

In order to assess hormone specificity for the labeling, the same 3-h incubation at 37 °C with 0.5 nM ^3H -DHT with the simultaneous presence of a 2000-fold excess of non-radioactive hormone, DHT, or diethylstilbestrol (DES), was carried out. As shown in figure 2,c, no appreciable labeling occurs in an incubation containing excess DHT. DES, on the contrary, elicits no change in the labeling of the cells, even at this excess (figure 2,d). These results indicate the DHT-specificity for the labeling.

The labeling of fibroblasts from each incubation was quantitated by counting the grains (table). The background as assessed from the labeling present on fibroblasts incubated without hormone was 5.34 ± 0.52 grains/cell (mean $\pm$ SE of determinations in 50 cells). Fibroblasts from an incubation containing ^3H -DHT had 15.86 ± 1.42 grains/cell (mean $\pm$ SE of determinations in 50 cells). Incubation of cells with excess DHT besides labeled hormone reduced the labeling to 7.26 ± 0.80 grains/cell (mean $\pm$ SE of determinations in 50 cells), a decrease by 81.7% from the value for an incubation containing labeled hormone alone when corrected for the background. On the contrary, the simultaneous presence of excess DES in an incubation gave the value of 13.15 ± 1.35 grains/cell (mean $\pm$ SE of determinations in 50 cells), showing no change in the labeling despite

a 25.8% decrease in terms of the values corrected for the background. These data add further verification to the findings obtained from photographs.

This study provides direct evidence for a cytoplasmic and nuclear localization of DHT binding sites in cultured human fibroblasts, in the cytosol of which the existence of DHT receptors was also confirmed by sucrose gradient analysis carried out in parallel.

- 1 Acknowledgments. Gratitude is extended to Dr K. Yasuhira and Mr T. Matsushita for pertinent advice and technical assistance, respectively, and to M. Ohara for criticism of the manuscript.
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A histochemical study of leucine amino peptidase activity in the testes of immature and mature guinea-pigs

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Summary. The distribution of the enzyme leucine amino peptidase (LAP) has been studied histochemically in immature and mature guinea-pig testes. Immature guinea-pig testis showed a very feeble LAP activity, while in mature ones strong LAP activity was noted. The present communication indicates a direct relationship between the activity of the enzyme LAP and sexual maturation.

Leucine amino peptidase (LAP) activity has been demonstrated in several endocrine glands¹⁻³ including the testis of different vertebrates⁴. Further it has been reported that the activity of the enzyme LAP is enhanced during HCG-induced hyperactivity of Leydig cells, while estrogen-induced blockage of pituitary gonadotropins diminished the activity of this enzyme in guinea-pig testis⁵. The present investigation has been undertaken to correlate histochemically the activity of the enzyme LAP with testicular maturity in guinea-pig.

Materials and methods. 8 mature and 8 immature male guinea-pigs were taken. The animals were killed and the testes were dissected out. Pieces of testes were fixed in Bouin's fluid for histological studies.

Testicular pieces were also fixed in chilled buffered formol (10%) for 3-4 h for histochemical demonstration of the LAP activity. Frozen sections of prefixed tissues were cut at 20 μm in a cryostat at -20 °C, collected over pre-cleaned coverslips, and immersed in an appropriate medium for LAP activity according to the improved method of Nachlas et al.⁶, using leucyl-4-methoxy-naphthylamide as substrate. Parallel sections, incubated in substrate free medium, served as controls. Paraffin sections were cut at 6 μm and stained with PAS-haematoxyline for histological studies.

Results. PAS haematoxyline stained sections of testes showed the presence of numerous spermatozoa and prominent Leydig cells in the mature ones and their absence in the immature ones, which confirmed the maturity and immaturity of the respective testes. Histochemical studies

revealed the presence of LAP activity in Leydig cells of both mature (figure 1) and immature (figure 2) guinea-pig testes, but the intensity of reaction of this enzyme was significantly higher in mature ones than in the immature ones.

Discussion. In the present investigation a marked rise in the LAP activity has been observed histochemically in Leydig

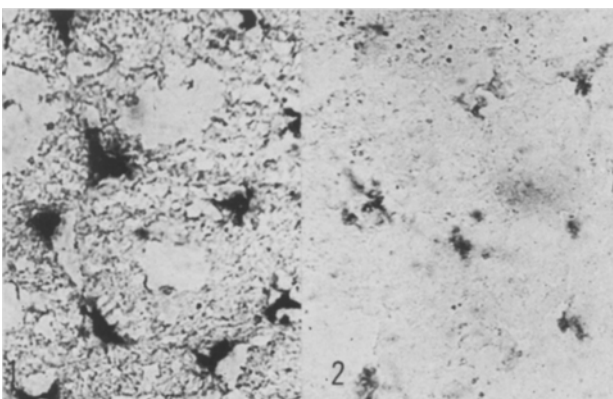


Fig.1. Leucine amino peptidase in testis of mature guinea-pig. $\times 69$.

Fig.2. Leucine amino peptidase in testis of immature guinea-pig. $\times 69$.

cells following sexual maturation. This finding is in positive correlation with the previous observations of Dey et al.⁵, that increased LAP activity is associated with HCG treatment and a fall of LAP activity following estrogen induced inhibition of gonadotropic hormones. Further it is now well known that the Leydig cells are stimulated during sexual maturation⁷⁻⁹ due to increased secretion of gonadotropins¹⁰.

Hence the present finding may further support the positive relationship between LAP activity and gonadotropin-regulated Leydig cell function in guinea-pigs. Therefore, on the basis of previous⁵ and the present experiments, the histochemical demonstration of LAP activity in the testis can be considered as a valuable parameter for evaluating the functional status of the Leydig cells in the guinea-pig.

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Effets de l'intoxication par le lindane sur les corpora allata de *Locusta migratoria* (Orthoptère)

Effects of intoxication by lindane on the corpora allata of *Locusta migratoria* (Orthoptera)

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Summary. The corpora allata of adult and 5th instar nymphs of the migratory locust exhibit several ultrastructural alterations after lindane intoxication. The type of cell degeneration observed here is very similar to ultrastructural changes obtained after the administration of precocene.

Dans nos travaux antérieurs¹, nous avons pu mettre en évidence diverses modifications ultrastructurales dans les cellules neurosécrétrices protocérébrales de mouches domestiques intoxiquées par le lindane. Nous avons montré, en outre, que l'intoxication par cet insecticide provoque des perturbations du développement ovarien avec stérilisation partielle ou totale selon le degré d'empoisonnement des femelles exposées à des doses subléthales. Récemment², nous avons examiné les effets cytopathologiques du lindane sur les corpora cardiaca du criquet migrateur, *Locusta migratoria* et décrit diverses lésions tant dans la partie nerveuse que dans la partie glandulaire de ces glandes. Nous complétons ces investigations par l'étude des effets de l'intoxication par le lindane sur les corpora allata. Les altérations ultrastructurales décrites dans la présente note suggèrent que le lindane, lors d'une intoxication aiguë, provoquerait une inhibition de l'activité glandulaire.

Matériel et méthodes. Des criques (*Locusta migratoria cinerascens*) de sexe femelle, au dernier stade larvaire (5e) et au début de la vie imaginaire, ont été intoxiqués par application topique d'une microgoutte d'une solution acétonique de lindane, déposée au niveau de la région collaire. Une dose subléthale provoquant environ 90% de knock-down en 24 h a été appliquée soit 6 µg par individu pour les larves du dernier stade et 8 µg par individu pour les imagos. L'action du lindane provoque des troubles neuromusculaires caractérisés par une série de symptômes cliniques se succédant de façon systématique et décrits chez *Schistocerca*³. On observe une phase prodromique typique, choreoataxique, clonique et psérique (ou paralytique). Les insectes étudiés ont atteint la phase paralytique. Les corpora allata ont été disséqués in situ après ouverture de la région

occipitale de la capsule céphalique et application d'une goutte de fixateur. Ce dernier est constitué par une solution de glutaraldéhyde à 2,5% dans un tampon cacodylate 0,1 M (pH 7,4) additionné de saccharose 0,15 M. Après rinçage, on réalise une postfixation dans du tétroxyde d'osmium à 1%. Les organes sont ensuite inclus dans l'épon 812. Les coupes ultrafines sont contrastées par l'acétate d'uranyle et le citrate de plomb.

Résultats. Les corpora allata de *Locusta* sont de type semi-centralisé⁴. Ils sont constitués de cellules glandulaires d'aspect identique enveloppées par une fine assise conjonctive ou «lamina basale» entre lesquelles s'insinuent des fibres nerveuses ordinaires et les prolongements des fibres neurosécrétrices émanant des cellules neurosécrétrices protocérébrales médianes (pars intercerebralis) incluses dans les nerfs paracardiaques internes (NCCI). L'ultrastructure des corpora allata chez les larves et les adultes de *Locusta* a été décrite par divers auteurs⁵⁻¹¹. Les cellules glandulaires présentent un cytoplasme d'opacité variable aux électrons. Toutefois, la majorité des auteurs semble convenir que ce caractère constitue plus un artefact qu'une différence structurale à mettre en rapport avec l'activité sécrétrice. Des travaux antérieurs¹¹ suggèrent qu'il existerait trois zones fonctionnelles structurellement différenciées dans ces cellules.

L'intoxication aiguë par le lindane de *L. migratoria*, tant au 5ème stade larvaire que chez les femelles en cours de premier cycle ovarien provoque des modifications ultrastructurales assez prononcées dans les corpora allata. Nous observons chez les individus intoxiqués une augmentation anormale du volume des espaces intercellulaires. Chez les larves du 5e stade, dont les corpora allata sont inactifs,