

phosphorylation by thyroxine, thus allowing greater amounts of calcium and strontium to cross the intestinal wall.

The relative importance of the duodenal active transfer mechanism and the metabolic block in the small intestine in the control of calcium and strontium absorption is not yet understood. However, the fact that the duodenal mechanism is markedly depressed in hyperthyroidism while overall calcium and strontium absorption is increased suggests that the metabolic block in the small intestine exerts the greatest influence on the absorption of these metals.

The presence of the duodenal active transport mechanism and the metabolic block to calcium and strontium transfer from the lumen of the small intestine have not yet been demonstrated in man. However, if they are present in the human intestine it seems likely that calcium,

and strontium, absorption will be increased in hyperthyroidism.

**Résumé.** Chez le rat, l'administration de L-thyroxine pendant les 3 jours précédant un dosage oral de  $^{47}\text{Ca}$  et  $^{85}\text{Sr}$  provoque une augmentation frappante de l'absorption des isotopes. On suggère que cette augmentation est le résultat de l'inhibition du bloc métabolique au passage du calcium et du strontium à travers l'intestin grêle par non assemblage de la phosphorylation oxydative par la thyroxine.

D. M. TAYLOR

*Department of Biophysics, Institute of Cancer Research, Surrey Branch, Belmont (Sutton, Surrey, England), 5 January 1968.*

### On the Innervation of the Prothoracic Glands in *Papilio demoleus* L. (Lepidoptera)

Innervation of the prothoracic glands has been reported in at least 5 insect orders (Apterygota, Orthoptera, Hemiptera, Coleoptera and Lepidoptera). But in many of these cases, the observations of one investigator have been contradicted by those of another, sometimes within the same species. In *Tenebrio molitor*, for example, ARVY and GABE<sup>1</sup> report innervation of the glands, SRIVASTAVA<sup>2</sup> contradicts them. In *Hyalophora cecropia*, WILLIAMS<sup>3</sup> has reported profuse innervation, while HERMAN and GILBERT<sup>4</sup> find it 'much more difficult to get a conclusive histological evidence of the same'. The main reason for these conflicting observations has been the fact that they are based on gross dissections alone and no attempt has been made to stain the nerves histologically or in situ.

We, therefore, attempted to stain the nerves in situ by employing the intra vitam leucomethylene blue nerve staining technique of ZACHARUK (as described by STAY and GELPERIN<sup>5</sup>) in the fifth instar larva of *Papilio demoleus*. The staining reveals that the prothoracic glands

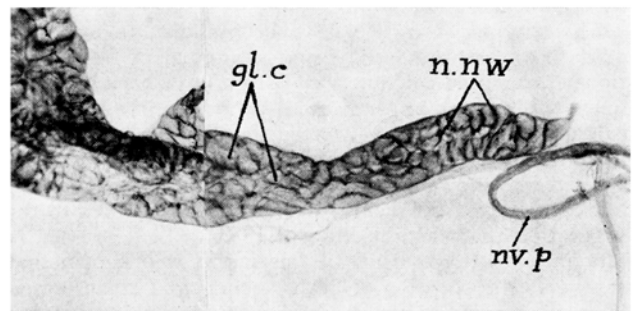
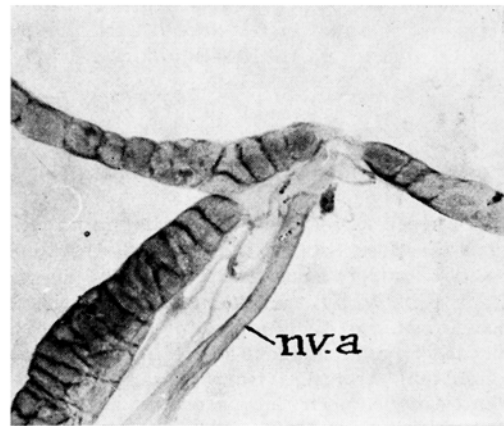


Fig. 2. Photomicrographs of the prothoracic gland stained in leucomethylene blue to show the nerve network. gl.c, gland cells; n.nw, nerve network. Remaining lettering same as in Figure 1.

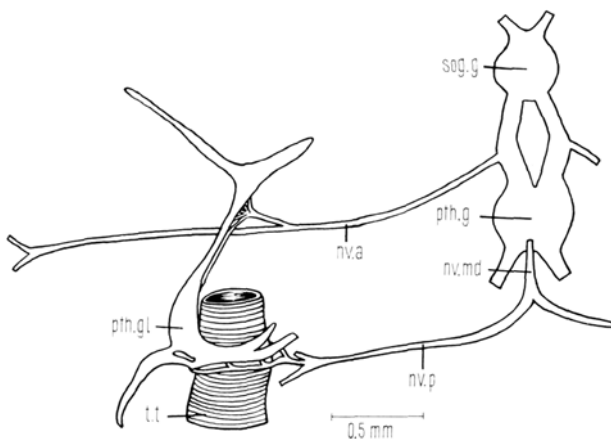


Fig. 1. Camera-lucida diagram showing the innervation of the prothoracic gland in *Papilio demoleus*. nv.a, nv.p, nv.md, anterior, posterior and median nerves respectively; pth.g, prothoracic ganglion; pth. gl, prothoracic gland; sog.g, suboesophageal ganglion; t.t, tracheal trunk.

<sup>1</sup> L. ARVY and M. GABE, C. r. hebd. Séanc. Acad. Sci., Paris 237, 844 (1953b).

<sup>2</sup> U. S. SRIVASTAVA, Q. Jl. microsc. Sci. 100, 51 (1959).

<sup>3</sup> C. M. WILLIAMS, Biol. Bull. 94, 60 (1948).

<sup>4</sup> W. S. HERMAN and L. I. GILBERT, Gen. comp. Endocr. 7, 275 (1966).

<sup>5</sup> B. STAY and A. GELPERIN, J. Insect Physiol. 12, 1217 (1966).

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 insects<sup>6-8</sup> 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 cells<sup>9</sup>.

**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.

K. P. SRIVASTAVA and HARI HAR SINGH

Department of Zoology, Banaras Hindu University, Varanasi-5 (India),  
29 February 1968.

- <sup>6</sup> L. ARVY and M. GABE, Bull. Soc. zool. Fr. 75, 267 (1950).
- <sup>7</sup> B. SCHARRER, Z. Zellforsch. mikrosk. Anat. 64, 301 (1964).
- <sup>8</sup> T. C. NORMAN, Z. Zellforsch. mikrosk. Anat. 67, 461 (1965).
- <sup>9</sup> 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 GRIESEWOOD<sup>1</sup> have claimed that the vagina of rats open precociously after irradiation of the ovarian region, but BEAUMONT<sup>2</sup> has failed to confirm this finding. DONOVAN and VAN DER WERFF TEN BOSCH<sup>3,4</sup> as well as other authors<sup>5-7</sup> 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

- <sup>1</sup> J. MANDEL and E. N. GRIESEWOOD, Proc. Soc. exp. Biol. Med. 32, 295 (1934).
- <sup>2</sup> H. BEAUMONT, J. Endocr. 24, 113 (1962).
- <sup>3</sup> B. T. DONOVAN and J. J. VAN DER WERFF TEN BOSCH, Nature 178, 745 (1956).
- <sup>4</sup> B. T. DONOVAN and J. J. VAN DER WERFF TEN BOSCH, J. Physiol. 147, 78 (1959).
- <sup>5</sup> M. ELWERS and V. CRITCHLOW, Am. J. Physiol. 198, 381 (1960).
- <sup>6</sup> R. J. GELLERT and W. F. GANONG, Acta endocr., Copenh. 33, 569 (1960).
- <sup>7</sup> S. HOROWITZ and J. J. VAN DER WERFF TEN BOSCH, Acta endocr., Copenh. 41, 301 (1962).