Tab. II. Assay of agar plates for juvenile hormone activity using chilled pupae of *A. polyphemus* as assay animals

Pupa	Material assayed	Days for assay pupa to molt	Effect	Juvenile hormone activity
1	1/2 of clear inhibitory zone	24	normal adult	—
2	1/4 of clear inhibitory zone	26	normal adult	—
3	1/3 of clear inhibitory zone	25	normal adult	—
4	3/4 material in well	15	pupal-adult intermediate	++++
5	1/2 material in well	14	pupal-adult intermediate	+++
6	1/2 material in well	14	pupal-adult intermediate	+++

spectra are smiliar, they are not identical, suggesting that the active substance from *cecropia* and *orizaba* differs from that found in royal jelly.

Using *Proteus vulgaris* as the test organism, slight inhibitory activity was found with extracts of adult wax moths (*Galleria mellonella*), male adult *cynthia* and *cecropia* pupae. No activity was found with female *cynthia* or with *cecropia* hemolymph.

Preliminary results indicated that smaller zones of inhibition and a different spectrum of antimicrobial activity were obtained with trypticase soy agar (pH 7.4) than with nutrient agar (pH 6.8). That this is due to the difference in pH of the media is indicated by the finding that the antibacterial activity increases proportionally upon acidification of the nutrient agar from pH 7.5 to pH 6.0. The results suggested that the active principle in these extracts was a fatty acid(s) and that with dissociation of the acid, the antibacterial activity declines. Further evidence supporting this view was obtained by allowing active extract to diffuse into agar containing bromthymolblue indicator. After 2 h an acid reaction occurred in an area approximating the inhibition zone found in a duplicate plate seeded with *P. vulgaris*. To substantiate further the fatty acid nature of the active principle, male *cecropia* extract was gently saponified with methanolic KOH. The ether soluble components were removed, the saponified material brought to a pH of 3 with HCl, and fatty acids extracted with ether. Both fractions were evaporated *in vacuo* and assayed with *P. vulgaris* in nutrient agar. Antibacterial activity was associated only with the saponifiable fraction, supporting the view that a free fatty acid(s) is responsible for the bacterial inhibition. These data exclude the juvenile hormone as the active substance since it is associated with the unsaponifiable fraction (SCHNEIDERMAN and GILBERT[9]). Further evidence was obtained for dissociating the antibacterial effect from the juvenile hormone component of the ether extract. The agar containing the inhibitory zone and the residual oil in the wells were separately removed from assay plates and assayed for juvenile hormone activity on chilled *Polyphemus* pupae (GILBERT and SCHNEIDERMAN[10]). Table II reveals that the juvenile hormone remained in the well since this caused the assay pupae to molt into pupal-adult intermediates while the clear agar of the inhibitory zone showed no juvenile hormone activity[11]).

Zusammenfassung. Extraktstoffe von *H. cecropia*, *S. cynthia* und *R. orizaba* zeigten antibakterielle Wirkung gegenüber mehreren Mikroorganismen. Ein schwächerer Effekt wurde auch mit den Extraktstoffen von *Galleria mellonella*, adulten Männchen von *Cynthia-* und von *Cecropia*-Puppen beobachtet. Die antimikrobielle Aktivität wurde mit dem Fettsäureanteil der Extraktstoffe in Beziehung gesetzt und von der Wirkung des Juvenilhormons abgegrenzt.

R. A. SLEPECKY and L. I. GILBERT

Department of Biological Sciences, Northwestern University, Evanston (Illinois USA), July 12, 1961.

[9] H. A. SCHNEIDERMAN and L. I. GILBERT, Anat. Rec. *128*, 618 (1957).

[10] L. I. GILBERT and H. A. SCHNEIDERMAN, Trans. Amer. Microsc. Soc. *79*, 38 (1960).

[11] *Acknowledgment.* We are grateful to Mr. R. B. WILSON, Royal Jelly Research Foundation, for a gift of Royal Jelly.

Hormonal Influences on Leucine Aminopeptidase in the Accessory Reproductive Tracts of the Rat

Effects of various sex hormones on the concentration of numerous enzymes in the seminal vesicles and prostates have been studied, e.g. acid and alkaline phosphatase[1], carbonic anhydrase[2], succinate dehydrogenase[3] etc. The behaviour of these enzymes after castration and hormonal treatments is not at all similar.

So far as is known, no corresponding studies concerning the changes observable in leucine aminopeptidase (LAP) in the accessory genital tract have been published. Such a study is described in the present work.

LAP is a proteolytic exopeptidase taking part in protein degradation and possibly also in protein synthesis. A great interest was aroused when it was observed to be increased in the blood during pregnancy and certain malignant tumours[4]. It is a widely distributed enzyme, being present in nearly all tissues so far studied.

In the present study, altogether 121 male rats aged 3–4 months were used. Castration was performed on 12 rats, which were killed 10–14 days later. The following subcutaneous hormone treatments were used: to 13 rats testosterone (Sustanon, N.V. Organon) was injected 1 mg daily, to 11 rats chorion gonadotrophin (Pregnyl, N.V. Organon) 40 IU daily, to 5 rats serum gonadotrophin (Gestyl, N.V. Organon) 46 IU daily, to 15 rats luteotrophic hormone (Prolactin, N.V. Organon) 10 IU daily, to 14 rats cortisone 0.5–2.0 mg (Adreson, N.V. Organon) daily, to 7 rats ACTH (Lääke Oy) 5 UI daily, and in addition to 11 rats reserpin (Lääke Oy) 0.05 mg daily. All these treat-

[1] R. O. STAFFORD, I. M. RUBINSTEIN, and R. K. MEYER, Proc. Soc. exp. Biol. Med. *71*, 353 (1949).

[2] T. MIYAKE and G. PINCUS, Endocrinology *65*, 64 (1959).

[3] A. TELKKÄ, A. KIVIKOSKI, and V. K. HOPSU, Acta endocr. (Kbh)., in press.

[4] J. A. GOLDBARG and A. M. RUTENBURG, Cancer *11*, 283 (1958).

ments were given for 10–14 days after which the rats were killed and the accessory sex organs were removed and weighed. Altogether 33 rats were used as controls.

Histochemically LAP was demonstrated by the method of NACHLAS et al.[5]. Quantitative enzyme determinations were made according to the method of GOLDBARG and RUTENBURG[4]. In both of these *L*-leucyl-*β*-naphthylamide was used as substrate. From the seminal vesicles a circular piece was taken from the middle of the vesicle. From the prostate gland a piece was always taken from the same place of the ventral lobe without paying any more attention to the constant differences in the enzymatic activity in various regions of the gland.

Histochemical localization of LAP is visible in Figure 1 which is from a normal prostate gland. It is clearly located mainly in the epithelium. The same was stated in the seminal vesicles. A clear increase in the enzyme activity can be seen after castration in Figure 2.

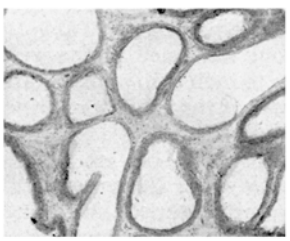

Fig. 1. LAP in the normal prostate gland of a rat.

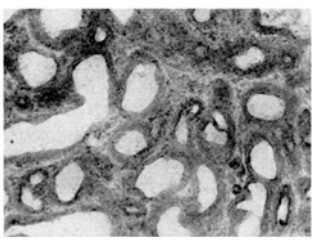

Fig. 2. LAP in the prostate gland of a castrated rat.

Changes in the weight of the seminal vesicles and in the LAP activity of the seminal vesicles and prostate gland after various hormone treatments.

Kind of treatment	Percentage changes of the control values		
	Weight of the seminal vesicles	Activity of LAP[a]	
		Seminal vesicles	Prostate gland
Castration	− 60.7	+ 20.4	+ 23.1
Testosterone	+ 85.2	− 15.0	− 21.2
Serum gonadotrophin	+ 40.8	− 33.5	− 22.1
Chorion gonadotrophin	+ 37.2	− 53.8	− 32.9
Luteotrophic hormone	− 2.3	+ 56.2	+ 60.3
ACTH	− 4.0	+ 22.2	+ 26.3
Cortisone	− 4.6	+ 15.2	+ 16.9
Reserpine	− 31.2	+ 25.7	+ 21.3

[a] All these changes were statistically significant at least at the level $P < 0.05$.

Numerical results from the quantitative enzyme determinations are presented in the Table. In this Table, only the percentage changes of the control values are presented both in the weight of the seminal vesicles and LAP concentrations.

It is obvious that marked changes in the concentration of LAP in the accessory sex organs can be induced with various hormonal treatments. As a general conclusion, it can be stated that treatments which caused a decrease in the weight of the seminal vesicles, e.g. castration, luteotrophic hormone, cortisone, ACTH and reserpine, have increased the activity of LAP both in the prostate gland and seminal vesicles. Treatments again having increased the weight of the vesicles, e.g. testosterone, serum and chorion gonadotrophins, have decreased the activity of LAP. Relatively, the greatest changes were observed after treatment with chorion gonadotrophin and luteotrophic hormone.

The weight of the seminal vesicles is known to be a good indicator of the activity of the seminal vesicles and prostate gland, and thus also an indicator of andromimetic and androlytic effects. So it can be concluded that all treatments which caused a decreased function in these glands, or had androlytic effects, caused an increase in the LAP activity, and those having andromimetic effects or increased the secretory activity, caused a decrease in LAP activity.

In those enzymes participating in functions of these glands, an opposite situation could be expected. Succinate dehydrogenase, for instance, correlates well with changes in the functional state of these glands, showing markedly increased values after andromimetic and decreased ones after androlytic treatments. Therefore, the conclusion can be drawn that LAP in the seminal vesicles and prostate gland has no significant role in the secretory functions of these glands.

Earlier LAP has been obtained to show an increased activity in the parathyroid glands after feeding rats on a Ca-deficient diet[6], and in the renal tubules soon after birth of the rat[7], as well as in the mammary gland tissue after weaning of the lactating rat[8]. A decreased activity is again observed in the parathyroids after treatment with AT 10[6], in the thyroid after treatment with thiouracil[9] or TSH[10], in the gastric mucosa after adrenalectomy[11], as well as in the mammary gland after beginning of the lactation[12]. From this summary, the conclusion can be drawn that LAP may participate in one way or other in many processes: hormone and enzyme production, tubular function and tissue digestion etc.

The present observations of an increased enzyme activity, always in connection with the atrophy of the accessory sex glands, may be an indication only of general digestive processes which occur during the regression and necrobiosis of cells[13]. WOLFF[14] has studied the digestive activity in the Müllerian duct of developing male chick embryos. Proteolytic enzymes were active when endogenous or exogenous androgens were at high level, which condition favours regression of the duct. LOBEL et al.[15] again observed no enzymatic LAP activity in the healthy follicles but a marked activity in the follicular epithelium when follicles appeared atretic. An increased activity was also observed in lutein cells prior to next ovulation before and during shrinkage of the corpora lutea.

On the base of the present observations, however, possible specific effects of one or some of these hormones on LAP in the accessory sex organs cannot be excluded. The quantitative LAP determinations in blood serum and in the adrenal glands made in the same experimental conditions showed no constant changes when compared with controls.

[5] M. M. NACHLAS, D. T. CRAWFORD, and A. M. SELIGMAN, J. Histochem. *5*, 264 (1957).
[6] A. G. E. PEARSE and G. TREMBLAY, Nature *181*, 1532 (1958).
[7] V. K. HOPSU and S. RUPONEN, Acta physiol. scand., in press.
[8] V. K. HOPSU, S. RUPONEN, and S. TALANTI, Ann. Med. exp. Fenn., in press.
[9] S. TALANTI and V. K. HOPSU, Acta endocr. (Kbh.) *35*, 481 (1960).
[10] V. K. HOPSU and S. TALANTI, Ann. Med. exp. Fenn. *38*, 427 (1960).
[11] S. TALANTI and V. K. HOPSU, Endocrinology *68*, 184 (1960).
[12] S. TALANTI and V. K. HOPSU, Nature *191*, 86 (1961).
[13] A. B. NOVIKOFF, Biol. Bull. *117*, 385 (1959).
[14] E. WOLFF, Exper. *9*, 121 (1953).
[15] B. I. LOBEL, R. M. ROSENBAUM, and H. W. DEANE, Endocrinology *68*, 232 (1961).

Zusammenfassung. Die Aktivität von Leucinoaminopeptidase in der Prostata und den Samenbläschen der Ratten wurde mit histochemischen und quantitativen Methoden nach verschiedenen Hormonbehandlungen bestimmt. Es wurde gefunden, dass alle androlytischen Behandlungen, die eine Atrophie in diesen Organen verursachten, die enzymatische Aktivität erhöhten. Nach allen Behandlungen, die aber andromimetisch wirkten, war sie immer erniedrigt.

V. K. Hopsu, P. Riekkinen, and E. Luostarinen

Department of Anatomy, University of Turku (Finland), July 19, 1961.

Isolation and Purification of the Penicillinase from Mycobacteria

Woodruff and Foster[1] described in 1945 that a rapidly growing avirulent strain of *Mycobacterium tuberculosis* destroys penicillin. A few years later, Iland and Baines[2] found that a species of *Mycobacterium tuberculosis* produces an extracellular penicillinase after a long incubation period. Ribeiro[3] described penicillinase production in a strain of BCG, and Soltys[4] reported penicillinase as a constitutional enzyme in *Mycobacterium phlei* and BCG strains, and as an inductive penicillinase in human and bovine *Mycobacteria tuberculosis*. He was not able to find penicillinase activity in avian type of *Mycobacteria tuberculosis*. Investigating a great number of species and strains of genus Mycobacteria, Bönicke and Dittmar[5] demonstrated penicillinase as endocellular enzyme in almost all the strains examined. The concentration of extracellular enzyme in liquid media is, according to these authors, proportional to the autolysis rate. By use of 'lysis inducing media' with low concentration of nitrogen substances, they were able to demonstrate that the concentration of extracellular penicillinase depends on the autolysis rate. *Mycobacteria tuberculosis* of the avian type, without apparent autolysis, produced endocellular penicillinase after cellwall disruption by ultrasonic treatment.

Mycobacterial penicillinase activity in various phases of isolation

Preparation	Penicillinase arbitrary units
1. Culture fluid centrifuged until cell free	2.5 PAU/ml
2. Supernatant fluid after benzoic acid precipitation	0
3. Distilled water washing of precipitation	0
4. *M*/1 Tris buffer pH 9.0 solution of butanol precipitate	150 PAU/ml
5. Approximate units per mg of dissolved precipitate	200 PAU/mg
6. Ethanol-phosphoric acid precipitate	500 PAU/mg
7. Protein fraction in supernatant after precipitation by PAS	800 PAU/mg

The arbitrary penicillinase units correspond to the amount of enzyme destroying 50% of sodium penicillin units in a batch of 2000 units in water bath at 37°C, after 10 min, at a pH 7.0 = PAU.

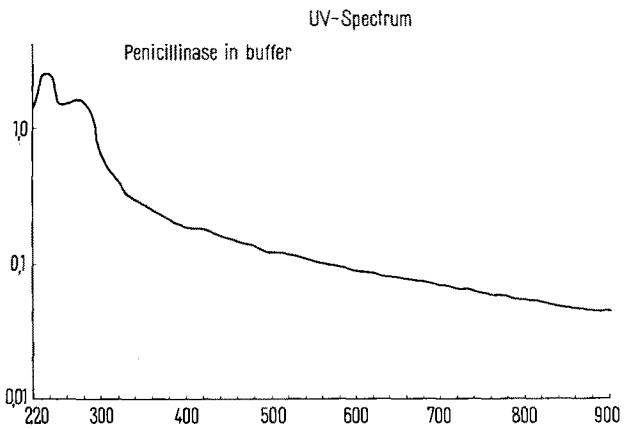

Fig. 1. Penicillinase 0.1 mg/ml of Tris buffer 2 *M* of pH 8.7. Zeiss Spectrophotometer.

Isolation and purification of the penicillinase from mycobacteria

For this purpose, we used *Mycobacterium smegmatis* (Borstel strain SN 2), which shows a great tendency to autolysis and at the same time produces a high yield of extracellular penicillinase. The enzyme was isolated according to the method of Gough[7] as modified by Herrmann[8], who used this method for isolation of high active tuberculins.

Mycobacterium smegmatis was obtained from Löwenstein-Jennsen medium, and inoculated by an enrichment carried out in Lockeman liquid medium. After 21 days of incubation at room temperature, cultures were filtrated through Glass filter (Schott G 5 M) and 5% of sodium benzoate dissolved in the filtrate. The solution was cooled to 0°C, and the benzoic acid was precipitated with 3 *n* HCl up to a pH 3.8. The precipitate was separated by filtration on Whatmann paper No. 3, and washed several times with distilled water saturated with benzoic acid, until the filtrate was free from HCl. All filtrates are free of proteins and contain nearly all the phosphate content of the original liquid medium. The filter cake was dried in vacuum over concentrated sulphuric acid, and then dissolved in *n*-butanol. The butanol suspension was filtered, and the thin filter cake was re-suspended in *n*-butanol and mixed with an equal volume of distilled water. Penicillinase was present in the insoluble protein fraction between the two liquid phases.

After discarding butanol and water, the insoluble fraction was washed by centrifuging with distilled water

[1] H. B. Woodruff and J. W. Foster, J. Bact. *49*, 7 (1945).
[2] C. N. Iland and S. Baines, J. Path. Bact. *61*, 329 (1949).
[3] L. Ribeiro, J. Soc. Cien. med., Lisboa, *119*, 145 (1955).
[4] M. Soltys, Tubercle *33*, 120 (1952).
[5] R. Bönicke and W. Dittmar, Zbl. Bakter. I orig. *170*, 366 (1957).
[6] R. Bönicke, Jahresber. Borstel *4*, 43 (1957).
[7] G. A. C. Gough, Brit. J. exp. Path. *15*, 237 (1934).
[8] R. Herrmann, Biochem. Z. *323*, 181 (1952).