Density banding experiments were performed using Renografin gradients[5]. Approximately 2×10^6 cells from biopsy samples or their respective source tumors were loaded onto each 14 ml preformed gradient (10-35% Renografin in Ringer's solution), centrifuged at $13{,}000 \times g$ for 30 min in a swinging bucket rotor (Beckman L5-50, SW 27.1 rotor) at 4 °C. Each gradient was fractionated into 1 ml samples. The refractive index (N_{24}) was determined for each fraction and the density calculated using the formula $\rho = (g/cm^3) = 3.4683 \times N_{24}$ 3.6267. The number and viability of cells recovered in each fraction was determined using a hemocytometer and a phase contrast microscope. The resulting data were presented as percent total and cumulative percent total of cells recovered per density increment.

Results and discussion. Thus far, 24 tumors have been biopsied using this procedure and there has been good agreement between the density profiles of the material from each of the biopsies and their corresponding solid tumors. Representative data are presented in the figure.

There is currently considerable interest in developing suitable biopsy procedures for application as prognostic indicators. It is unclear, however, as to how well biopsy material reflects the source tumor. In particular, it is of interest to know the minimum requirement of biopsy material required for a given size of tumor to accurately reflect both the relative composition and proportion of classes of tumor cells present. In this study, the Tru Cut needle was used because it allowed for the largest and least fragmented tissue samples[9]. The adequacy of a single biopsy sample as being suitably reflective of a 8 mm in diameter tumor is demonstrated by the close correlation of density distributions presented in the figure. It is most probable, however, that larger size tumor masses will require proportionally larger sized biopsy samples. The applicability of this procedure to other tumor systems requires the development of adequate single cell suspension methods. Currently this approach has been applied successfully to an L-P59 sarcoma and a fibrosarcoma of spontaneous origin. Its applicability to carcinomas, however, has not as yet been tested.

While the density of each FSa tumor subpopulation remains relatively constant from tumor to tumor, the relative number of cells comprising each subpopulation will vary in each individual tumor. In addition, normal cell populations present in the tumor can be assayed. By combining density gradient centrifugation with centrifugal elutriation, nontumor cells can be selectively isolated from the tumor and enriched to over 80%[2]. Earlier studies have indicated that relatively radiation resistant FSa cells are collected at densities greater than 1.12 g/cm^3[3,5,6]. With this as a reference density the relative proportion of sensitive vs resistant FSa cells in each tumor can be approximated using the Renografin density gradient system. In conclusion, the good correlation between the density profiles of biopsies and their corresponding disaggregated tumors thus allows the consideration of the biopsy technique together with density centrifugation as a prognostic indicator system for FSa tumor response.

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Electrophysiological evidence of nervous involvement in the control of the prothoracic gland in *Periplaneta americana*[1]

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Summary. The nervous activity of the prothoracic gland nerve in the last larval instar of *Periplaneta americana* was investigated by means of suction electrodes. Nervous activity is low immediately before and after the last larval molt, and increases in the middle of the last intermolt phase. The level of nervous activity is in remarkable coincidence with the course of the ecdysteroid titer in the hemolymph. An involvement of nervous control mechanisms in the regulation of the prothoracic gland function should be taken into account.

It is well known that molting in insects is induced by the prothoracicotropic hormone of the neurosecretory cells of the brain, stimulating the prothoracic gland. However, some physiological indications of nervous influences on prothoracic gland activity[2-5] as well as anatomical evidence of prothoracic gland innervation from the suboesophageal, prothoracic, and mesothoracic ganglia in different species[6-14] have remained mostly disregarded. Recently the prothoracic gland of *Periplaneta americana* was demonstrated by cobalt chloride-iontophoresis to be innervated by 4 neurons of the prothoracic ganglion via a side branch of nerve 4 rlb[15,16], emerging from the 4th segmental nerve of the prothoracic ganglion (fig. 1). It enters the prothoracic gland on each side at the gland's posterior distal end. These findings raise the question of the involvement of nervous control in prothoracic gland function.

Material and methods. Registration of the activity of the prothoracic gland nerve of *Periplaneta americana* in the last larval instars and, in some cases, in penultimate instars just before molt was performed after opening the prothoracic region of the animals dorsally. The prothoracic gland was dissected by removing the oesophagus and the main tracheae. After cutting the prothoracic gland nerve near the prothoracic gland the nerve was drawn into a suction

electrode to its basis on nerve 4 rlb for activity registration (fig. 1). The animals were selected from the rearing population immediately after molt, they were stored at 27 °C and used at different days in the intermolt of the last instar. Though the animals were treated alike and timing was started on the day of molting, the length of the last larval instar was not uniform; this is known in *Periplaneta*[17]. Under the present conditions, the mean length of the last larval instar is 30 days, that of the penultimate instar is 25 days.

Results. The combined results of the measurements of nervous activity during the whole last larval instar are demonstrated in fig. 2. It is evident that nervous activity is very low before and after the penultimate molt. It remains low until the 13th and 14th days of the last instar. It starts to increase with the 15th day after molt; up to the 21st day we could observe a further increase in this nervous activity.

Due to the variability of the length of the molting interval in *Periplaneta* larvae, the standard deviation on the 15th, 16th and 21st days is relatively large. But just this indicates the fact that in the middle of the molting interval the increase of a physiologically active phase of the prothoracic nerve has to be supposed.

These activity changes in different periods during the molting interval are very instructive in comparison with the level of ecdysteroids in the hemolymph[18] (fig. 2). This comparison shows that the nervous activity of the prothoracic gland nerve during the first 21 days of the last intermolt is correlated with the hemolymph titer of ecdysteroids or the ecdysone production of the prothoracic gland. Therefore, an involvement of nervous control in the regulation of the prothoracic gland's functional activity, besides the hormonal control, should be suggested.

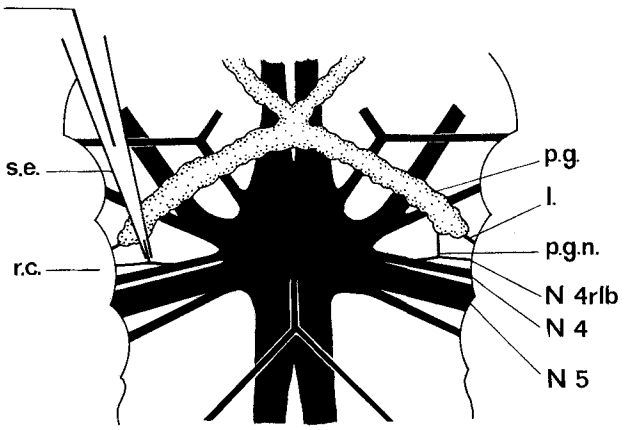

Figure 1. Diagram of the only innervation of the prothoracic gland from the prothoracic glanglion in *Periplaneta americana*. Dorsal view. l., ligament; N 4 rlb, nerve 4 rlb; N 4, nerve 4; N 5, nerve 5; p.g., prothoracic gland; p.g.n., prothoracic gland nerve; r.c., region of coxal muscles and sternal leg muscles; s.e., suction electrode.

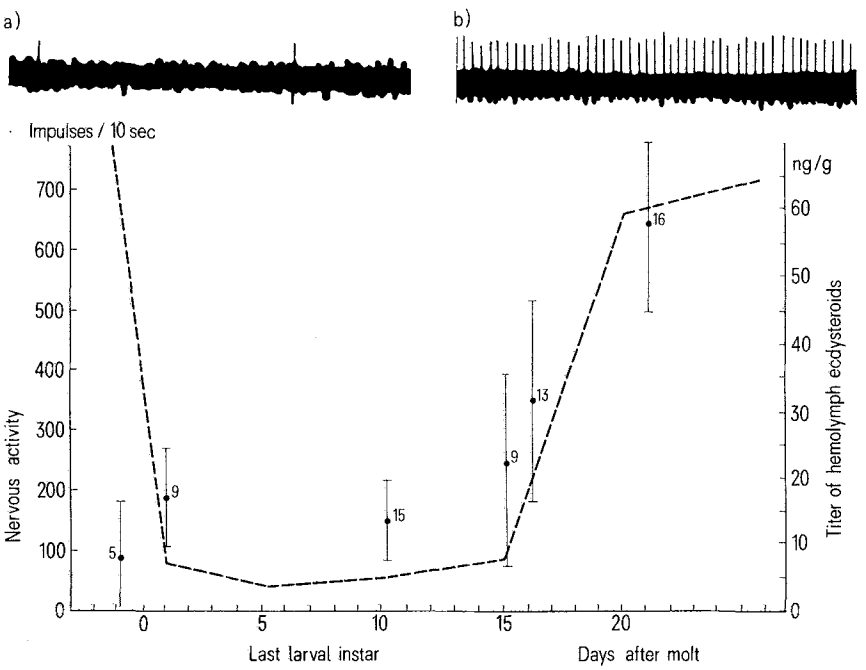

Figure 2. Nervous activity in the prothoracic gland nerve at the end of the penultimate larval instar and during different times in the last larval instar. Each point is a mean of the indicated number of samples with ± SEM, shown as vertical bars. The dashed line shows the hemolymph ecdysteroid titer, given by Eibisch et al.[18], for comparison. The recordings show typical nervous activities in the prothoracic gland nerve at the 9th (a) and 16th (b) day after molt. Calibration 50 µV, 200 msec.

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