cells are more abundant (Figure 5) which may indicate resorption of colloid from the follicles (Figure 2). Zymogen-like granules and lysosomes are evident, features which would also indicate the resorption of colloid (Figures 3 and 4). No 'light' cells[7] (also called parafollicular cells) were seen. Intercellular channels, apical vesicles and desmosomes were also noted (Figures 3 and 5).

Discussion. Embryonic thyroid glands have been cultured for 4 days and the method used to study the effect of iodine metabolism[8]. The results of the present study clearly demonstrate that the ultrastructure of the rat thyroid gland can be maintained in an essentially normal condition for at least 8 days in organ culture. Some of the histological changes which occur appear to be the direct result of 1. cessation of synthetic activity by the follicle cells, and 2. resorption of colloid from the follicles. These changes would be expected since the level of TSH in the support medium (6 IU/ml) might be insufficient to maintain the synthetic activity of the gland, and essential precursors (leucine, mannose, galactose, etc.[3]) were not added. However, the presence of apical vesicles in Figure 4 would be evidence of secretion into the colloid[6]. Small quantities of amino acids are included in the formula of Eagle's medium, and it is possible that the calf serum used as a supplement to the medium may contain some important factors.

The present results confirm and extend those of PETROVIC and PORTE[1,2]. It is conceivable that the high gas pressures that we used during culture increased the partial pressure of dissolved oxygen. We were thus able to avoid the use of high concentrations of oxygen in the gas phase (e.g. 95% O_2, WHUR and MERSCOVICS[3]) since these levels may be toxic to some cells.

Our results thus clearly show that the technique of organ culture of the thyroid gland is eminently suitable for studies of biosynthetic pathways within the gland by means of ultrastructural radioautography.

Zusammenfassung. Die Ultrastruktur der Rattenschilddrüse wird bis zu 8 Tagen in Organkulturen erhalten, was für radioautografische Studien der Ultrastruktur vorteilhaft ist.

T.G. BAKER and B.A. YOUNG

Hormone Laboratory,
Department of Obstetrics and Gynaecology,
University of Edinburgh,
Edinburgh (Scotland),
and
University of Cambridge,
Department of Anatomy,
Downing Street,
Cambridge CB2 3DY (England),
6 July 1973.

[7] B. A. YOUNG and C. P. LEBLOND, Endocrinology *73*, 669 (1963).
[8] B. M. NATAF, E. M. RIVERA and I. L. CHAIKOFF, Endocrinology *76*, 35 (1965).

Ecdysone: An Antagonist of Juvenile Hormone in the Control of Cuticle Synthesis in the German Cockroach (*Blattella germanica*)

In addition to their well known morphogenetic effects, juvenile hormone (JH) and its analogues (JHA) possess the capacity to inhibit ecdysis. This effect causes death during the larval moult of all but the last larval instar of the German cockroach[1]. The length of the last larval instar can be prolonged idefinitely by continuous application of high dosages of JH (methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; *cis/trans* mixture) or JHA (6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl 2-octene; *cis/trans* mixture[2])[3]. The synthesis of the new cuticle in these permanent larvae can be induced by injection of ecdysone[3]. This is the first report of antagonism between JH and ecdysone in vivo[4].

10 freshly hatched last stage larvae of *Blattella germanica* were confined in 200 cm^3 plastic cups with two 10 cm^2 paper discs each folded to form a tunnel. The JH active substance was applied in an acetone solution either to

[1] W. HANGARTNER and P. MASNER, Experientia, in press (1973).
[2] Stauffer compound No. R-20458.
[3] All compounds were kindly provided by F. Hoffmann-La Roche, Ltd. Basel.
[4] Results presented to members of the A.R.C. Unit of Invertebrate Chemistry and Physiology at Brighton (Prof. A. W. JOHNSON, Director) and Cambridge (Dr. J. E. TREHERNE, Deputy Director) on March 19 and 20, 1973.

Table I. Effect of JH and JHA on freshly emerged last stage larvae. The penultimate column contains the mean length of the last larval instar of insects which hatched (20 animals were tested in each experiment)

Treatment				No. of dead last stage larvae	No. of insects hatching to the next stage				Length of the last stage (days)	No. of permanent larvae
Substance	Application	Dosage	Frequency (day)		Perfect adults	Deformed adults	Extralarvae	Dead in old cuticle		
Acetone control	Topical	1 µl	2, 4, 6, 8, 10, 12, 14		20				16	
JH	Topical	10µg	2, 4, 6, 8, 10, 12, 14	2		1	17		21	
JH	Topical	100 µg	2, 4, 6, 8, 10, 12, 14	1			7		27	12
JHA	Topical	10 µg	2, 4, 6, 8, 10, 12, 14						> 31	20
JHA	Topical	100 µg	5, 10	2			9	9	31	
JHA	paper	100µg/cm²	permanent						> 31	20

Table II. Effect of ecdysone tested alone and in combination with JHA on development of freshly emerged last stage larvae. (20 animals were tested in each experiment)

Treatment		No. of dead last stage larvae	No. of insects hatching to the next stage			Lenght of the last stage (days)	No. of permanent larvae
JHA (μg/cm² paper)	Ecdysone (μg)		Adults	Extralarvae	Dead in old cuticle		
–	–		20			16	
–	5			20		11.8	
–	10	2			18	11	
–	50				20	9.5	
100	5						20
100	10				20	10.1	
100	50	1			19	8.5	

the paper or to the surface of the uninjured abdominal tergites. 5 μl of a 10% ethanol solution of α-ecdysone were injected using a Hamilton syringe.

The last larval stage lasts approximately 16 days (25 °C, 45% r.h.). Treatment with JH active substances delays further development and the time at which ecdysis may take place depends on the compound, the time and the dosage applied (Table I). The larvae treated with 10 μg of JH every other day during a 2 weeks period moult into perfect extralarvae one week after the last application. A dosage 10 times higher prevents the whole moulting process (cuticle synthesis and ecdysis) in more than 50% of the treated larvae which remain permanently in this stage. The dosage of 10 μg of JHA applied in the same way prevents the moulting process in all larvae. The same effcet is accomplished by permanent exposure of last stage larvae to filter paper treated with 100 μg JHA/cm². The larvae exposed to such treated paper after the 6th day of this instar synthesize the new cuticle but die during ecdysis as do the larvae of earlier stages.

The permanent larvae remain in the last instar for months showing no signs of poisoning. The ovaries, which are inactive in control insects during the entire last instar, contain numerous activated oocytes in an advanced stage of vitellogenesis. The length of the terminal oocyte reaches 1.4 mm, which is about 2/3 of the ripe egg. The prothoracic glands appear to be normal and ready to secrete in a few days after the permanent larvae were transferred to uncontaminated jars. The moult into perfect or deformed adults follows within 2–3 weeks. In some way JH appears to cause an ecdysone deficiency which is reflected in the inhibition of cuticle synthesis. At the same time JH induces vitellogenesis, which is made possible again by deficiency of ecdysone, the supposed inhibitor of ovarian function in insects[5,6].

In contrast to JH treatment, the injection of ecdysone into freshly moulted last stage larvae shortens the instar considerably (Table II). The epidermis is forced to synthesize the new cuticle and the larvae moult, presumably without having a chance to re-program the existing genetical information[7]. Larvae injected with 5 μg moult after 12 days into an extra larval stage as has been described for Holometabola[7,8]. Still higher dosages accelerate cuticle snythesis even more and also disturb the mechanism of ecdysis. The pharate extralarvae die trapped inside the old cuticle. The effect of 5 μg of ecdysone is counteracted by simultaneous JHA treatment and the larvae remain permanently in the last stage. Injection of 10 μg of ecdysone into larvae simultaneously treated with JH or JHA, at a dose high enough to prevent the whole moulting process, stimulates the synthesis of the new cuticle (Table II). Ecdysone fails, however, to restore normal ecdysis and the larvae die in the old skin, as do JH-treated younger instars.

The extralarvae induced by JH are recognized by their dark pigmentation. Within 15–18 days they attempt to moult into the next extralarval stage, but they die during ecdysis. The extralarvae induced by ecdysone show the normal last stage colour pattern. Within 2–3 weeks they moult into perfect adults. This indicates the normal functioning of the prothoracic glands, which should, however, degenerate after a secretory cycle in the absence of JH[9]. Preservation of the glands seems to be the result of abnormal conditions of accelerated development after injection of ecdysone into the last stage larvae. The prothoracic glands might stay inactive or they could be protected by remaining traces of JH.

A high titer of JH prevents ecdysis in any larval stage of the german cockroach. The beginning of the last stage, however, is the only moment during the whole post-embryonic development of this hemimetabolous species when the actual synthesis of the new cuticle can be blocked, as was already demonstrated in many holometabolous insects. The reversal of this JH effect by ecdysone is consistent with the hypothesis of an antagonism between these 2 hormones proposed by Benz[10]. This concept is supported by several in vitro results[11–13], and our findings provide the first evidence in vivo. Clearly, JH has 2 effects on larvae of *Blattella*: it blocks ecdysis in all stages and it blocks cuticle synthesis during the last larval instar. Whether these 2 effects of JH on moulting are peripheral effects on the epidermal cells or central effects on the neuroendocrine system remains to be proved[14].

[5] W. E. Robbins, J. N. Kaplanis, M. J. Thompson, T. J. Shortino, C. F. Cohen and S. C. Joyner, Science *161*, 1158 (1968).
[6] M. J. Thompson, J. A. Svoboda, J. N. Kaplanis and W. E. Robbins, Proc. R. Soc., Lond., Ser. B, *180*, 203 (1972).
[7] J. Žďárek and K. Sláma, Biol. Bull. *142*, 350 (1972).
[8] C. M. Williams, Biol. Bull. *134*, 344 (1968).
[9] V. B. Wigglesworth, Adv. Insect Physiol. *2*, 247 (1964).
[10] G. Benz, Experientia, 29, in press. (1973).
[11] L. F. Congote, C. E. Sekeris, R. Karlson, Expl Cell Res. *56*, 338 (1969).
[12] M. Lezzi and L. I. Gilbert, Proc. natn. Acad. Sci., USA *64*, 498 (1969).
[13] H. Laufer and T. K. H. Holt, J. exp. Zool. *173*, 341 (1970).
[14] We are greatly indebted to Prof. H. A. Schneiderman, University of California, Irvine, for helpful criticism.

Zusammenfassung. Behandlung von *Blattella germanica* im letzten Larvenstadium mit hohen Dosen von Juvenilhormon verhindert ausser der Morphogenese auch die Bildung der neuen Cuticula und die Häutung. Die Prothoracaldrüsen der entstandenen Dauerlarven sind normal; durch anschliessende Injektion von α-Ecdyson wird Cuticulasynthese induziert, die Tiere sterben jedoch in der alten Haut. Diese Befunde unterstützen die Hypothese der antagonistischen Wirkung von Juvenilhormon und Ecdyson.

P. MASNER and W. HANGARTNER

Biological Laboratory, Dr. R. Maag, Ltd., CH-8157 Dielsdorf (Switzerland), 25 April 1973.

Effect of Dopaminergic Blocking Agents on Plasma Luteinizing Hormone Releasing Hormone Activity in Hypophysectomized Rats

The releasing factor (RF) mechanisms of the hypothalamus are regulated by dopaminergic systems of the diencephalon[1,2]. Numerous RFs are detectable in plasma after hypophysectomy[3], thus providing a model for evaluating the effects of pharmacologic agents on the hypothalamic neurotransmitter/RF system. The present report describes preliminary experiments on the effect of two dopamine receptor blocking agents, pimozide and fluspirilene, on the plasma luteinizing hormone releasing hormone (LRF) activity in hypophysectomized rats.

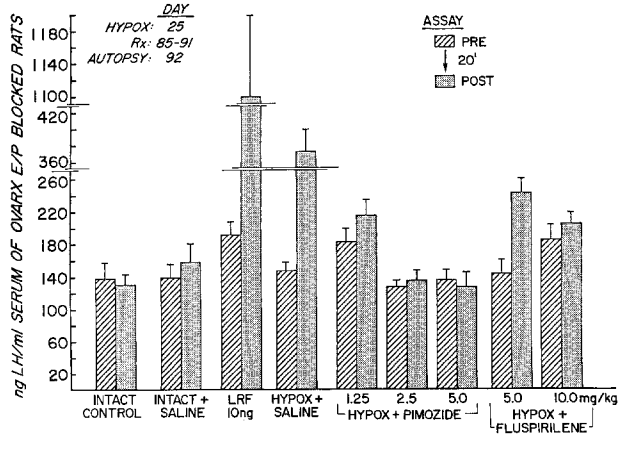
Methods and materials. Female Sprague-Dawley (S-D) rats were hypophysectomized when 25 days old and used 60 days later. Rats (5/group) received various i.p. doses of pimozide or fluspirilene[4] from days 85 to 91. On the following day the rats were sacrificed and plasma obtained for LRF assay. Plasma also was obtained from intact rats of the same age.

Plasma LRF activity was evaluated by measuring the quantity of LH released into the serum of adult ovariectomized S-D rats that had been treated 72 h earlier with s.c. doses of 50 μg estradiol benzoate (E) plus 25 mg progesterone (P) (5 rats/group). A blood sample was obtained before and 20 min after i.v. injection of 1.0 ml of the donor plasma. Serum LH levels were determined by the double antibody radioimmunoassay technique of NISWENDER et al.[5] and are expressed in terms of NIAMD-Rat LH-RP-1.

Results and discussion. The data are presented in the Figure and demonstrate that plasma derived from intact adult female rats does not possess any detectable LRF activity. The responsiveness of the ovariectomized assay rats was established by injection of 10 ng of synthetic LRF[6], which produced a very dramatic and significant ($P \leq 0.01$) rise in serum LH. In contrast to the intact rats, plasma derived from female rats that had been hypophysectomized for 60 days and that received only saline, produced a prominent and significant increment in serum LH ($P \leq 0.01$), attesting to the presence of LRF activity. Treatment of hypophysectomized rats with pimozide or fluspirilene eliminated or reduced plasma LRF activity; the former drug demonstrated the greater potency.

Pimozide and fluspirilene are clinically effective neuroleptics[7,8] and have been shown to be central nervous system dopamine receptor blockers[9]. Various studies support the concept that dopaminergic systems within the hypothalamus play a significant role in subserving the gonadotropin-releasing hormone (GnRH) mechanism resident in the hypophysiotropic area[10,11]. It follows therefore, that pharmacologic agents blocking this neurotransmitter system should inhibit the hypothalamic GnRF/pituitary LH-FSH axis. The results of the present study support those of OJEDA and MCCANN[12] who demonstrated that pimozide can partially inhibit the postcastration rise of plasma FSH, and show that pimozide's antigonadotropic action is via inhibition of hypothalamic GnRH. In addition, pimozide impedes the secretion of hypothalamic prolactin-inhibitory factor and leads to prolactin hypersecretion[13].

Our preliminary clinical studies with pimozide are relevant to the central theme discussed herein. The drug



Effect of CNS agents on plasma LRF activity in hypophysectomized female rats.

[1] T. HÖKFELT and K. FUXE, in *Brain-Endocrine Interaction* (Eds. K. M. KNIGGE, D. E. SCOTT and A. WEINDL; S. Karger, Basel 1972) p. 181.
[2] S. M. MCCANN, P. S. KALRA, A. O. DONOSO, W. BISHOP, H. P. G. SCHNEIDER, C. P. FAWCETT and L. KRULICH, in *Brain-Endocrine Interaction* (Eds. K. M. KNIGGE, D. E. SCOTT and A. WEINDL; S. Karger, Basel 1972), p. 224.
[3] A. CORBIN, E. L. DANIELS and J. E. MILMORE, Endocrinology *86*, 735 (1970).
[4] Janssen Pharmaceutica, Beerse, Belgium.
[5] G. D. NISWENDER, A. R. MIDGLEY JR., S. E. MONROE and L. E. REICHERT JR., Proc. Soc. exp. Biol. Med. *128*, 807 (1968).
[6] Wyeth Compound No. 16, 558.
[7] P. JANSSEN, J. BRUGMANS, J. DONY and V. SCHUERMANS, J. Clin. Pharmac. *12*, 26 (1972).
[8] G. CHOUINARD, T. A. BAN, H. E. LEHMANN and J. V. ANANTH, Curr. ther. Res. *12*, 604 (1970).
[9] N. E. ANDEN, S. G. BUTCHER, H. CORRODI, K. FUXE and U. UNGERSTEDT, Eur. J. Pharmac. *11*, 303 (1970).
[10] R. I. WEINER, R. A. GORSKI and C. H. SAWYER, in *Brain-Endocrine Interaction* (Eds. K. M. KNIGGE, D. E. SCOTT and A. WEINDL; S. Karger, Basel 1972), p. 236.
[11] J. C. PORTER, I. A. KAMBERI and J. G. ONDO, in *Brain-Endocrine Interaction* (Eds. K. M. KNIGGE, D. E. SCOTT and A. WEINDL; S. Karger, Basel 1972), p. 224.
[12] S. OJEDA and S. M. MCCANN, Fedn. Proc. *32*, 227 (abstr.) (1973).
[13] J. RAUS, Janssen Pharmaceutica, Beerse, Belgium, personal communication.