEAUMONT, J. Endocr. 24, 113 (1962).

³ B. T. DONOVAN and J. J. VAN DER WERFF TEN BOSCH, Nature 178, 745 (1956).

⁴ B. T. DONOVAN and J. J. VAN DER WERFF TEN BOSCH, J. Physiol. 147, 78 (1959).

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⁶ R. J. GELLERT and W. F. GANONG, Acta endocr., Copenh. 33, 569 (1960).

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widening of the interval was accompanied by a high degree of individual dispersity. For that reason we have decided to withhold the publication of our data until more information is obtained on a larger number of animals.

The effect of irradiation in infancy on the occurrence of opening of the vagina in rats with and without hypothalamic lesions

	No. of rats	Age (days)	Body weight (g)
Without hypothalamic lesions			
Normal	83	44.0 $\pm$ 4.75	108 $\pm$ 14.1
Irradiated with 200 R	13	43.2 $\pm$ 3.17	109 $\pm$ 10.3
Irradiated with 400 R	48	46.5 $\pm$ 3.96	89 $\pm$ 16.2
Irradiated with 500 R	8	50.6 $\pm$ 6.42	83 $\pm$ 6.9
With hypothalamic lesions			
Non-irradiated	32	31.8 $\pm$ 4.09	55 $\pm$ 18.7*
Irradiated with 200 R	9	35.4 $\pm$ 0.83	84 $\pm$ 11.5
Irradiated with 400 R	25	37.5 $\pm$ 4.00	72 $\pm$ 11.8

* The mean and the standard deviation estimated for 27 animals.

The results presented suggest that lesions in the hypothalamus effected through microcoagulation accelerate the onset of puberty in the total body X-irradiated rats analogous to what they do in normal animals, but that the exposure to X-rays delays the puberty proportionally to the dose whether a microcoagulation has been performed or not. Further work on the effects of irradiation of the head only of animals with and without hypothalamic lesions is in progress.

Résumé. Les résultats obtenus suggèrent que la lésion de l'hypothalamus provoquée par la microcoagulation accélère l'apparition de la puberté des rats totalement irradiés aux rayons X, ce qui est le cas aussi chez des rats normaux. Mais l'apparition de la puberté est retardée proportionnellement aux doses de rayons X appliquées, indépendamment de la microcoagulation.

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La voie du pentose phosphate dans le métabolisme du glucose et son interaction avec la glutathion-réductase dans les noyaux de foie de rat

La présence des enzymes du cycle de l'hexose monophosphate dans les noyaux cellulaires est très controversée¹⁻⁵. Cette incertitude tient à plusieurs causes d'erreur provenant de la méthode d'isolement des noyaux (perte en protéines, contamination par des cellules entières ou par des débris cytoplasmiques). Récemment, nous avons réussi à isoler des noyaux de foie de rat dans un milieu de saccharose tamponné, en utilisant des tensio-actifs pour faire éclater les cellules. Le fait que par cette méthode, les causes d'erreur citées ci-dessus sont éliminées, nous a incité à étudier le métabolisme du glucose par la voie du pentose phosphate en le comparant à celui de la fraction cytoplasmique soluble. Les résultats présentés ici concernent l'activité de la glucose-6-phosphate-déshydrogénase (G-6-PD) (EC 1.1.1.49) et de la 6-phosphogluconate-déshydrogénase (6-PGD) (EC 1.1.1.44) des noyaux de foie de rat, ainsi que l'interaction de ces enzymes avec la glutathion-réductase (GSSG-réductase) (EC 1.6.4.2), interaction qui pourrait être un mécanisme de réoxydation du NADPH dans les noyaux cellulaires.

La méthode de préparation des noyaux sera décrite dans une publication ultérieure⁶. Les extraits solubles et les homogénats de noyaux, préparés dans le *Tris* 0,05 M, $MgCl_2$ 0,5 mM (pH 7,4), n'ont montré que des traces d'activité de G-6-PD, par la mesure directe au spectrophotomètre (méthode décrite par GLOCK et McLEAN⁷), sans doute à cause de l'interférence de la GSSG-réductase⁸. Nous avons donc mesuré les activités de G-6-PD et de 6-PGD ensemble, par la formation de $^{14}CO_2$ à partir du glucose-1- ^{14}C ou du glucose-1- ^{14}C 6-phosphate. Les conditions expérimentales sont décrites dans les légendes des Tableaux. Les protéines sont dosées selon la méthode décrite par FISZER⁹.

Les résultats du Tableau I montrent que les noyaux de foie de rat transforment le glucose-1- ^{14}C en $^{14}CO_2$, par la voie oxydative de l'acide 6-phosphogluconique. Cependant, l'activité est plus forte avec le glucose-1- ^{14}C 6-phosphate (exp. 2). Les extraits solubles de ces noyaux ne contiennent que des traces d'hexokinase, bien que les autres enzymes glycolytiques s'y trouvent en quantités appréciables⁶. Il n'est donc pas surprenant que l'activité nucléaire, en présence d'ATP et de glucose-1- ^{14}C , soit faible. Le Tableau I montre également que le surnageant cytoplasmique transforme facilement le glucose-1- ^{14}C en $^{14}CO_2$, et que l'activité est beaucoup plus forte avec le glucose-1- ^{14}C 6-phosphate. Avec le glucose-1- ^{14}C comme substrat, l'addition de noyaux à la fraction cytoplasmique provoque, dans les conditions de l'expérience, une inhibition de 75% du $^{14}CO_2$ formé. Une telle inhibition n'est pas observée avec le glucose-1- ^{14}C 6-phosphate. En supprimant l'ATP et en augmentant la concentration du glucose-1- ^{14}C 6-phosphate on obtient, d'une part, une augmenta-

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