blood glucose levels attained in the unprotected animals (figure 1). In fact, the levels of plasma glucose attained in the animals which received SZ but not nicotinamide did not significantly differ, except in the case of the highest SZ dose (80 mg/kg b.wt). However, different amounts of nicotinamide were required to provide full protection in the different experimental groups (70–140 mg/kg in 50 mg/kg SZ given rats; 210 or 280 mg/kg in rats given 60 or 70 mg/kg SZ respectively). When 80 mg/kg SZ were given, even the largest dose of nicotinamide provided partial protection only.

Most likely, the fact that protection changes together with the relative dosages of both nicotinamide and SZ is not a consequence of an interaction between these 2 molecules at the level of cell receptors. Probably, the obtainable protection depends on the extent of the beta cell damage, which, in its turn, depends on the size of SZ dose. Evidence supporting this statement comes from our second experiment (figure 2): younger animals (70 g b.wt) – which are less sensitive to SZ[11] – are protected by nicotinamide much better than older animals (130 g b.wt) given the same dose (80 mg/kg b.wt) of SZ. Incidentally, this finding can help to explain conflicting results previously reported[7, 9, 10].

Finally, we explored whether similar experimental designs could let us time when the pancreatic lesions induced by SZ become irreversible. 0, 70, 140 or 240 mg/kg nicotinamide were given to 130 g rats 75, 45, 15 min before or 45, 75 min after the injection of a medium dose (60 mg/kg b.wt) of SZ (figure 3). Changes of blood glucose were similar and full protection obtained at 140–210 mg/kg b.wt nicotinamide in all cases except in that of the latest nicotinamide administration (75 min after SZ injection). At this interval, the protective effect of nicotinamide is remarkably reduced (but still significant at the highest nicotinamide dosage). If this is the lag that SZ-induced lesions require to become irreversible in most beta cells, we should comment that no-return changes appear much later than the earliest signs of cell lesion detectable at the electron microscope (Z. Gori, personal communication). Also, they seem to be simultaneous with (or shortly follow) NAD depletion[4] and are accompanied by the first significant impairment of insulin secretion[12].

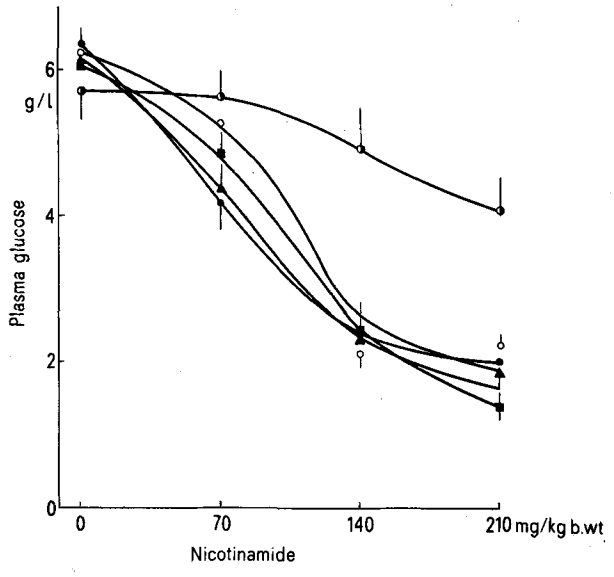

Fig. 3. Plasma glucose concentration in 130 g male Wistar albino rats 1 day after the injection of 60 mg/kg b.wt streptozotocin. Nicotinamide was administered at the dosage indicated on the abscissa respectively 75 min (▲), 45 min (●) or 15 min (■) before or 45 min (○) or 75 min (◑) after the streptozotocin injection. Means of 6 cases are given. Vertical bars represent 1 or 2 × SEM but are omitted when symbols overlap.

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Endocrine organs of different larval instars of Spodoptera (Prodenia) litura Fabricius (Lepidoptera: Noctuidae)

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Summary. A_1 and A_2 cells of the median neurosecretory group are filled with PF stained neurosecretory material between the first 24 to 48 h of the earlier moult in each instar followed by immediate release before the next moult at 72 h, while loaded posterior and lateral neurosecretory cells also appear from the 3rd and 5th instars onwards. Corpora cardiaca, corpora allata and prothoracic glands increase in size in each instar.

Various lepidopterous larvae such as Hyalophora cecropia[1, 2], Bombyx mori Linn[3, 4]. and its race, nistari[5], Ostrinia nubilalis[6] Heliothis zea[7] and 13 genera of 8 families[8] have been studied in respect to the morphology and histology of the retrocerebral complex and prothoracic glands. However, this paper presents the changes in the form of the endocrine organs in various larval instars of Spodoptera litura ultimately leading to moulting in them.

Material and methods. The larvae of Spodoptera litura were reared on soybean leaves from the eggs laid in the

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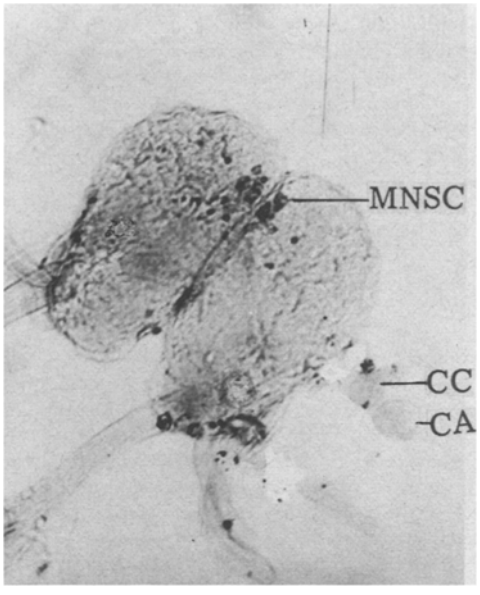

Fig. 1. Brain of larva of Spodoptera litura showing median neurosecretory cells (MNSC) on either side of median furrow dividing the brain into 2 hemispheres: corpora allata (CA) and corpora cardiaca (CC) are also seen. ×36.

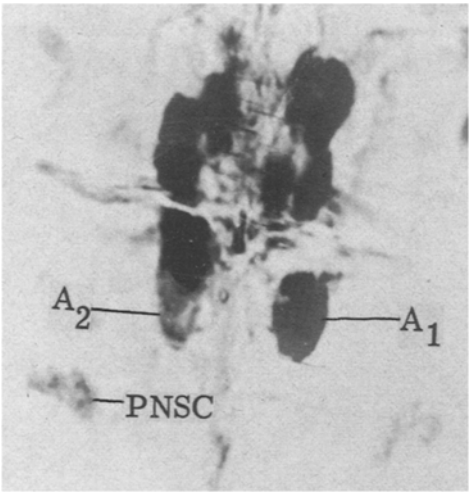

Fig. 2. Heavily loaded A_1 and A_2 median neurosecretory cells (MNSC) in the 5th instar larva of S. litura at 24 h after previous moult. ×250.

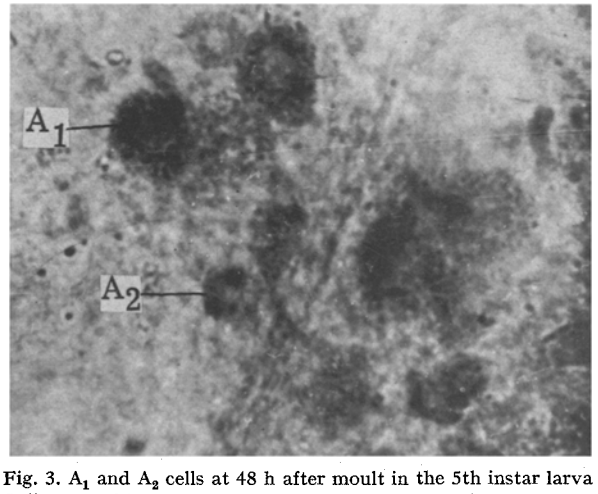

Fig. 3. A_1 and A_2 cells at 48 h after moult in the 5th instar larva of S. litura. ×250.

laboratory by the adult moths. Each larval instar was dissected on each day to remove the brain along with corpora cardiaca, corpora allata and the prothoracic glands for staining, respectively, with paraldehyde fuchsin (PF)[9] and eosine before mounting in Canada balsam.

Results and discussion. Endocrine organs. The endocrine organs of the larva of S. litura include the neurosecretory cells of the brain, corpora cardiaca, corpora allata and the prothoracic glands. The neurosecretory cells are located in 3 groups, median, lateral and posterior in either hemisphere of the brain. The median groups of cells are closely apposed to each other in the pars intercerebralis region near the median furrow dividing the brain into 2 hemispheres (figure 1). The lateral groups of the neurosecretory cells are situated on the lateral edges of the corpora pedunculata, while the posterior groups (PNSC) are present in the posterio-dorsal region of the brain (figure 2). The corpora cardiaca and corpora allata lie independently on either side of the aorta and oesophagus in the head, while the prothoracic glands are located in close vicinity of the prothoracic spiracles where they are richly supplied with the tracheae.

Neurosecretory cells: The median cells are not visible in the 1st and 2nd instar larvae, even with the PF stain. They become prominent only from the 3rd instar onwards. The lateral and posterior cells are smaller than the median cells and are visible, respectively, from the 3rd and 5th instars of the larva of S. litura when they take up faint PF stain, suggesting some secretory activity as observed in Adelphocoris lineolatus[10]. Each median, lateral and posterior group consists of 12, 3, and 3 neurosecretory cells, thus totalling to 36 as against 4 in Bombyx mori and 40 in Ostrinia nubilalis. The median neurosecretory cells of the larvae of S. litura appear morphologically to be of 2 subtypes, A_1 and A_2. A_1 cells are round and become greatly swollen in form within about 24 h of the previous moult, due probably to the heavy filling in of the neurosecretory material, and assume oval shape in about 48 h (figure 2) when the presence of neurosecretory material in the axons of the cells signified the flow of the same. Loaded posterior and lateral neurosecretory cells are aslo seen at this time. The larval moult always took place after 24 h of the flow of neurosecretory material in the axons. This kind of cycle of filling and release of the neurosecretory material of the median cells was seen in each larval instar before moult. A_2 cells filled with neurosecretory material appear lightly stained with PF (figure 3) stain and are always smaller in size, more or less irregular in shape and become prominent during or after the emptying phase of the A_1 cells in the 4th, 5th and 6th instars of the larva of S. litura. Their release was not seen. During the 6th instar, the filling in of the A_1 cells is delayed by about 24 h and therefore the flow of the neurosecretory material begins at about 72 h or later, resulting in delay in the final moult before pupation.

Corpora cardiaca: They are paired oblong organs which increase in size from 0.039 mm in length and 0.036 mm in width in the 3rd instar to 0.068 mm in length and 0.052 mm in width in the 6th instar. No PF stain is taken by them. The nervi corporis cardiaci externi clearly enter the outer walls of corpora cardiaca.

Corpora allata: They are paired round structures increasing in size from 0.039 mm in length and 0.036 mm in width in the 3rd instar larva to 0.068 mm in length and 0.063 mm in width in the 6th instar larva of S. litura. They also do not take up any PF stain.

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Prothoracic glands: The prothoracic glands of S. litura are semi-transparent white and compact, as reported by Lee[8], with about 51 to 62 bead-like cells in each gland which has a small flattened body made up of a single row of 5 to 16 cells in the middle and double or more rows of cells towards the extremities which bifurcate anteriorly and posteriorly. 2 small-sized branches of anterior extremity bear 2 to 3 tiers of bead-cells which touch the posterior margin of the head. The branches of the posterior extremity are larger in size; the lateral one reaching the spiracle and the inner one terminating near the prothoracic ganglia. The prothoracic glands also increase in length in each larval instar from 0.702 mm in the 3rd instar to 1.503 mm in the 6th instar larva of S. litura, but they do not reveal any changes in their from either before or after a moult.

Cellular and subcellular localization of ^{3}H-diethylstilbestrol in the pituitary, oviduct and uterus of the female rat[1]

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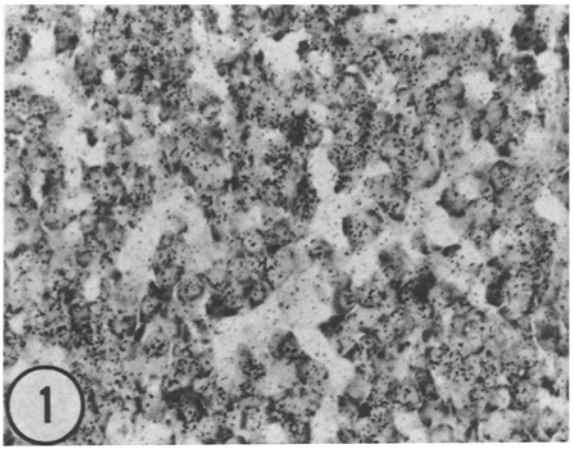
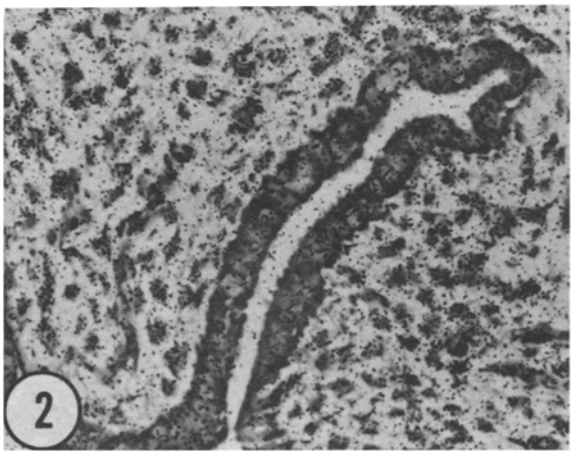
Summary. Female rats were injected with ^{3}H-diethylstilbestrol. The pituitaries, oviducts, and uteri were removed and processed for autoradiography. The nuclear uptake and retention in these tissues appeared similar to that seen after the injection of ^{3}H-estradiol except in the pars nervosa of the pituitary gland.

Numerous studies using biochemical techniques have demonstrated nuclear uptake and retention of ^{3}H-estradiol-17β by the pituitary gland[2,3], the oviduct and the uterus[4]. Autoradiographic techniques have revealed that the nuclear uptake and retention occurs in well over 60% of the cells of the pars distalis, a smaller number in the pars nervosa, of the pituitary gland[5], and in the connective tissue cells, muscle cells and epithelial cells of the oviduct and uterus[6]. Since the commonly used synthetic estrogen, diethylstilbestrol, has been shown to bind to the estrogen receptor in all of these tissues[3,7], it has been generally assumed that the same cells which take up estradiol-17β also take up diethylstilbestrol. The following study was conducted to determine whether the nuclear uptake and retention of this commonly used synthetic estrogen is the same as that of its natural counterpart.





Figs. 1 and 2. Autoradiograms of the pituitary (figure 1), pars distalis, and uterus (figure 2), from 25-day- old female rats, killed 2 h after the injection of ^{3}H-diethylstilbestrol. Exposure time 8 weeks. 4 µm. Stained with methylgreen-pyronin. ×400.

Methods. 25-day- old female rats (n = 5/group) were injected i.v. with 1.0 µg of ^{3}H-diethylstilbestrol (62.4 Ci/mM)/100 g b.wt only or in combination with a 15-fold greater dose of unlabeled estradiol-17β or unlabeled testosterone. The animals were killed 2 h after the injection, and the tissues processed for autoradiography. The autoradiographic procedure is described in detail elsewhere[8]. Briefly, the pituitaries, oviducts and uteri were excised, placed on brass tissue holders and simultaneously frozen and mounted by immersion in liquefied propane at about −180 °C. 4 micrometer sections were cut at −30 °C knife temperature in a Wide-Range Cyrostat (Harris Manufacturing Co., Cambridge, Massachusetts, USA). The frozen sections were then freeze-dried with a cyropump for 24 h, and then dry mounted by pressure with a teflon support on emulsion coated slides, previously stored over drierite. The slides were exposed at −15 °C for 8 weeks, then developed for 45 sec at 18 °C in Kodak D-19 developer, rinsed, and fixed for 5 min in Kodak fixer, and finally rinsed and stained with methylgreen-pyronin.

Results. Nuclear concentration of radioactivity after the injection of ^{3}H-diethylstilbestrol was found in well over 70% of the cells in the pars distalis, (figure 1) but not in either the pars intermedia or pars nervosa of the pituitary gland. Nuclear concentration of radioactivity was found in the connective tissue cells, muscle cells, and epithelial

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