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DIFFERENTIATION OF THE FAT BODY CELLS IN THE PRECOCIOUS ADULTS OF *SCHISTOCERCA GREGARIA:* REGULATION OF POLYPLOIDY BY JUVENILE HORMONE

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Precocious adults from 2nd and 3rd instar larvae of the desert locust Schistocerca gregaria *were used to assess the competence of their fat body to synthesize DNA in response to a juvenile hormone analog (JHA), hydroprene. Autoradiographic studies show that JHA stimulates DNA synthesis since a significant proportion of the fat body nuclei are labelled after treatment with 100 or 200 µg of JHA. Maximum DNA synthesis occurs 24 h after treatment with 100 µg of JHA. The nuclear ploidy classes of the precocious adults from 3rd larvae are similar to those of 1-d-old normal adults, but treatment of these precocious adults with 100 µg of JHA doubles the DNA content resulting in enhanced ploidy classes which resemble those of 10-d-old normal females. In the precocious adults that emerged from 2nd instar larvae the ploidy classes are higher than those of 1-d-old normal adults, and treatment of these precocious adults with JHA results in a further increase in the DNA content of the fat body nuclei leading to the formation of high percentages of 16C and 32C nuclei. The results of these studies suggest that any model on the mode of action of JH should recognize this phenomenon of JH-induced polyploidization in the fat body nuclei.*

Studies conducted in our laboratory have established that in locusts differentiation of the fat body during adult maturation involves DNA synthesis, and that it is expressed in endocycles (Nair et al., 1981a) Since we could induce *Locusta migratoria* this process of polyploidization is regulated by juvenile hormone (JH) (Nair et al., 1981a). Since we could induce precocious metamorphosis of *Schistocerca gregaria* larvae by exposure to precocene II (Unnithan et al. 1980) it was of interest to determine whether the fat body cells of these precocious adults whose corpora allata have been shown to be degenerate, have attained a level of nuclear ploidy similar to those of 1-d-old normal adults, and whether JH could stimulate the fat body cell to synthesize DNA resulting in enhanced nuclear ploidy classes seen in normal mature adults (Kooman & Nair, 1982). A brief account of some aspects of this work was reported earlier (Nair et al., 1981b).

MATERIALS AND METHODS

^{3}H-thymidine incorporation into fat body nuclear DNA

Precocious adults of *S. gregaria* were obtained by treating 2nd and 3rd instar larvae, less than 6-8 hours old, with precocene II by the method described by Unnithan et al., (1980). The insects were maintained at 35°C during the 16 hr photophase and at 31°C during the 8 hr scotophase. Only the female precocious adults (Fig. 1) were used in the present study. To determine the effects of various doses of a juvenile hormone analogue (JHA), hydroprene (ZR-512, Zoecon Corporation, Palo Alto, Calif.), on DNA synthesis in the fat body nuclei, 10-15-day-old precocious adults that emerged from precocene-treated 3rd instar larvae (Fig. 1) were treated topically on the abdomen with 25, 50, 100 and 200 µg of the JHA in acetone solution. 24 hours after treatment, 10 µCi of methyl^{3}H-thymidine (5 Ci / mmole, Amersham Corporation) was injected into each insect through the arthroidal membrane at the base of the 3rd pair of legs. Four hours after the injection of the radioisotope, squashes of the abdominal fat body were fixed in alcohol-acetic acid (3:1 vol.), Feulgen-stained and processed for autoradiography as described by Kooman & Nair (1982).

Since a dose of 100 µg of JHA induced DNA synthesis in a significant proportion of the fat body nuclei, this dose was chosen for the time-course study to determine the temporal increments in DNA synthesis as a result of JHA treatment. 10-15-day-old precocious adults from 3rd instars were treated topically on the abdomen with 100 µg of JHA in acetone solution. Four, 8, 24, 48 and 72 hours after treat-

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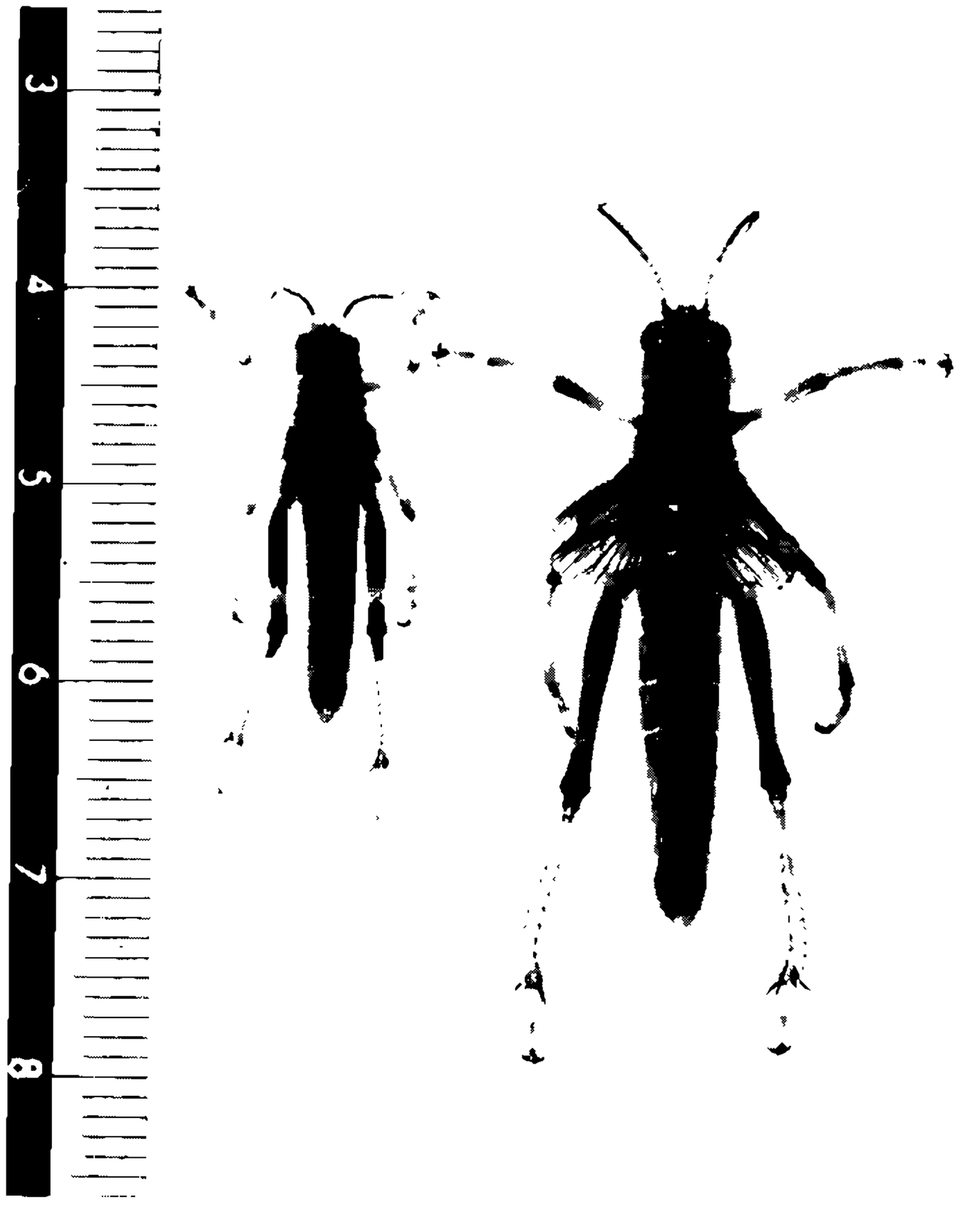

Fig. 1: Precocious adults from precocene II-treated 2nd instar (left) and 3rd instar (right) larvae. Scale: cm.

ment, 10 μCi of methyl^{3}H-thymidine was injected into each insect as described earlier. Four hours after injection, fat body squashes were processed for autoradiography. In both the above experiments 5 insects were used in each treatment group, and a minimum of 500-9000 nuclei from each group were examined for labelling.

DNA content

10-15-d-old precocious adults that emerged from precocene-treated 2nd and 3rd instar larvae (Fig. 1) were used to study the influence of JHA on the fat body nuclear ploidy classes. Acetone solutions containing doses of 100 or 200 μg of JHA were applied topically on the abdomen. The control insects were treated with 10 μl of the solvent only. 48 hours after treatment, fat body squashes were fixed in alcohol-acetic acid (3:1 vol.) for 1 hour and processed for Feulgen-staining (For details, see Kooman & Nair, 1982). In addition,for purposes of comparison, fat body squashes from 3-day-old 2nd and 3rd instar larvae, 1-day- and 10-day-old normal female adults were fixed and Feulgen-stained. Extinction measurements of these nuclei were made with the Scanning Microscope Photometer (Carl Zeiss, W. Germany) at 570_{nm} at 1 μm intervals. A total of 4 insects were used in each group and 100 to 300 nuclei from each group were measured for $E_{s\,570\,nm}$ values. The diameter of the scanning apperture was 1.4 μm. The references standards were obtained by measuring Feulgen-stained early spermatid nuclei (1C) and neuronal nuclei (2C) from testis and brain squashes respectively.

RESULTS

The results of the JHA dose-response study (Table I) shows that even the lowest dose used (25 μg) stimulated DNA synthesis, but the maximum responses are seen in the groups that were treated with 200 μg of JHA. The data on the time-course study (Table II) reveal that a significant percentage of nuclei are labelled 24 hr after JHA treatment ($p<0.05$), and thereafter it declined to lower levels when ^{3}H-thymidine was injected 48-72 hr after JHA treatment.

TABLE I

The effects of various doses of hydroprene on DNA synthesis in the fat body nuclei of *Schistocerca greraria*

Dose in μg	No. of Nuclei	% Labelled Nuclei ($\bar{X} \pm$ S.E.)
25	9037	7.7 ± 2.1 a
50	5949	15.7 ± 3.4 ab
100	5803	27.5 ± 5.2 b
200	4730	40.3 ± 4.9 c

10-15-day-old *S. gregaria* precocious adults from 3rd instar larvae were treated topically on the abdomen with the above doses of hydroprene in acetone solution. 24 h after JHA treatment 10 μCi of ^{3}H-thymidine was injected into each insect. 4 h later the fat body squashes were processed for autoradiography. 5 insects were used for each treatment. The data were analysed for significance using ANOVA statistics and the Duncan's Multiple Range test. Means followed by the same alphabet are not significantly different from each other at 95% level.

TABLE II

Time course study on DNA synthesis in the fat body nuclei of precocious adults of *Schistocerca gregaria* after treatment with 100 μg of hydroprene

Time (Hr)	No. of Nuclei	Labelled Nuclei ($\bar{X} \pm$ S.E.)
4	654	1.5 ± 0.5 a
8	722	6.7 ± 1.8 a
24	908	25.7 ± 4.8 b
48	945	11.9 ± 3.8 c
72	502	14.6 ± 4.6 c

10-15-day-old *S. gregaria* precocious adults from 3rd instar larvae were treated topically on the abdomen with 100 μg of hydroprene in acetone solution. Four h after the injection of ^{3}H-thymidine the fat body suashes were processed for autoradiography. 5 insects were used for each treatment and the data were analysed for significance by ANOVA statistics and Duncan's Multiple Range test. The means followed by the same alphabet are not significantly different from each other at 95% level.

TABLE III

The average DNA content/fat body nucleus expressed as total extinction in the various groups of *Schistocerca gregaria*

Groups	Treatment	Total Extinction ($\bar{X} \pm$ S.D.)	
1. 2nd instar larvae	None	74.3 ± 9.8	
2. 3rd instar larvae	None	73.9 ± 6.3	1 vs 2 N.S.
3. 1-d-old adults	None	102.4 ± 9.2	
4. 10-15-old adults	None	315.7 ± 33.3	3 vs 4 $p < 0.05$
5. *Precocious adults	10 μl acetone	167.3 ± 40.3	
6. *Precocious adults	100 μg JHA	255.1 ± 17.2	5 vs 6 $p < 0.05$
7. *Precocious adults	200 μg JHA	359.3 ± 62.7	5 vs 7 $p < 0.05$
8. **Precocious adults	10 μl acetone	192.6 ± 9.1	
9. **Precocious adults	100 μg JHA	538.4 ± 23.2	8 vs 9 $p < 0.05$

*10-15-d-old precocious adults from precocene-treated 3rd instar larvae. **10-15-d-old precocious adults from precocene-treated 2nd instar larvae. N.S. Not statistically significant.

TABLE IV

Percentages of nuclear ploidy classes in the various groups of *Schistocerca gregaria* ($\bar{X} \pm$ S.E.)

Groups	Treatment	2C	4C	8C	16C	32C	n
A. 2nd instar larvae	None	45.0 ± 5.8	47.0 ± 4.8	8.0 ± 5.0	0	0	100
B. 3rd instar larvae	None	30.0 ± 2.5	61.0 ± 7.2	9.0 ± 3.5	0	0	100
C. 1-d-old adults	None	0	73.0 ± 2.0	27.0 ± 2.0	0	0	200
D. 10-15-d-old adults	None	0	< 2.0	44.0 ± 9.5	53.0 ± 2.5	3.0 ± 2.0	200
E. *Precocious adults	10 µl acetone	0	53.0 ± 2.5	47.0 ± 9.5	0	0	200
F. *Precocious adults	100 µg JHA	0	14.5 ± 2.4	52.0 ± 4.8	34.0 ± 1.0	0	300
G. *Precocious adults	200 µg JHA	0	0	37.0 ± 7.7	57.0 ± 7.5	6.0 ± 3.8	200
H. **Precocious adults	10 µl acetone	0	38.0 ± 3.3	33.0 ± 4.4	29.0 ± 1.5	0	100
I. **Precocious adults	100 µg JHA	0	0	< 2.0	52.0 ± 6.2	46.0 ± 4.4	100

*Precocious adults from 3rd instar larvae **Precocious adults from 2nd instar larvae

The DNA content per nucleus expressed as total extinction$_{570\,nm}$ units, is presented in Table III. It shows that the average total extinction units per nucleus (DNA content) in the normal 1-day-old adults and 10- to 15-day-old control precocious adults are 102.4 and 167.3 respectively. Maturation of the normal adults is accompanied by a doubling of the DNA content. The data also reveal that the nuclei of the precocious adults treated with JHA have a higher DNA content than those of the untreated precocious adults.

Although in the 2nd and 3rd instar larvae 30-45% of the fat body nuclei are diploid (Table IV), in the precocious adults that emerged from precocene-treated 3rd instar larvae there are no diploid nuclei; their ploidy classes are similar to those of 1-d-old normal adults. Treatment of these precocious adults with 100 µg of JHA increases the DNA content per nucleus and enhances their ploidy classes, mostly in the 8C and 16C classes. Further increases in the ploidy classes are seen in the group that received 200 µg of JHA; the predominant ploidy classes are 8C and 16 C with a few 32C nuclei. In the precocious adults that emerged from precocene-treated 2nd instars the nuclear ploidy classes are 4C, 8C and 16C. Treatment with JHA elevates the ploidy classes to 16C and 32C. Thus the highest increase in the ploidy classes and the DNA content per nucleus are seen in the nuclei of JHA treated precocious adults from 2nd instars.

DISCUSSION

During maturation of adult *S. gregaria* the DNA contents of the fat body nuclei double by day 8 (Kooman, 1980; Kooman & Nair, 1982). A similar event occurs also during the maturation of another species of locust, *L. migratoria*, and it has been suggested that this increase in nuclear ploidy levels is dependent on JH (Nair et al., 1981a). In the precocious adults that emerged from precocene-treated 3rd instar larvae the ploidy classes are similar to those of 1-day-old normal adults (Table IV) which suggests that the internal organs such as the fat body of precocene-treated larvae have undergone a process of rapid differentiation towards the adult form. This increase in ploidy from the larval level to the adult level occurs in the absence of JH since the CA of the larvae exposed to precocene undergo degeneration. Treatment of these precocious adults with JHA induces DNA synthesis in the fat body cells resulting in enhanced ploidy classes which resemble those of mature adults.

The average DNA content as well as the levels of ploidy in the fat body nuclei of precocious adults that emerged from precocene-treated 2nd instar larvae are higher than those of 1-day-old normal adults or control precocious adults from 3rd instars (Tables III, IV). Whether the enhanced ploidy classes in these untreated precocious adults are due to an increase in the frequency of fat body nuclear fusion which occurs under certain conditions (Wigglesworth, 1967) or whether the fat body cells of these small adults have compensated for the presumed reduction in cell number by increasing the DNA content remains to be established. Whatever may be mechanisms involved in the formation of these large nuclei with high DNA content, treatment of these adults with JHA also stimulates DNA synthesis resulting in the formation of a high percentage of 32C nuclei. These results are, therefore, in conformity with those of Chen et al. (1976, 1979) and Nair et al. (1981a) who showed that treatment of allatectomised *L. migratoria* with a JHA induced

replication of the DNA content of the fat body, leading to an elevation of the nuclear ploidy classes. Our results also show that maximum DNA synthesis occurs within 24 h of JHA treatment and that it precedes yolk deposition in the oocytes (unpublished). A similar obligatory DNA synthesis occurs prior to the synthesis specific proteins has been observed in *Xenopus* liver (Green & Tata, 1976; Tata & Smith, 1979), in the chick oviduct (Socher & O'Malley, 1973) and in the fat body of the mosquito *Aedes aegypti* (Hagedorn, personal communication). Nair et al. (1981a) suggest that the JH-dependent DNA synthesis and the resultant increase in the ploidy of the fat body cells of *L. migratoria* permits accelerated production of mRNA for vitellogenin and possibly other proteins. In *L. migratoria* hormonal induction of vitellogenin synthesis after primary stimulation with JHA occurs after a lag of 48 h, whereas a second application of the hormone (secondary stimulation) after 10 days renewed vitellogenin synthesis with very little initial lag (Chen et al., 1979; Chen & Hillen, 1983). We tentatively suggest here that the establishment of highly polyploid cells may be one of the reasons for the initial lag in vitellogenin production after the primary stimulation. During the secondary stimulation the latent period may be reduced since the fat body cells have already doubled their DNA content.

Whether this JH-induced fat body polyploidization seen in *L. migratoria* and *S. gregaria* is a universal phenomenon in insects or whether it is restricted to locusts remains to be established, but available evidence indicates that it occurs in other species of insects as well. Quan & Chen (1983) observed an increase in the nuclear ploidy classes of fat body cells of *Coccinella septempunctata* after *in vivo* treatment with a JHA. Similarly in *A. aegypti* treatment of the females with a JHA stimulates DNA synthesis in the fat body cells leading to higher nuclear ploidy classes (Ditmann & Hagedorn, 1984). JH has been shown to stimulate DNA synthesis in other tissues viz. the salivary glands of *Drosophila* (Sinha & Lakhotia, 1983) and ovarian follicle cells of *S. gregaria* (Nair et al., 1981b) and of *Leucophea maderae* (La Pointe et al., 1985).

A model for the endocrine control of vitellogenin synthesis and vitellogenesis has been proposed by Engelmann (1979). He postulates that JH acts on the fat body genome for mRNA production, and it also acts at the cytoplasmic level to stimulate the proliferation of RER. In the light of the results reported we suggest that any

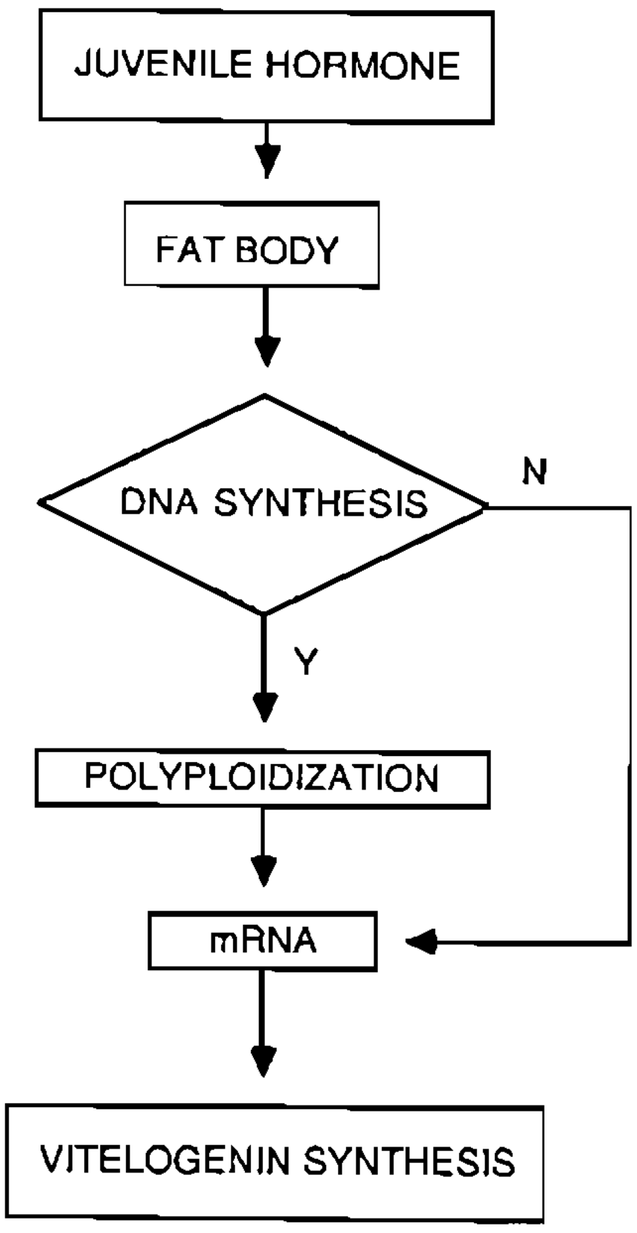

Fig. 2: Action of juvenile hormone on fat body cells of locusts. N, No; Y, Yes.

model on the mode of action of JH should recognize this phenomenon of JH-stimulated polyploidization in the fat body nuclei (Fig. 2).

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REFERENCES

BRODSKY, Y. & URYVAEVA. I. V., 1977. Cell Polyploidy; its relation to tissue growth and function. *Int. Rev. Cytol., 50* :275-332.

CHEN, T.T.; COUBLE, P.; NAIR, K. K. & WYATT, G. R., 1977. Juvenile hormone-induced DNA synthesis in the adult locust fat body. *Canad. Fed. Biol. Sci. Proc., 20* :181.

CHEN T. T.; COUBLE, P.; ABU-HAKIMA, R. & WYATT, G. R., 1979. Juvenile hormone-controlled vitellogenin synthesis in *Locusta migratoria* fat body: Hormonal induction *in vivo*. *Develop. Biol., 69* :59-72.

CHEN, T. T. & HILLEN, L. J., 1983. Expression of the vitellogenin genes in insects. *Gamete Res., 7* :179-196.

DITMANN F. & HAGEDORN, H. H., 1984. DNA synthesis in the fat body nuclei after hormonal application on isolated abdomens of *Aedes aegypti*. XVII Intl. Congress of Entomology, Hamburg, p. 234.

ENGELMANN, F., 1979., Insect vitellogenin: Identification, biosynthesis and role in vitellogenesis. *Adv. Insect Physiol., 15* :49-108.

GREEN, C. D. & TATA, J. R., 1976. Direct induction

by estradiol of vitellogenin synthesis in organ cultures of male *Xenopus laevis* liver. *Cell, 7* :131-139.

KOOMAN, C. J., 1980. Polyploidization in the fat body cell of the desert locust *Schistocerca gregaria*. M. Sc. Thesis, Simon Fraser University.

KOOMAN C. J. & NAIR, K. K., 1982. Polyploidization in the fat body of the desert locust *Schistocerca gregaria. Cytobios, 34* :25-34.

NAIR, K. K.; CHEN, T. T. & WYATT, G. R., 1981a. Juvenile hormone stimulated polyploidy in adult locust fat body. *Develop. Biol., 81* :356-360.

NAIR, K. K.; UNNITHAN, G. C.; WILSON, D. R. & KOOMAN, C. J., 1981. Cytochemistry of corpora allata, fat body and follicle cells of precocene-treated *Schistocerca gregaria*. p. 389-404. In: F. Sehnal, et al. (eds.) *Regulation of Insect Development and Behaviour*. Wroclaw Technical University Press.

QUAN, X-c & CHEN, E-y., 1983. Polyploidy in the fat body cells of adult *Coccinella septempuctata L. Acta Entomol. Sinica., 26* :368-374.

SINHA, P. & LAKHOTIA, S. C., 1983. Replication in *Drosophila* chromosomes. IX. Stimulation of initiation of polytene replication cycles in vitro by juvenile hormone. *Cell Differentiation, 12* :11-17.

SOCHER, S. H. & O'MALLEY, B. W., 1973. Estrogen mediated cell proliferation during chick oviduct and its modulation by progesterone. *Develop. Biol., 30* :411-417.

TATA, J. R. ? SMITH, D. F., 1979. Vitellogenesis: A versitile model for hormonal regulation of gene expression. *Recent Progr. Hormone Res., 35* :47-95.

UNNITHAN, G.C.; NAIR, K. K. & SYED, A., 1980. Precocene-induced metamorphosis in the desert locust *Schistocerca gregaria. Experientia, 36* :135-136.65.

WIGGLESWORTH, V. B., 1967. Polyploidy and nuclear fusion in the fat body of *Rhodnius* (Hemiptera). *J. Cell Sci., 2* :603-616.