An Autoradiographic Study of Neurosecretory Cell Activity of Allatectomized Females of the Grasshopper, *Melanoplus sanguinipes* (Fab.)

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Neurosecretory cell activity was studied autoradiographically in the allatectomized females of *Melanoplus sanguinipes* on every alternate day and in the normal control females on every fourth day for a period of 34 days. Experimental females exhibited five activity cycles against the two recorded by the control females. There was a comparatively high uptake of [^{3}H]cystine by the neurosecretory cells of the experimental females on a particular day. During the first 12 days there was no accumulation of stainable colloid in their system. By the 24th day there was a slow and gradual accumulation of the colloids in the cells and their tracts so that on 34th day the system was well loaded with these colloids. In control females there was good accumulation of the stainable material during the first 12 days and then it declined so that 20 day old females had very little material in their system. This was followed by a massive accumulation of the stained colloids by 32nd day. The uptake of [^{3}H]uridine was very high in the nerve-regenerate of the experimental females, and there were six cycles of secretory activity during the period of experimentation. The corpus allatum of control females, like their neurosecretory cells, exhibited only two activity cycles.

Since the activity of the neurosecretory cells parallels the RNA synthesis in the nerve-regenerate, it is postulated that the neurosecretory cells produce factors for nerve-regeneration. On the basis of high uptake of isotopes by the neurosecretory cells of those allatectomized females in which the oocytes were mature it is suggested that the hormone(s) produced by the neurosecretory cells are perhaps responsible for oocyte maturation in these allatectomized females.

Introduction

It has been repeatedly demonstrated that egg laying in insects is controlled by the neurosecretory cells of the pars intercerebralis and the corpus allatum; the former apparently controls the synthesis of vitellogenic protein in the fat body and the latter facilitates its incorporation into the developing oocytes (Engelmann [1]; Doane [2]). Nevertheless, there are quite a few species where allatectomy does not seem to effect egg maturation and oviposition. As for example, in the phasmid *Carausius morosus* (Pflugfelder [3]) and in the lepidopterans *Bombyx mori* (Bounhiol [4]), *Phryganidia californica* (Bodenstein [5]) and *Hyalophora cecropia* (Williams [6]) allatectomy performed even during the larval period had little effect on the fecundity of these females. On the other hand, in the flesh fly *Calliphora erythrocephala* (Thomsen [7]) and in the bug *Rhodnius prolixus* (Davey [8]) the operation reduced the total number of eggs produced, but did not completely prevented oocyte maturation. Thus the relative importance of these endocrine glands varies from species to species.

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In the grasshopper *Melanoplus sanguinipes* allatectomy inhibits egg production in two-third females by the resorption of the developing oocytes and the remaining one-third females produce eggs at a rate of about 50 to 60 per cent of the normal females (Dogra and Even [9]). In the operated females there was an excessive growth of the nervous and the connective tissue either from the cut end of the nervi corporis allati or *de novo* from the corpora cardiaca and it was suggested that the regenerative type of growth of the nervous and connective tissue perhaps stimulates the cerebral neurosecretory cells and that the cerebral hormone(s) may be responsible for the maturation and deposition of eggs (Dogra and Ewen [9]).

Endocrine activity in the normal virgin females of *M. sanguinipes* through extended period of time has been reported earlier (Gillott and Dogra [10]), and the present investigation was aimed to study autoradiographically the changes in the synthetic activity of the cerebral neurosecretory cells of the allatectomized females in relation to nerve regeneration and oocyte maturation.

Material and Methods

Adult females were collected within 1 hour of emergence from the stock population maintained

in the laboratory according to the method of Pickford [11]. More than one hundred females were allatectomized within 24 hours of their emergence following the technique of Strong [12] and previously used by us (Dogra and Ewen [9]). The wound was sealed with paraffin wax after putting few crystals of the antibiotics streptomycin sulphate and penicillin G. Survival rate was very good and more than 90% females sustained the operation. All the operated animals were allowed to recover from the shock of the operation for 48 hours and thus in the beginning of the experiment all the animals were 72 hours old. The operated females were kept without males at 30 ± 1 °C with 12 L; 12 D photoperiod. Food was supplied *ad lib.* and jars of moistened sand were also supplied for egg laying. Normal virgin females subjected to similar conditions served as control.

It was apparently difficult in the experimental population to separate egg laying from the non-egg laying females particularly during the maturation of the first batch of eggs. Attempts to separate them on the basis of their body weight was not much profitable because even within the non-egg laying population some individuals were heavier than the others, perhaps due to a relatively higher percentage of fat in their body. Therefore, the only criteria which could be followed was to fix them at regular intervals and record the regenerative growth and the state of the gonadial tissue individually. This makes it difficult to analyse the results statistically.

In each case six females were sampled, three were used for neurosecretory cell activity and three for corpus allatum activity or activity in the regenerative type of growth from the NCA or de novo from the corpus cardiacum. The experimental females were sampled on every alternate day and the control on every fourth day, for a period of 34 days. The dose and time interval for the isotope was the same as has been used in previous studies on this grasshopper (Gillot *et al.* [13]; Dogra and Gillott [14]; Gillott and Dogra [10]). For neurosecretory cell activity the animals were injected with 2.5 μl of L-[^{3}H]cystine (sp. act. 630 mCi/mmol, conc. 1.0 mCi/ml in 0.01 N HCl; Schwarz BioResearch Inc., New York); and for RNA synthesis in the corpus allatum and/or in regenerative type of growth 2.5 μl of [^{3}H]uridine (sp. act. 6700 mCi/mmol; conc. 1.0 mCi/ml in distilled water; Amersham-Searle Corp., Toronto) was injected. The animals were sacrificed after 1 hour and their heads were fixed overnight in buffered formalin. After paraffin embedding and serial sectioning at 4 μm they were stained with performic acid/Victoria blue without counterstain (Dogra and Tandan [15]) and then air-dried. Autoradiographs were prepared in the manner described earlier (Dogra and Gillott [14]) by using Kodak AR 10 stripping film. After exposure for six weeks the autoradiographs were developed, fixed and were then examined under oil immersion lens. Grain counts were made from 10 A-type neurosecretory cells from each specimen and incorporation of [^{3}H]uridine by the corpus allatum and the regenerative tissue was measured as the number of grains in an area of known size projected through a drawing tube. The grain counts obtained in each case after correction of background activity were expressed per unit area (100 μm^2). Lateron they were examined for the presence of NSM in the system.

Results

Neurosecretory cells

The rate of incorporation of [^{3}H]cystine in the neurosecretory cells of the control and experimental females is shown in Fig. 1. In 4 day old control females the rate of incorporation was high which gradulally declined to a low level by the 16th day. A rapid increase in the rate of incorporation of isotopes occurred thereafter and 20 day old females

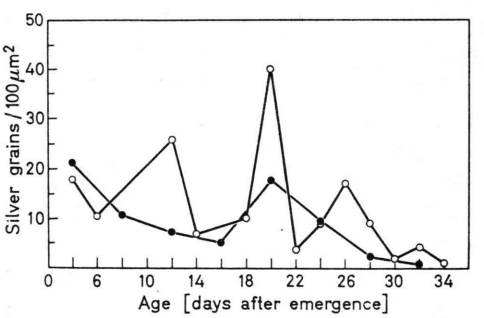

Fig. 1. Uptake of L-[^{3}H]cystine by A-type neurosecretory cells of the pars intercerebralis of the allatectomized and control females of *M. sanguinipes*. —●—, normal; —○—, allatectomized.

exhibited a second cycle peak. This was followed by a gradual decline in activity so that by 32nd day there was negligible uptake of [^{3}H]cystine by the neurosecretory cells. In the neurosecretory cells of the experimental females the over all grain count was much higher and there were many more peaks of activity. Four day old females showed a rate of incorporation which declined to a low level by the 6th day. The rate was increased to a relatively high level by 12th day and then declined so that 16th day old females had a low grain count. This was

followed by a rapid increase in activity and 20 day old females showed a very high grain count which was the highest both for the experimental as well as the control females during the course of experiment. A rapid decline followed and 22 day old females incorporated very little isotope. Again there was an increase in activity and 26 day old females exhibited a high activity which declined by the 30th day. After a small hike on 32nd day the grain count went down to a very low level so that 34 day old females incorporated negligible amounts of [^{3}H]cystine.

The stainable material in the neurosecretory cells of the control and experimental females was fairly uniform on a particular day. In 4 day old control females there was very small amounts of colloids in the neurosecretory cells but it was gradually accumulated so that in 12 day old females there was fairly good amounts of material visible in the system. This was followed by a decrease in the intensity of colloids for next one week, and then gradually accumulated again so that in 32 day old females there was massive material visible in the neurosecretory cells, their tracts and the corpora cardiaca. There was no such correlation in the stainable material in the neurosecretory system of the allatectomized females in which during the first 12 days there was no material in the neurosecretory cells and very little was seen in the corpora cardiaca. Subsequently, a slow and gradual accumulation of colloids occurred and 24 day old females exhibited fairly good amount of stainable colloids in the neurosecretory cells, their tracts and corpora cardiaca. By 34 days the system of these females was loaded with NSM.

Corpus allatum and nerve-regenerate

The corpus allatum of the control females, like their neurosecretory cells, showed two peaks of secretory cycles (Fig. 2). In 4 day old females the grain count was very high which gradually declined to a very low level by the 16th day. An increase in the rate of incorporation of [^{3}H]uridine occurred and in 24 day old females the grain count was considerably high. This was followed by a decline in activity so that in 32 day old females the uptake of isotopes was reduced to a very low level. In the experimental females there was very high uptake of [^{3}H]uridine throughout the period of experiment and there were six recognisable activity cycles.

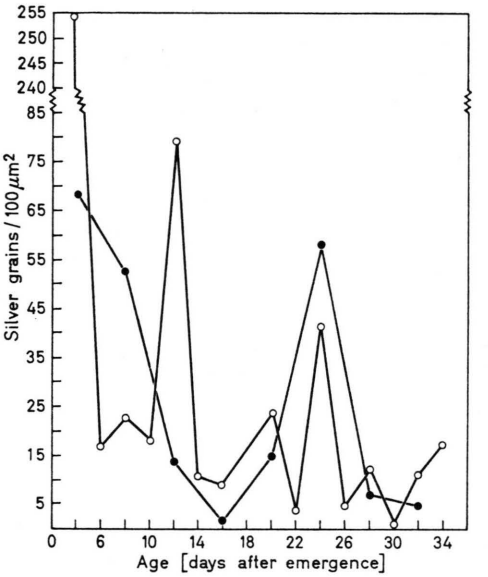

Fig. 2. Uptake of [^{3}H]uridine by the nerve-regenerate of the allatectomized and the corpus allatum of the control females of *M. sanguinipes*. —●—, normal; —○—, allatectomized.

In 4 day old allatectomized females there was an enormously high grain count in the nerve-regenerate. Such a high grain count was neither observed in these females on any other day nor in the corpus alatum and nervi corporis allati of the control females. The activity declined by the 10th day but a sudden increase in the rate occurred and 12 day old females exhibited another peak of activity which declined to a sufficiently low level by the 16th day. This was followed by a rise and 20 day old females had a moderately high rate of incorporation. Twenty-two day old females incorporated very small amount of [^{3}H]uridine, but then suddenly the rate of uptake increased on 24th day. On 28th day there was a short peak and thereafter a decline on 30th day when there was negligible uptake of the isotope. The activity finally raised to a high level on 34th day.

The details of the process of nerve regeneration in the allatectomized females was reported earlier (Dogra and Ewen [9]). There are two types of growth — a more common slender nerve growth and a globular growth of the connective tissue and the nerve fibres. The regeneration of the tissues is fairly bilaterally symmetrical. The growth starts within 4 days after allatectomy and during the next one week there was a rapid growth of the tissues from the cut end of the nervi corporis allati I or *de dovo*

from the corpora cardiaca when the nervi corporis allati was completely removed during allatectomy. The growth continues and by the third week there is a fairly long and thick nerve-regenerate. In case of globular growth it forms a large globular mass adjoining to the corpora cardiaca, the position and cellular organisation of this growth is different than that of the normal corpus allatum. As stated earlier the slender nerve-regenerate acquires the shape of nervi corporis allati similar to that of the normal control females and likewise divides into two branches (Dogra and Ewen [9]).

In the control females the first batch of eggs was deposited on day 13.2 (range 7 – 17 days) and subsequent eggs at a rate of 1.4 pods/female/week. On the other hand, in the experimental animals two females of each of 20 and 34 days from [^{3}H]cystine injected batch and one each from 22, 26, 28 and in 2 females of 34 days old [^{3}H]uridine injected batch had mature eggs in their oviducts. Of these in [^{3}H]cystine injected females the grain count was 37.54 and 42.58 grains/unit area in 20 day old females and 0.66 and 1.62 grains/unit area in 34 day old females. In case of [^{3}H]uridine treated animals 22 and 26 days old females had a grain count of 1.25 and 4.76 grains/unit area in the nerve regenerate; while in the two 34 day old females the grain count in the nerve-regenerate was 52.90 and 14.46 grains/unit area respectively. Excepting 22 day old cystine injected females where there was globular growth, other females showing mature oocytes had a slender nerve-regenerate.

Discussion

A comparison of [^{3}H]cystine uptake by the neurosecretory cells of the experimental and control females suggest that there are five activity cycles in the neurosecretory cells of the experimental females against the two in the normal control females, during the period of experimentation. The over all grain count in the operated females is by far the more as compared to the control animals showing thereby that the neurosecretory cells of the experimental females are more intimately involved in physiological activities than their counterpart in the control females. An examination of the allatectomized females suggests that there could be two main requirements at this stage – one for regeneration of the nervi corporis allati damaged during allatectomy and the other is the demand for the production of metabolites for oocyte maturation.

The uptake of [^{3}H]uridine by the nerve-regenerate is very much more than that by the corpus allatum of the control females. While there are only two activity cycles in the corpus allatum, the allatectomized females exhibit six cycles in the nerve-regenerate during the same period. The cyclic uptake of [^{3}H]uridine by the corpus allatum is related to the oocyte maturation (Gillott and Dogra [10]); on the other hand, the uptake of [^{3}H]uridine by the experimental females is undoubtedly concerned with the regeneration of the nervi corporis allati damaged during allatectomy.

Furthermore, in normal *M. sanguinipes* females there is a parallelism between the activity cycles of the neurosecretory cells and the corpus allatum, because both the endocrine glands exhibit only two activity cycles almost at the same time during the course of the experiment. More or less a similar relationship exists between the activity cycles of the neurosecretory cells and the RNA synthesis in the nerve-regenerate of the allatectomized females (compare Figs 1 and 2). For the normal females it was stated that the activation hormone from the neurosecretory cells controls the production of gonadotrophic hormone by the corpus allatum, as well as the production of metabolites required for oocyte maturation (Gillott and Dogra [10]). On the basis of high activity in the neurosecretory cells of the allatectomized females and a parallelism between the neurosecretory cell activity and RNA synthesis in the nerve-regenerate it could be postulated here that the neurosecretory cell activity is related to the regeneration of the damaged nervi corporis allati as well as for the production of metabolites in the fat body. The fat body of these allatectomized females are always well developed.

It has been reported many times that wound healing and regeneration of lost parts of the body is controlled by the endocrine glands (Doane [2]). Almost nothing is known about the role of endocrine glands in the regeneration of internal organs including the nervous system, although it is definitely known that nervous system of insects has a great capacity of regeneration (Edwards [16]). Like nervous system some of the endocrine glands also regenerate; Stumm-Zollinger [17] and more recently De Wilde and De Boer [18] reported the regeneration of the nervi

corporis cardiaci in *Platysamia cecropia* and *Leptinotarsa decemlineata*. In the present study which is in continuation to our previous report (Dogra and Even [9]) and perhaps a singular attempt to quantitatively study the endocrine activity in relation to nerve-regeneration, there exists a definite relationship in the regeneration of the nervi corporis allati and incorporation of [^{3}H]cystine by the cerebral neurosecretory cells. After about 3 weeks when the nerve-regeneration slows down the uptake of the isotope by the neurosecretory cells declines.

There is yet another point which needs elaboration and that is the maturation of eggs by the allatectomized females. In normal *M. sanguinipes* females there is no parallelism in the activity of the endocrine tissues and oocyte maturation, except in the production of the first batch of eggs. Because during the 4 week period these females laid 5 batches of eggs but their endocrine tissues exhibited only two activity cycles. The first cycle of activity is undoubtedly related to the maturation of the first batch of eggs, the second cycle with the hormonal level in the blood (Gillott and Dogra [10]). During the maturation of the first batch of eggs the medial group of neurosecretory cells of the control females have a maximum grain count of 20.99 grains/unit area. As against this the neurosecretory cells of the allatectomized females showing first batch of mature eggs has a grain count of 37.54 and 42.58 grains/unit area which is the level neither attained by these females at any other stage nor by the normal females. Furthermore, the high grain count in these experimental females is at day 20 post-operative when most of the regeneration is completed. This may mean to interpret that the high activity exhibited by the neurosecretory cells of these females at this time is perhaps concerned with the maturation of first batch of oocytes. The other two females showing mature eggs at day 34 post-operative has a very low grain count of 0.66 and 1.62 grains/unit area. These eggs could well be second or subsequent cycle eggs and the high titre of hormone produced during the maturation of the first batch of eggs is responsible for their maturation somewhat similar to that reported for the normal females (Gillott and Dogra [10]).

It is pointed out here that the present results do not clearly show whether the various peaks obtained for the incorporation of the isotopes are the falling peaks or the rising peaks of the curves, but the mere fact that there exists a relationship between the uptake of the isotopes, the nerve-regenerate and the oocyte maturation suggests that in the absence of corpus allatum the cerebral neurosecretory cells produce factors for nerve-regeneration and oocyte maturation.

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