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Juvenile Hormone and Protein Synthesis in Adult Female Cockroaches *

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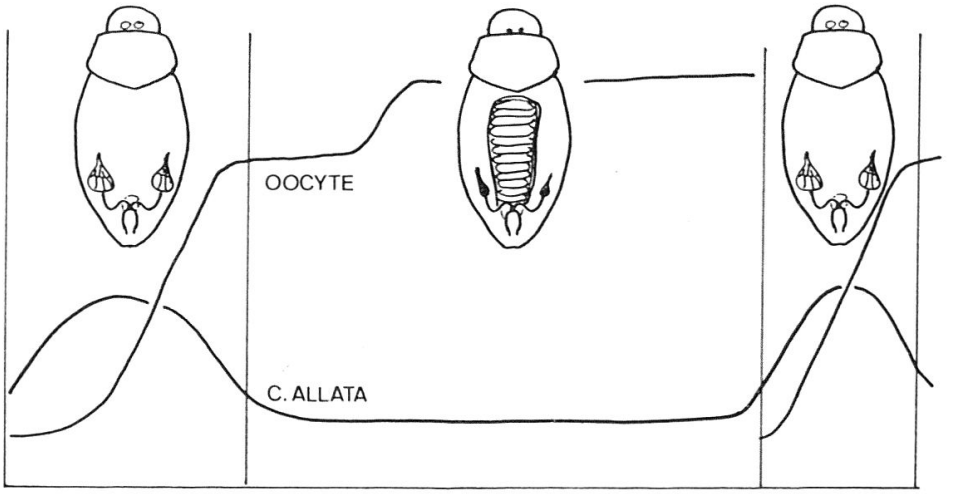
Females of the viviparous cockroaches *Leucophaea maderae* and *Nauphoeta cinerea* show a characteristic cycle of activity of the corpora allata, which is correlated to the sexual cycle of alternating oocyte maturation and gestation periods (Fig. 1). Some time after emergence the corpora allata become active and secrete juvenile hormone which causes oocyte maturation (Engelmann 1957, Lüscher 1968). When the oocytes have matured, the corpora allata become inactive and remain



LEUCOPHAEA	30 days	65 days	20 days
NAUPHOETA	15 days	45 days	12 days

Fig. 1. — The sexual cycle of adult female ovoviviparous cockroaches. The curves represent oocyte length and corpora allata volume. Partly after Engelmann 1957.

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so during the whole gestation period. Just after parturition they become active again and cause the maturation of a next set of oocytes. The clear alternation of high activity and inactivity of the corpora allata makes these insects very suitable for the study of metabolic processes which are influenced by juvenile hormone. For some time now we have made use of them for the study of the influence of hormones on protein synthesis.

Since the yolk which is incorporated in the oocytes under the influence of juvenile hormone consists to a great extent of proteins, it seemed likely that juvenile hormone initiates or stimulates the synthesis of specific yolk proteins. In order to test this possibility we have determined the haemolymph protein concentration during the sexual cycle. We have then investigated the rate of protein synthesis in different tissues at various periods of the cycle and under experimental conditions. Finally we have tried to identify the single proteins which are specifically influenced by hormones and especially by juvenile hormone. It must be emphasized here that our research is not entirely original and that a number of research groups have investigated similar problems at the same time. The limited time does however not allow for an extensive review of the literature.

The total amount of haemolymph proteins per insect *(Leucophaea)* with its variations during the sexual cycle is shown in Figure 2. It is

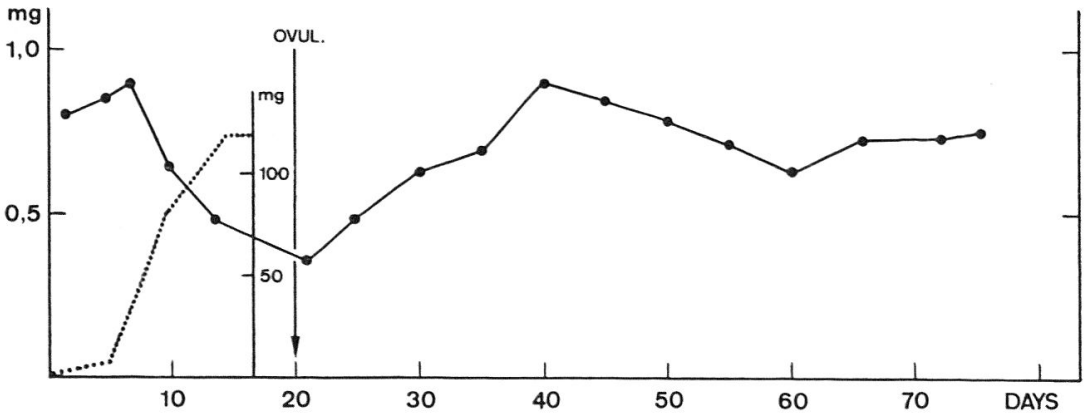

Fig. 2. — The total haemolymph proteins per insect during the sexual cycle of *Leucophaea* (solid line). The values were calculated from haemolymph protein concentration and haemolymph volume determinations. The dotted line represents the dry weight of both ovaries. After Scheurer and Leuthold 1969.

clear from this figure that proteins must be synthesized at the beginning and probably throughout the oocyte maturation period and also at the beginning of the gestation period. The tissues responsible for the synthesis of yolk proteins being most probably the fat body and the ovary tissues, we have measured the rate of synthesis in fat body, follicles and ovarial connective tissue by incubating these tissues for 4 hours in Ringer containing C^{14}-labeled amino acids. The activity

of the precipitated and washed proteins was then determined in a liquid scintillation counter (for details of the method see Wyss-Huber and Lüscher 1966).

The course of protein synthesis in such an experiment is shown in Figure 3 for fat body tissue of *Leucophaea*. The radioactivity of the total proteins increases at a constant rate for 4 hours whereas the activity of the proteins extracted from the tissue itself reaches an equilibrium after 1½ hours when the synthesized proteins begin to be released into the incubation medium. In order to estimate the total protein synthesis after 4 hours it was therefore necessary to measure the radioactivity of the

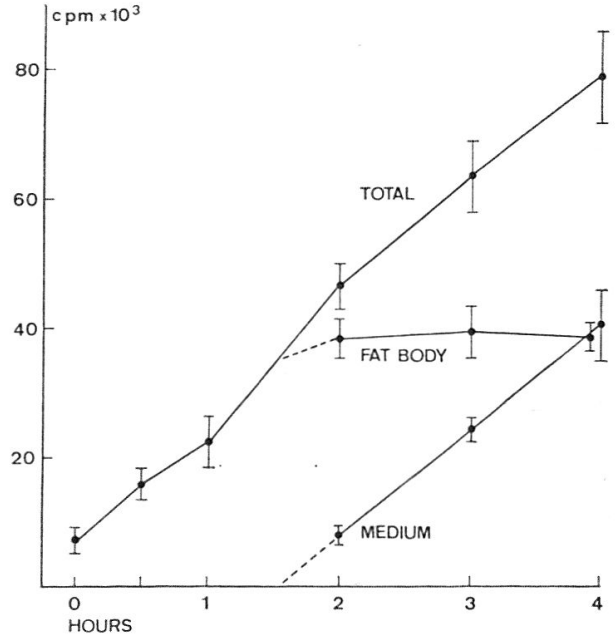

Fig. 3. — Course of incorporation of C^{14}-alanine into the proteins synthesized in vitro by fat body tissue of female *Leucophaea*. The fat body was taken on the 5th day of the second sexual cycle. After Lüscher et al. 1966.

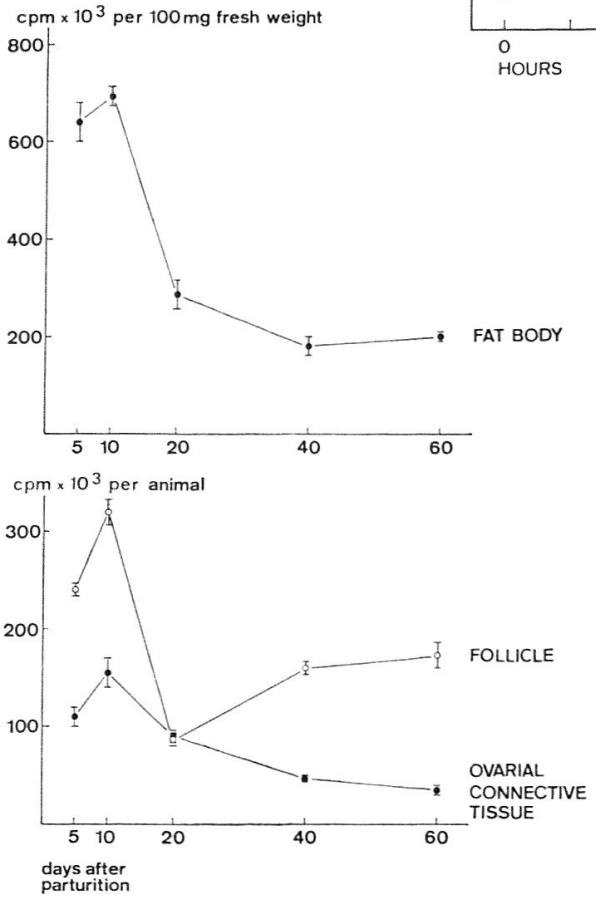

Fig. 4. — Protein synthesis in fat body, follicle tissue (containing oocytes) and ovarial connective tissue during the sexual cycle of *Leucophaea*. (Unpublished data).

proteins extracted from the tissue as well as of those contained in the incubation medium.

In all tissues a high rate of protein synthesis was found during oocyte maturation and a relatively low rate during gestation (Fig. 4). Most of the proteins are synthesized by the fat body, but if the synthesis rate per gram tissue is being considered, the follicle cells and the ovarial connective tissue are much more active. The connective tissue is during oocyte maturation at least 30 times more active in protein synthesis than fat body tissue. The fact that all tissues produce more proteins during oocyte maturation indicates a possible stimulatory action of juvenile hormone in all three tissues.

In order to find out if the hormones influence directly the rate of protein synthesis we have incubated fat body tissue of pregnant and not pregnant *Leucophaea* together with isolated endocrine organs, namely corpora allata, corpora cardiaca and brains (Fig. 5 ; Lüscher

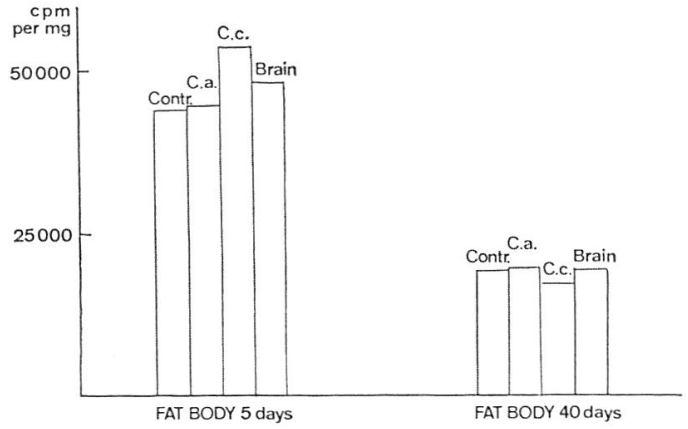

Fig. 5. — Protein synthesis in fat body of *Leucophaea* in vitro, incubated together with corpora allata (C.a.) or corpora cardiaca (C.c.) or brain. The fat body was taken 5 or 40 days after parturition. The endocrine organs were dissected from females on the 5th day after parturition. After Lüscher et al. 1966.

et al. 1966). The rate of synthesis could only be increased significantly in fat body of oocyte maturing females and only by corpora cardiaca, not by corpora allata. This shows that the fat body is not at all times competent to react to hormones and that a corpus cardiacum hormone can have an immediate effect on protein synthesis. The fact that the corpora allata had no influence does not necessarily mean that juvenile hormone does not stimulate protein synthesis. It is possible that the corpora allata do not release hormone in Ringer solution.

We have then investigated the effects of implanted glands on protein synthesis *in vivo* in *Nauphoeta* (Lüscher 1968). The females were decapitated just before the beginning of oocyte growth. Three series of decapitated females received implants of corpora allata, corpora cardiaca or of both glands. Four days later C^{14}-labeled amino acids were injected and after one hour the insects were dissected in ice-cold Ringer. The proteins of the fat body were precipitated, washed and tested for radioactivity. In this experiment (Fig. 6) the rate of protein synthesis was about 3 times lower in decapitated than in normal females.

The normal rate of synthesis could be restored by implanted corpora allata, but not by corpora cardiaca. It can therefore be concluded that juvenile hormone stimulates protein synthesis enormously, but since this could not be demonstrated *in vitro*, it seems not to be an immediate effect.

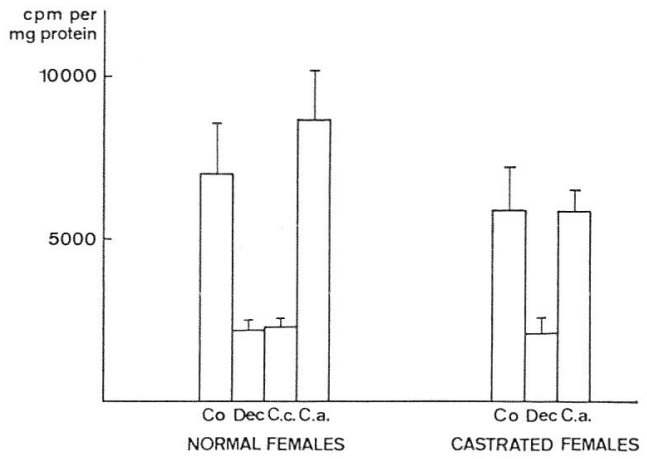

Fig. 6. — Protein synthesis in the fat body of *Nauphoeta* in vivo, 4 days after decapitation and implantation of endocrine organs. C^{14}-amino acids were injected and one hour later the proteins were recovered from the fat body. Co = normal female; Dec = decapitated female; C.c. and C.a. = decapitated females with implanted corpora cardiaca (C.c.) or corpora allata (C.a.). After Lüscher 1968.

It was now of interest to see if different tissues reacted in the same way to juvenile hormone *in vivo*. We have therefore performed the same experiment in *Leucophaea* and investigated the rate of protein synthesis in fat body, muscle tissue and midgut epithelium. The latter was not separated from its surrounding conncective tissue sheath. Instead of implanting corpora allata, Miss B. Lanzrein who carried out these experiments, injected the juvenile hormone analogue farnesylmethylester (FME) into the decapitated females. It can be seen in Figure 7 that the rate of synthesis is highest in the midgut epithelium and extremely low in muscle tissue. The juvenile hormone analogue stimulated protein synthesis significantly in the fat body but not in the other tissues. This shows that different tissues react differently to the hormone. Thus the differentiated tissues are so programmed that they react to the hormones in a tissue-specific way.

So far we have seen that protein synthesis can be stimulated under certain circumstances by corpora cardiaca and to a much greater extent by corpora allata or by juvenile hormone. The question now arises if these hormones act on the synthesis of specific proteins or if they have a more general stimulating effect. This problem was first studied in our laboratory by Scheurer (1969). Females of *Leucophaea* were decapitated one day after the first parturition. Immediately after decapitation corpora cardiaca, brains or corpora allata were implanted. Seven days after the operation the haemolymph was subjected to electrophoresis on polyacrylamide gels (disc electrophoresis). The relative amount of the individual proteins was determined by densitometry of the stained pherograms. The proteins which can be found in female haemolymph

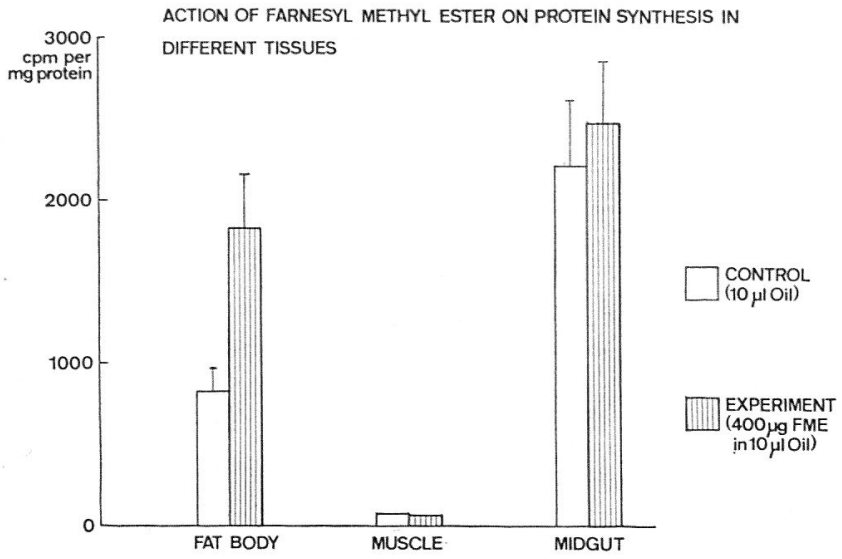

Fig. 7. — Protein synthesis in different tissues of *Leucophaea* in vivo under the influence of farnesylmethylester (FME). All insects were decapitated 4 days previous to an injection of C^{14}-labeled amino acids. The proteins were isolated from the tissues one hour after this injection. Unpublished observations by B. Lanzrein.

were denominated by letters (Fig. 8) and those which showed consistent differences between the experimental insects and the controls were compared. Figure 9 gives the result for three selected proteins. The synthesis of one protein (D) is stimulated by brain, that of another (B) by corpora cardiaca. The most interesting is protein G which disappears almost completely from the haemolymph after decapitation and which is synthesized de novo under the influence of corpora allata. Brain and corpora cardiaca do not influence the synthesis of protein G. This protein has been shown to be a typical *female protein* which is present only in adult females during the oocyte maturation period and which seems to be transferred quantitatively into the yolk of the oocytes (Scheurer and Lüscher 1968). It is absent from the haemolymph during gestation and it is also absent in larvae, nymphs and adult males. It is probably identical with the female protein demonstrated by Engelmann and Penney (1966). A similar female protein could also be demonstrated in *Nauphoeta*.

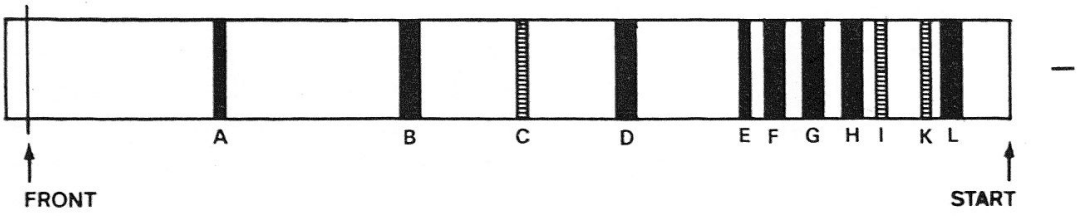

Fig. 8. — Protein fractions from haemolymph of female *Leucophaea*, separated by disc electrophoresis on polyacrylamide gel. After Scheurer and Lüscher 1968.

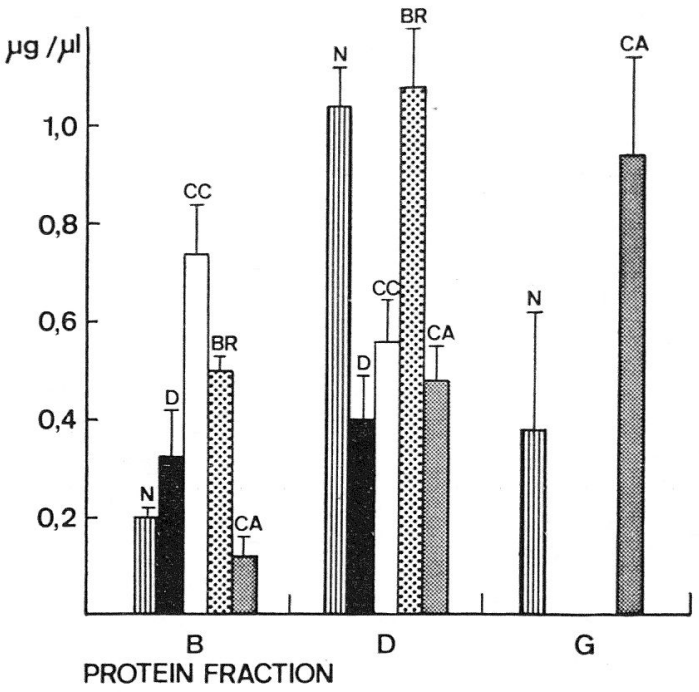

Fig. 9. — The concentration of some haemolymph proteins in decapitated females of *Leucophaea* under the influence of implanted endocrine organs. N = normal females ; D = decapitated females ; CC, BR, CA = decapitated females with implanted corpora cardiaca (CC), brains (BR) or corpora allata (CA). The concentrations are given in albumin units (µg serum albumin / µl haemolymph). After Scheurer 1969.

It can be seen in Figure 9 that the corpora allata also have another interesting effect : they inhibit the synthesis of protein B. Their action in this respect seems to be antagonistic to that of the corpora cardiaca. The effects of juvenile hormone, namely the initiation of the synthesis of protein G and the inhibition of the synthesis of protein B could recently be demonstrated more securely by autoradiography of haemolymph electropherograms which were prepared from the females of *Leucophaea* which served to show the differential action of farnesylmethylester on different tissues. In Figure 10 the amidoblack stained

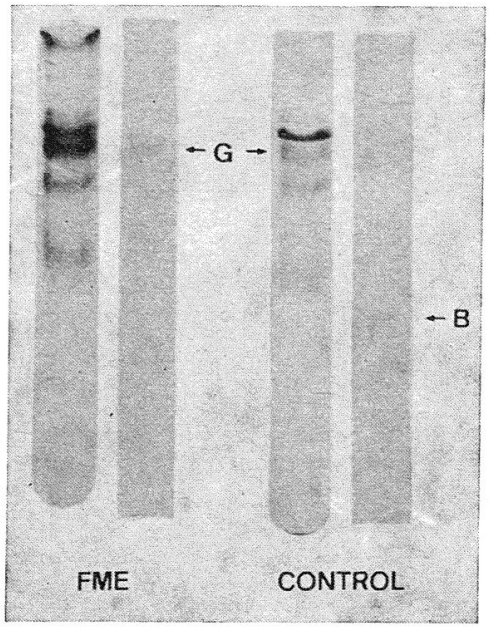

Fig. 10. — Synthesis of proteins B and G in *Leucophaea* under the influence of farnesylmethylester (FME). The figure shows the stained electropherograms and their autoradiographs. For further details see text. Unpublished results.

pherograms of decapitated controls and JH-analogue treated insects are compared with the corresponding autoradiographs. These show clearly that the synthesis of protein B is apparent in the controls and that it is inhibited and replaced by synthesis of protein G in the juvenile hormone-analogue (FME) treated insects [1].

After it became clear that the juvenile hormone is responsible for the synthesis of protein G, it seemed important to investigate if this specific protein is present in the different tissues which are probably connected with yolk protein formation and if it is synthesized in all these tissues. In order to test the presence of protein G we have prepared a protein G-specific antibody which precipitates only this protein in tissue extracts in the agar diffusion test. For evaluating the specific synthesis of protein G we have incubated the tissues together with radioactive amino acids. We have then prepared electropherograms of the medium and of the extract of the tissue and we have finally studied autoradiographs of these pherograms. The results of this investigation are shown in Figure 11. Protein G is present in the fat body and in the

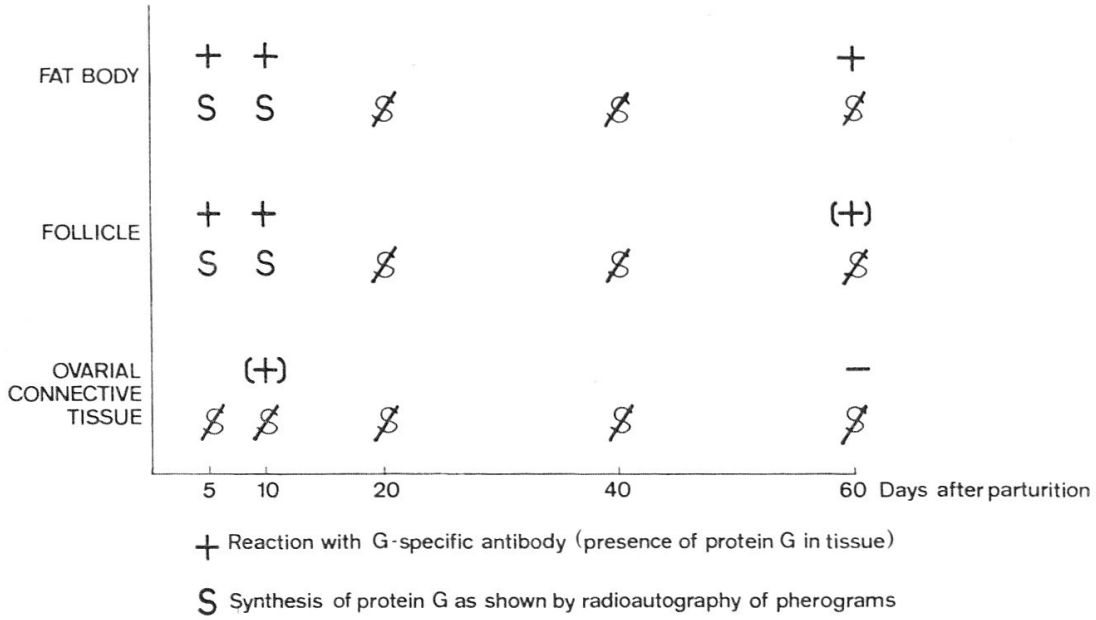

Fig. 11. — Presence and synthesis of protein G in different tissues of *Leucophaea* during the sexual cycle. Synthesis occurs only during oocyte maturation and only in two of the investigated tissues. Unpublished data.

follicles or oocytes at all times, but in greater quantities during oocyte maturation. The ovarial connective tissue contains very small amounts of protein G during oocyte maturation only. Synthesis of protein G

[1] Our protein G corresponds probably to the longday protein in *Leptinotarsa*, while protein B might be compared with the shortday protein of this insect (see lecture by J. de Wilde in this Symposium).

can only be demonstrated during oocyte maturation in follicles containing oocytes and in fat body. No synthesis of protein G occurs at any time in the ovarial connective tissue, although this tissue is very active in protein synthesis in general as we have seen above. The synthetic activity of the ovarioles which were separated from the connective tissue is certainly due to the activity of the follicle cells, since no endoplasmatic reticulum can be found within the oocytes. The ovarioles were therefore denominated as follicle in Figure 4 and 11.

This experiment has shown that not all tissues which synthesize proteins are able to synthesize the specific female protein. It has also shown that some protein G remains in the synthesizing tissues also during the periods of inactivity and that it then is not released from these tissues. Fat body and follicle cells are almost certainly the most important sites of synthesis of the specific protein.

When a hormone like juvenile hormone is responsible for the synthesis of a specific protein, then the mechanism of action could possibly be an activation or derepression of one or more specific genes which would be responsible for producing the specific messenger RNA. If this is so it should be possible to prevent the synthesis of the specific protein with actinomycin D and to demonstrate an immediate specific synthesis in the appropriate tissues under the influence of the hormone. In fact we could show that injection of actinomycin D in females of *Nauphoeta* prevents the synthesis of protein G and that oocyte maturation is completely blocked. But the earliest instance when protein G could be detected with antiserum in the haemolymph of decapitated females after treatment with hormone was 20 hours later. Furthermore we have so far only in one case shown a significant stimulation of protein synthesis in *vitro* by juvenile hormone. It was the action of the synthetic juvenile hormone (provided by Dr. H. Röller) on ovaries of *Leucophaea* (Wyss-Huber and Lüscher 1969). The autoradiographs of the electropherograms prepared during this experiment revealed no apparent synthesis of protein G. It therefore cannot yet be decided if the synthesis of the female specific protein is due to an activation of the synthesis of a specific messenger RNA or to an indirect action on the cytoplasm.

In conclusion it can be stated that in both species, *Leucophaea* and *Nauphoeta*, juvenile hormone induces the synthesis of one specific protein in certain tissues and that at least in one species *(Leucophaea)* it inhibits the synthesis of another specific protein. These actions are sex-specific and tissue-specific. They can be shown only in the adult and they depend on a competence of the responding tissues which changes consistently within the sexual cycle.

REFERENCES

ENGELMANN, F. 1957. Die Steuerung der Ovarfunktion bei der ovoviviparen Schabe *Leucophaea maderae* (Fabr.). *J. Insect Physiol.* 1, 257–278.

ENGELMANN, F. and D. PENNEY 1966. Studies on the endocrine control of metabolism in *Leucophaea maderae (Blattaria)*. — I. The haemolymph proteins during egg maturation. *Gen. comp. Endocrinol.* 7, 314–325.

LÜSCHER, M. 1968. Hormonal control of respiration and protein synthesis in the fat body of the cockroach *Nauphoeta cinerea* during oocyte growth. *J. Insect Physiol.* 14, 499–511.

LÜSCHER, M., K. MOESCH, R. SCHEURER and M. WYSS-HUBER 1966. Hormonal control of protein synthesis in the fat body of *Leucophaea maderae*. Internat. Symp. on Insect Endocrines, Brno 1966. *Endocrinologia experimentalis*, Bratislava, 5, 69–72 (1971).

SCHEURER, R. 1969. Endocrine control of protein synthesis during oocyte maturation in the cockroach *Leucophaea maderae*. *J. Insect Physiol.* 15, 1411–1419.

SCHEURER, R. and R. LEUTHOLD 1969. Haemolymph proteins and water uptake in female *Leucophaea maderae* during the sexual cycle. *J. Insect Physiol.* 15, 1067–1077.

SCHEURER, R. und M. LÜSCHER 1968. Nachweis der Synthese eines Dotterproteins unter dem Einfluss der *Corpora allata* bei *Leucophaea maderae*. *Rev. suisse Zool.* 75, 715–722.

WYSS-HUBER, M. und M. LÜSCHER 1966. Über die hormonale Beeinflussbarkeit der Proteinsynthese in vitro im Fettkörper von *Leucophaea maderae*. *Rev. suisse Zool.* 73, 517–521.

WYSS-HUBER, M. and M. LÜSCHER 1969. Influence of juvenile hormone on ovarian protein synthesis in *Leucophaea maderae*. *Gen. comp. Endocrinol.* 13, 542.

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