Labeling indices of acinar and islet cells at various intervals, starting from 24 h after partial hepatectomy (No. of labeled nuclei/1000 cells)

		Time interval 24	30	36	42	48	54	60	66	72	84	96	120
Acinar cells	PH	0.2 ± 0.2[1]	0.2 ± 0.2	0.7 ± 0.5	9 ± 4	32 ± 6^b	71 ± 3^b	50 ± 17^c	43 ± 9^a	68 ± 17^c	70 ± 18^d	37 ± 16	4 ± 2
	Sham				4 ± 1	21 ± 1		18 ± 6		6 ± 3	2 ± 1	8 ± 3	4 ± 2
Islet cells	PH	11 ± 6[1]	29 ± 2	35 ± 7	34 ± 17	19 ± 9	10 ± 7	21 ± 5	10 ± 3	11 ± 2	6 ± 2	6 ± 1	6 ± 0
	Sham				8 ± 1	3 ± 1		7 ± 1		7 ± 1	10 ± 1	9 ± 3	4 ± 2

[1] mean ± SE. (In PH group, 4 animals are sacrificed at 24, 30, 36, 42, 66, 72 and 96 h and at each remaining interval 3 animals are sacrificed. In sham group, 4 animals are sacrified at 42, 60, 96 and 120 h and 3 animals at each remaining period.) $^a p < 0.02$, $^b p < 0.001$, $^c p < 0.05$, $^d p < 0.01$. Labeling indices in acinar and islet cells of control hamsters were 2 ± 1 and 13 ± 3 respectively. The labeling index in PH and sham operated groups are compared with the control animals. The labeling index in acinar cells of PH group is significantly greater at 48, 54, 60, 66, 72 and 84 h and in sham group at 48 h, whereas the increase in labeling index of islet cells in PH and sham groups is not significant.

ing indices of islet cells was also noted from 30–60 h, and this increase was not statistically significant. Cells of duct system also showed a minimal increase in labeling indices. However, the labeling indices of the duct cells varied tremendously from one segment of the duct to the other (i.e. interlobular, intralobular and intercalated ducts). To obtain meaningful statistical data on the indices of the duct system, we plan to examine a large number of animals and will be reported in a separate communication. Interestingly, in sham operated animals, acinar cells showed a significant increase in the number of labeled nuclei at 48 h ($p < 0.01$) over the control values. No increased DNA synthesis was seen in islet cells of sham operated animals.

The regenerative response in PH animals is qualitatively similar to that observed in hamsters after ethionine induced degeneration and necrosis[6]. However, the magnitude of response was lower in PH animals. In pancreatic regeneration induced after ethionine treatment, the maximum labeling index was 226 ± 25/1000 cells as compared to 71 ± 3 after PH.

From PH rats, serum factors capable of stimulating hepatocyte growth have been isolated by various investigators[12–14]. These growth factors, in addition to stimulating liver growth, are also capable of inducing pancreatic DNA synthesis as shown here[2]. This response is not surprising, since both liver and pancreas are derived from gut entoderm embryologically. In this regard, it is pertinent to note that hepatocytes can be induced in the pancreas of hamsters and rats after carcinogen administration and dietary modulation[15–17]. Similarly, pancreatic acinar tissue was induced in livers of rats treated with polychlorinated biphenyls[18].

The results of this study clearly demonstrate that the growth stimulatory effect of serum factor(s) released after PH is not organ specific and can stimulate both liver and pancreas. However, the effect of this serum factor on other organs remains to be examined.

1 This work is supported by NIH grant CA 36043.
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High molecular transport proteins for JH-III in insect hemolymph

C.A.D. de Kort and A.B. Koopmanschap

Laboratory of Entomology, Agricultural University, P.O. Box 8031, NL-6700 EH Wageningen (The Netherlands), 23 September 1985

Summary. Using two independent techniques, ultracentrifugation in a KBr-gradient, and native pore polyacrylamide gel electrophoresis in combination with [^{3}H]-epoxyfarnesyldiazoacetate photoaffinity labeling, we showed that in the hemolymph of *Periplaneta americana,* and probably also in *Leptinotarsa decemlineata* JH-III binds to the lipophorin, whereas in *Locusta migratoria* JH-III binds to a different protein.
Key words. Juvenile hormone binding protein; lipophorin; juvenile hormone I; juvenile hormone III.

In the hemolymph, juvenile hormone (JH) occurs in association with specific bindings proteins (JHBP). The first JHBP to be isolated and characterized was that of *Manduca sexta.* It appeared to be a single chain polypeptide of 28,000 mol. wt, capable of binding one molecule of JH ($Kd = 4.4 \times 10^{-7}$ M). Its affinity for JH-I was higher than that for JH-III and it displayed stereoselectivity[1]. However, JH-I only occurs in *Lepidoptera,* and in most other orders of insects JH-III is the principal hormone[2]. In a number of species containing only JH-III, a JHBP with a higher affinity for JH-III ($Kd < 10^{-7}$ M) than for JH-I was found[3–5]. This BP appeared to have a high molecular weight ($\sim$ 500,000) and it was also shown to be stereoselective[4,6]. An

interesting question which still needs clarification concerns the similarities and differences between the JH-III binding proteins in various species. In this paper we identified and compared the JH-III BP in the hemolymph of *Locusta migratoria, Periplaneta americana* and *Leptinotarsa decemlineata*.

Hemolymph was fractionated in a KBr-gradient by the method of Shapiro and Law[7], which separates lipoproteins (lipophorins) from the other proteins by ultracentrifugation. Hemolymph (50 µl) was mixed with 6.5 ml 44% KBr in phosphate buffer pH 7.2, and overlayered with 6.4 ml 0.9% NaCl. After centrifugation at 37,000 rpm for 22 h at 4°C in a Beckman SW 41 rotor, the lipophorins were visible as distinct bands in all three species. Using an ISCO model 272 density gradient fractionator, 750 µl fractions were collected from the top and analyzed for protein content (absorbance at 280 nm), density and JH-III binding activity. For the binding assay, 5 and 10 µl aliquots from each fraction were incubated in 200 µl of TMK-buffer (10 mM Tris-HCL pH 7.4, 0.15 M KCl and 5 mM $MgCl_2$), fortified with 5 mg/ml γ globulin and containing 17,000 dpm [^{3}H] JH-III (NEN, specific activity 672 µCi/µmol). After 1 h, the JH-protein complex was precipitated with PEG (polyethylene glycol, MW 6000) and counted for radioactivity as previously described[4]. Under these conditions, the amount of radioactivity in the precipitate is proportional to the size of the aliquots and therefore to the number of binding sites. In addition, most of the radioactivity can be displaced by excess (10^{-6} M) unlabeled JH-III, indicating that the binding is specific[5].

Figure 1 shows that the peak of JH-III binding activity with hemolymph of the Colorado potato beetle and the cockroach occurred at fraction 8 (density 1.080 g/ml), while with locust hemolymph it was in fraction 13 (density 1,250 g/ml). This strongly indicated that the nature of the JH-III binding protein may differ among insect species.

Tritiated epoxyfarnesyldiazoacetate ([^{3}H] EFDA), a photoaffinity label, has been successfully used to identify the JH-binding sites in hemolymph from *Leucophaea maderae* and *M. sexta*[8,9]. Recently, using this photoaffinity label, we were able to separate the JHBP from the lipophorin in locust hemolymph by native gradient polyacryl amide gel electrophoresis (PAGE)[8]. Since the JH-III binding activity in the Colorado potato beetle and the cockroach appeared to be associated with lipophorins, we decided to check this with [^{3}H] EFDA and PAGE. Attempts to label the JH-III binding sites of beetle hemolymph with EFDA were unsuccessful. However, the JH-binding sites of *P. americana* could be labeled using very diluted hemolymph. As shown in figure 2, the presence of excess unlabeled JH-III prevented 75–80% of the [^{3}H] EFDA from binding, indicating that the photoaffinity label binds to the JH-III specific sites.

After labeling hemolymph of *P. americana* with EFDA under optimal conditions the sample was concentrated by ultrafiltration and subjected to gradient PAGE[10]. Thereafter, the gels were stained and the radioactivity in the various bands analyzed as described previously[10]. As shown in figure 3, the lipophorin of *P. americana* could easily be identified by comparison of gel A (whole hemolymph) and gel B (containing lipophorin isolated by KBr-gradient ultracentrifugation).

The molecular weight of this protein as compared with high molecular weight standards (gel C) ranged between 610,000 and 690,000. The lipophorin from *Locusta* had a molecular weight of 690,000–850,000 in the same gel (gel D). These values are close to those previously reported[10] but higher than values reported by Chino[11] using the sedimentation equilibrium method. However, native gel electrophoresis of lipophorins may be affected by reduced solubility of the proteins, due to the low ionic strength of the buffer-systems used. This may also explain the irregular shape of the band, its tailing and the fact that relatively high amounts of the protein remain in the slot.

Nevertheless, the distribution of radioactivity peaked in the lipophorin band. Hemolymph incubated with [^{3}H] EFDA but without UV-illumination resulted in low labeling, indicating that the radioactivity recovered from gels after UV-treatment is covalently bound.

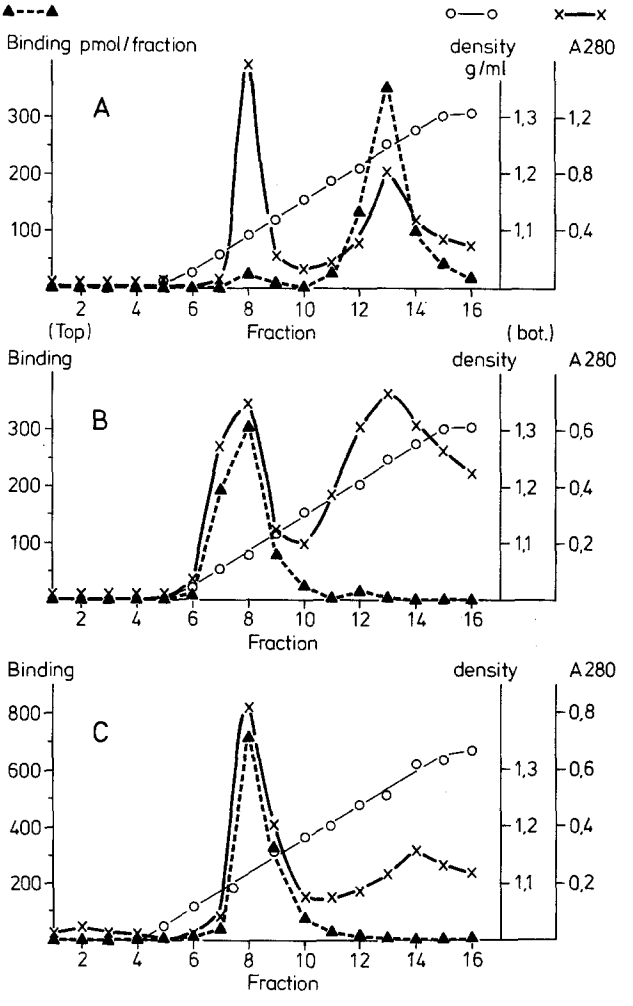

Figure 1. Ultracentrifuge density profiles of hemolymph from adults of *L. migratoria* (A), *L. decemlineata* (B) and *P. americana* (C). Insects and collection of hemolymph were as previously described[5]. Only males were used except for the Colorado potato beetle. Gradients were fractionated from the top into 0.75 ml fractions and analyzed individually for density (gravimetric), protein content (absorbance at 280 nm) and JH-III binding activity (see text).

These results differ essentially from those obtained with locust hemolymph[10], where the JH-III binding protein was distinct from the lipophorin (fig. 3, gel D). Taken together, the present data strongly suggest that binding of JH-III in hemolymph of *P. americana* and probably also *L. decemlineata* occurs with the lipophorins, whereas in *L. migratoria* a different protein of 575,000 mol. wt and density of 1.25 g/ml (fig. 1.[10]) is responsible for binding. It is well documented that insect lipophorins contain at least two subunits, a heavy chain subunit (apolipophorin I) 250,000 mol. wt and a light chain subunit (apolipophorin II), 85,000 mol. wt[11,12]. Whether binding occurs to either of these two subunits is now under investigation.

The gift of [^{3}H] EFDA from Dr G. D. Prestwich (Stony Brook NY) was greatly acknowledged. We thank Dr M. A. Khan for critically reading the manuscript.

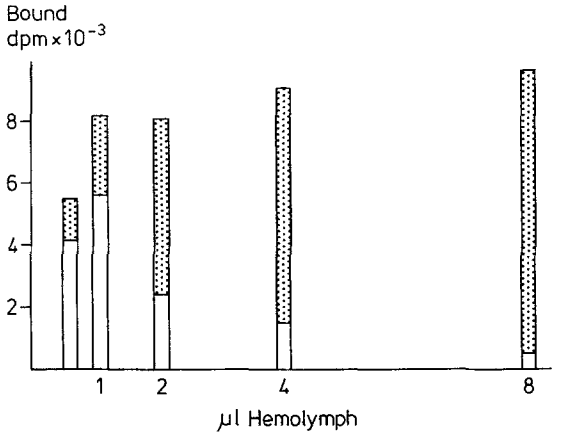

Figure 2. Relation between [^{3}H] EFDA-labeling and the amount of hemolymph from the American cockroach. [^{3}H] EFDA (50,000 dpm, spec. act. 11.0 Ci/mol) was incubated for 1 h with increasing amounts of hemolymph in 200 µl phosphate-buffered saline. After illumination by UV-light as described previously[10], bovine serum (25 µl) was added and the covalently bound [^{3}H] EFDA was precipitated with equal volumes of 10% TCA. After centrifugation, the pellets were washed with 500 µl 5% TCA, 500 µl ether/ethanol 1:1 v/v (2×) and 500 µl ether, dried and the radioactivity counted as described[10]. Whole columns, total amount of covalently bound radioactivity; shaded columns, amount of radioactivity after co-incubation with 10^{-6} M unlabeled JH-III; open columns, amount of [^{3}H] EFDA specifically bound to the JH-III binding sites.

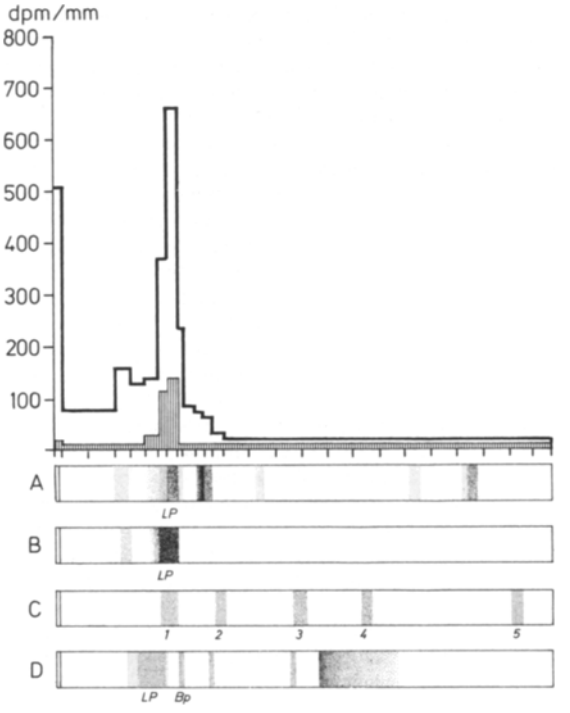

Figure 3. Electrophoretogram (A) and distribution of radioactivity (graph) in a native gradient gel of hemolymph from adult male *Periplaneta* treated with [^{3}H] EFDA (for details see fig. 2). After concentration of the sample, equivalents of 5 µl of hemolymph were applied to each slot, and gels were run and stained as described before[10]. Gels were subsequently sliced as indicated on the horizontal axis of the graph. Slices from two runs were pooled and counted[10]. The shaded graph represents the radioactivity when UV-illumination is omitted. The same slab-gel contained samples of lipophorin from *Periplaneta* (B) isolated by ultracentrifugation[7]; high molecular weight standard proteins (C): 1) Thyroglobulin (669,000 mol. wt), 2) Ferritin (440,000 mol. wt), 3) Catalase (232,000 mol. wt), 4) Lactate dehydrogenase (140,000 mol. wt), 5) Albumin (67,000 mol. wt); and a sample of *Locusta* hemolymph (D). Lp, lipophorin; BP, binding protein.

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Location of allatostatic centers in the pars lateralis regions of the brain of the Colorado potato beetle

M. A. Khan, H. M. Romberg-Privee and A. B. Koopmanschap

Department of Entomology, Agricultural University, Binnenhaven 7, NL-6709 PD Wageningen (The Netherlands), 30 September 1985

Summary. Selective lesions in the pars intercerebralis and pars lateralis areas of the brain of the adult Colorado potato beetle were produced by radiofrequency cautery. The effect of these lesions on the corpus allatum activity, determined by the short-term in vitro radiochemical assay, revealed that gland inhibitory centers are located in the pars lateralis.
Key words. Colorado potato beetle; corpus allatum control; corpus allatum activity; radio-frequency cautery; pars intercerebralis; pars lateralis; lateral neurosecretory cells; allatostatin.