mitochondrial marker, confirming the results of HOLZBAUER.

Two distribution patterns for corticosterone are presented. After both kinds of homogenization, the distribution pattern is identical with the same high concentration of the steroid in the postmicrosomal supernatant. 3 explanations are possible: 1. Corticosterone is present in the cytosol and during centrifugation reaches the Snt compartment with weak secondary absorption on several subcellular components. 2. This molecule stays in the cell inside the tubules forming the endoplasmic reticulum (Er) and is released in the Snt when the transformation in microsomal vesicles occurs. 3. The binding of the corticosterone on any organelle is extremely weak and the release in the homogenization medium is instantaneous after the beginning of this procedure.

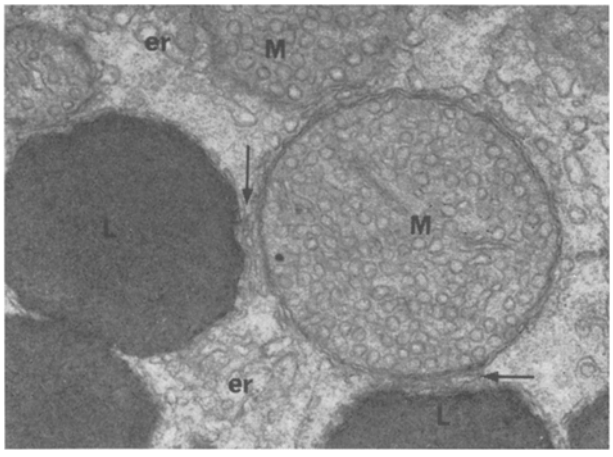

Fig. 2. Rat adrenal cortex. External fasciculata zone. Liposomes (L), mitochondria (M) and tubular elements of the endoplasmic reticulum (er). Arrows: er-cisternae between mitochondria and liposomes, in close contact with both organelles. All required enzyme-systems for the steroid pathway, the main steroids and cholesterol are present in this reduced space.
OsO_4-H_2O-fixative, reduced dehydration technique, lead-citrate stain[4]. × 30,400.

Lastly, the present steroid distribution pattern is not in agreement with the distribution suggested by the hydroxylating system; although both localization-scheme must not necessarely be identical. Enzyme systems fixed on (or in) organelle-membranes could enter into contact and react with the nearest steroid molecules which are either in the cytosol surrounding the organelles, or even fixed on another near-lying membrane system.

As can been seen in Figure 2, in the adrenocortical cells of the rat, there is a close relation between liposomes, mitochondria and endoplasmic reticulum, with a very important contact interface especially between the Er and the other organelles. This morphological fact, joined to the results of the steroid localization study reported here, suggest a partially new conception to resolve the problem of correlation between morphology and function in the adrenal cortex.

Biosynthesis of α- and β-Ecdysone by the Crayfish *Orconectes limosus* in vivo and by its Y-Organs in vitro

A. WILLIG and R. KELLER

Abteilung Biologie I der Universität Ulm, Oberer Eselsberg, D–7900 Ulm (German Federal Republic, BRD),
19 January 1976.

Summary. α- and β-ecdysone were synthesized from labelled cholesterol by premolt crayfish in vivo and by their Y-organs in vitro.

Molting in crustaceans – as in insects – is controlled by ecdysones (for review see[1]). From ablation-implantation experiments[2,3] it seems evident that in brachyuran crabs the sites of ecdysone biosynthesis are the paired Y-organs which were first described by GABE[4]. This assumption is confirmed by recent findings[5] which demonstrate a selective uptake of injected radioactive cholesterol, a biochemical precursor of ecdysones in insects, into the Y-organs of the crab *Hemigrapsus nudus* and by the demonstration[6] of α-ecdysone production by in vitro cultured Y-organs of *Pachygrapsus crassipes*.

However, reports on the Y-organs of Macrura are conflicting. Several distinctly different structures of macrurans have been described as 'Y-organs' but no evidence of their functional role as molting glands was given by ablation-implantation experiments or metabolic studies[7–10] (for reviews see[11,12]). In a recent report from our laboratory[13], the location and cyclic histological alterations during the intermolt cycle of a glandular structure of the crayfish *Orconectes limosus* were described. Preliminary extirpation experiments indicate the possible role of this organ as the molting gland of *Orconectes*.

The ability of putative Y-organs to synthesize ecdysones from cholesterol in vitro can be taken as an indication for their functional role as molting glands. We therefore investigated a) the ability of intact premolt crayfish to synthesize ecdysones in vivo as was recently demonstrated for α-ecdysone synthesis in molting lobsters[14],

[1] A. WILLIG, Fortschr. Zool. *22*, 55 (1974).
[2] G. ECHALIER, C. r. Acad. Sci., Paris *240*, 1581 (1955).
[3] L. M. PASSANO and S. JYSSUM, Comp. Biochem. Physiol. *9*, 195 (1963).
[4] M. GABE, C. r. Acad. Sc., Paris *237*, 1111 (1953).
[5] E. SPAZIANI and S. B. KATER, Gen. comp. Endocr. *20*, 534 (1973).
[6] W. E. BOLLENBACHER and J. D. O'CONNOR, Am. Zool. *13*, 1274 (1973).
[7] J. CHAUDONNERET, Ann. Sci. Nat., Ser. 11, Zool. Biol. Anim. *18*, 33 (1956).
[8] J. B. DURAND, Biol. Bull. mar. biol. Lab. Woods Hole *118*, 250 (1960).
[9] N. F. PATERSON, Ann. S. Afr. Mus. *51*, 1 (1968).
[10] D. B. CARLISLE and R. O. CONNICK, Can. J. Zool. *51*, 417 (1973).
[11] J. B. SOCHASKY, D. E. AIKEN and N. H. F. WATSON, Can. J. Zool. *50*, 993 (1972).
[12] J. B. SOCHASKY and D. E. AIKEN, Can. J. Zool. *52*, 1251 (1974).
[13] F. BURGHAUSE, Z. Morph. Tiere *80*, 41 (1975).
[14] R. B. GAGOSIAN, R. A. BOURBONNIERE, W. B. SMITH, E. F. COUCH, C. BLANTON and W. NOVAK, Experientia *30*, 723 (1974).

and b) the ability of the described[13] Y-organs to synthesize ecdysones in vitro. From the molting hormone titer curve of *Orconectes*, which exhibits a steep increase to its maximum during the premolt stage D_2[15], a maximum metabolic activity of the organs is expected during this stage.

Experimental and results. 40 µCi (4-^{14}C)-cholesterol (59.2 mCi/mmole) were emulsified in 250 µl of VAN HARREVELD's saline[16] with 10 mg of Tween 20 by sonication. 50 µl of this solution were injected in each of 5 male crayfish (*Orconectes limosus* Rafinesque) at premolt stage D_2 through the arthrodial membrane of a chela. The animals were kept at 15 °C in 500 ml each of tap water for 20 h. The total radioactivity of the water was then 0.71 µCi indicating that only little activity was lost by bleeding or excretion during the incubation. The animals were killed and homogenized in 60% methanol in water. Non-radioactive carrier α- and β-ecdysone were added to the homogenate and the hormones isolated by counter-current distribution between *n*-butanol and water[17] and thin-layer chromatography (TLC) on Kieselgel GF_{254}-plates developed with chloroform-*n*-propanol (9:5, v/v)[18] as described for molting hormone isolation from crayfish material[15]. After the 3rd TLC, the α-ecdysone fraction had a radioactivity (corrected to 100% efficiency) of 9.9×10^3 dpm (0.011% of that of the incorporated substrate) and the β-ecdysone fraction had 7.8×10^3 dpm (0.009%). Each fraction was acetylated with acetic acid anhydride–pyridine for 6 h at room temperature and the resulting tetra- and triacetates separated by TLC on Kieselgel GF_{254}-plates developed with ethyl acetate-hexane (4:1, v/v)[19]. The UV-absorbing acetates were eluted and their radioactivity determined. 84% of the radioactivity which was employed for the acetylation of α-ecdysone were found to coincide with α-ecdysone-2, 3, 22, 25-tetra-acetate (Rf 0.64) and 6% coincided with α-ecdysone-2, 3, 22-tri-acetate (Rf 0.35). 73% of the radioactivity which was used for the acetylation of β-ecdysone coincided with β-ecdysone-2, 3, 22, 25-tetra-acetate (Rf 0.48) and 9% with β-ecdysone-2, 3, 22-tri-acetate (Rf 0.25).

22 Y-organs of stage D_2 premolt crayfish were extirpated as described[20]. The organs were incubated for 20 h at 25 °C with 100 µCi of tritiated cholesterol (12.1 Ci/mole) in 1 ml medium consisting of 500 µl VAN HARREVELD's saline[16], 500 µl of a 10.000 *g* supernatant of hemolymph from premolt crayfish, 2 mg glucose, and 0.1 mg Aureomycin®. Additionally, the medium contained 200 ng of each α- and β-ecdysone in order to protect eventually formed labelled ecdysones from oxydation. It was attempted to obtain Y-organs and hemolymph aseptically. The other constituents of the medium were heat-sterilized. The incubation was terminated by addition of methanol, the organs were homogenized and the mixture analyzed for the presence of labelled ecdysones as described. After the 3rd TLC, the α-ecdysone fraction had a radioactivity of 32.5×10^3 dpm (0.015% of the total initial radioactivity) and the β-ecdysone fraction had 23.8×10^3 dpm (0.011%). After brief acetylation (2 h) of each fraction and separation of the resulting mixtures by TLC, 4 radioactive products were found which co-chromatographed with the acetylation products of authentic α- and β-ecdysone respectively. The acetates of the α-ecdysone fraction contained 77% of the radioactivity which was employed for the reaction, those of the β-ecdysone fraction contained 80%.

Discussion. The results demonstrate that the Y-organs of *Orconectes* described by BURGHAUSE[13] synthesize molting hormones. Molting is prevented by extirpation of these glands indicating that they are the sole organs of crayfish which are capable of molting hormone biosynthesis. Other organs of macrurans which have been described as 'Y-organs'[7–10] probably do not function as molting glands and require further investigation.

The finding that the Y-organs of *Orconectes* synthesized both α- and β-ecdysone in vitro differs from the result of BOLLENBACHER and O'CONNOR[6] who obtained only α-ecdysone with in vitro cultured Y-organs of a crab. Since α-ecdysone is rapidly converted to β-ecdysone by crustacean tissues[18], we cannot exclude the possibility that the labelled α-ecdysone produced in our incubations is eventually hydroxylated to β-ecdysone by small pieces of epidermal tissue adhering to the explanted Y-organs.

[15] A. WILLIG and R. KELLER, J. comp. Physiol. *86*, 377 (1973).
[16] A. VAN HARREVELD, Proc. Soc. exp. Biol. Med. *34*, 428 (1938).
[17] P. KARLSON and E. SHAAYA, J. Insect Physiol. *10*, 797 (1964).
[18] D. S. KING and J. B. SIDDALL, Nature, Lond. *221*, 955 (1969).
[19] N. J. DE SOUZA, E. L. GHISALBERTI, H. H. REES and T. W. GOODWIN, Biochem. J. *114*, 895 (1969).
[20] R. KELLER and A. WILLIG, J. comp. Physiol., in press.

Ein Progesteron-abhängiges Pheromon der weiblichen Maus

A Progesterone-Dependent Pheromone of the Female Mouse

SUZANNE BLOCH

Universitäts-Frauenklinik, Schanzenstrasse 46, CH-4056 Basel (Switzerland), 30. Januar 1976.

Summary. Female mice kept in groups exhibit less oestrous smears than females kept singly. Ovariectomized females have only slightly reduced number of oestrous smears, but ovariectomized females injected with progesterone act on other females like intact ones. It is concluded that a progesterone-dependent pheromone excreted by dioestrous females acts oestrus-depressing on other females.

Das Pheromon, das im Urin des Mäusemännchens ausgeschieden wird, und seine vielfachen Wirkungen auf das Weibchen: Blockierung der Gravidität[1], Aktivierung und Synchronisierung der Östren[2, 3], ist vielfach untersucht und beschrieben worden. Weniger bekannt sind Pheromone der weiblichen Maus. Zwar ist die Wirkung des östrischen Weibchens auf das Männchen wohl bekannt, doch legen verschiedene Beobachtungen die Annahme nahe, dass das Weibchen auch im Dioestrum ein Pheromon ausscheidet, das vom Progesteron abhängt und auf andere Weibchen wirkt.

[1] H. BRUCE, J. Reprod. Fert. *1*, 96 (1960).
[2] W. K. WHITTEN, J. Endocr. *13*, 399 (1956).
[3] W. K. WHITTEN, J. Endocr. *18*, 102 (1959).