that the antibody specific for the $C_{18}$ compound constituted no more than 4% of the total population.

We have thus shown that the present JH antibodies are highly specific for the structure of the immunogen. High antibody specificity was demonstrated in the competition experiments, in that the only major competitors of either the radioactive $C_{18}$-JH or $C_{16}$-JH (derivative 11) were the $C_{17}$ and $C_{16}$ epoxy compounds. The antisera used in these experiments were of comparatively low binding affinities; however the affinity should increase with increasing time of immunization and this aspect is currently being investigated. With better sera and a $C_{16}$ radioligand of higher specific activity, the sensitivity of the radioimmunoassay should increase well beyond that reported in this paper. It is obvious that these techniques will be useful in quantifying and screening for structurally related juvenile hormones in insects[16].

*Résumé.* Les auteurs ont produit des anticorps contre l'hormone juvenile du parasite du tabac *Manduca sexta* ($C_{16}$ JH) par immunisation avec un conjugé haptène-protéine. Les antisérums ont montré une forte spécificité pour le $C_{16}$ JH et le $C_{17}$ JH et ils contiennent très vraisemblablement un groupe d'anticorps ayant une affinité plus grande pour le $C_{17}$ JH que pour le $C_{16}$ JH.

R. C. LAUER[17], P. H. SOLOMON[18], K. NAKANISHI[18], and B. F. ERLANGER[17]

*Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York (New York 10032, USA), and Department of Chemistry, Columbia University, New York (New York 10027, USA), 17 December 1973.*

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[17] Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York 10032.

[18] Department of Chemistry, Columbia University, New York, New York 10027.

## Antibodies to the Insect Moulting Hormone β-Ecdysone

The moulting hormone of insects and crustaceae, β-ecdysone *1*, in conjunction with the juvenile hormone, regulates metamorphosis[1], and together with similar compounds is widely distributed in various insects, crustaceae and plant species[2]. A rapid and sensitive method of detection and quantification of β-ecdysone would be a valuable tool in further investigations concerning its physiological role. To this end we have sought to develop an immunochemical assay for β-ecdysone and here report the successful preparation of antibodies highly specific for β-ecdysone and capable of distinguishing it from closely related compounds.

*Methods and materials.* A hapten-protein conjugate of a hemisuccinate derivative of β-ecdysone with human serum albumin was used as the antigen. The hemisuccinate was prepared by treating 40 mg of β-ecdysone in 20 ml of tetrahydrofuran containing 25 µl of pyridine with 25 µl of succinyl chloride (freshly distilled) at 0 °C for 16 h. Excess succinyl chloride was decomposed with water and the material was purified by preparative layer chromatography on silica gel. The α, β-unsaturated carbonyl system showed a UV maximum at 242 nm (14,500) and NMR ($d_5$ pyridine, $d_5$ pyridine + conc. HCl) showed the product to be an equimolar mixture of 2, 3, 22-monohemisuccinates *2*.

Treatment of the hemisuccinates with N-hydroxysuccinimide and dicyclohexylcarbodiimide in tetrahydrofuran for 24 h at 4 °C followed by 48 h at room temperature yielded the N-hydroxysuccinimide ester[3] *3*.

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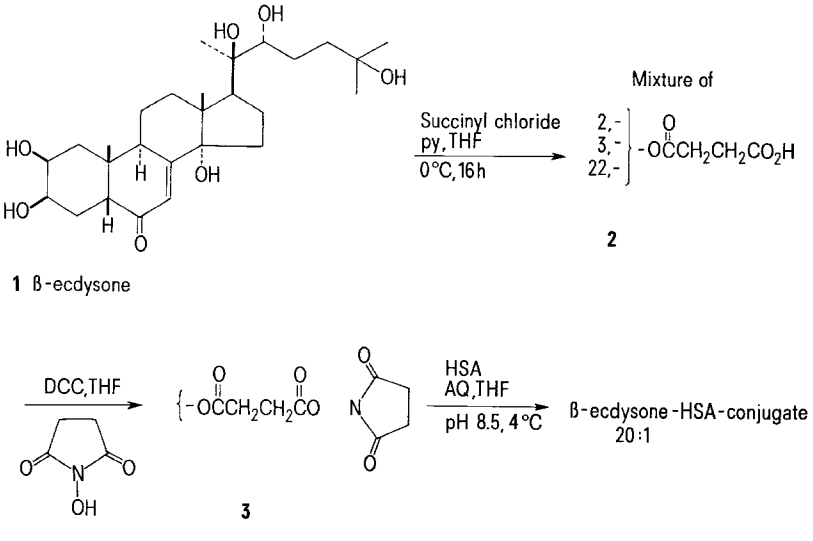
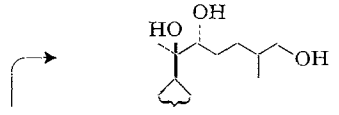
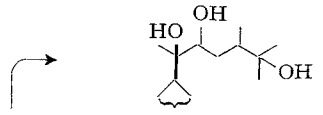
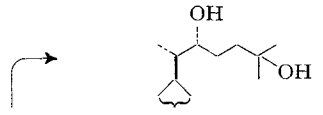


Competition experiments 50% inhibition of binding of $^3H$-β-ecdysone

| Compound | Picomoles | Factor[a] |
|---|---|---|
| β-Ecdysone | 4.6 | 1.0 |
| 5-β-OH-ecdysterone | 7.5 | 1.6 |
|  Inokosterone | 18.0 | 3.9 |
|  Makisterone A | 31.0 | 6.8 |
|  α-Ecdysone | 90.0 | 19.6 |
| 5-β-Ecdysone-2,3,22-triacetate | 100 | —[b] |
| 3-β-20,22-trihydroxy-5α-cholestane | 100 | —[b] |

[a] β-ecdoysone is taken as 1. [b] 50% inhibition was not reached.

After removal of dicyclohexylurea by filtration, 0.2 ml of filtrate containing the 2 mg active ester were added directly to 10 mg of human serum albumin in 1 ml of 50% aqueous tetrahydrofuran at pH 8.5 (adjusted with 0.1 *N* NaOH). After 24 h at 4 °C the solution was dialyzed against running water. The conjugate was recovered by lyophilization and contained 20 hapten groups per molecule of protein as estimated by measurement of the remaining free protein amino groups[4].

Three New Zealand white rabbits were immunized with conjugate emulsified with complete Freund's adjuvant to give a final concentration of 1 mg/ml. The immunization schedule was as follows: all 3 rabbits were injected with 0.1 ml/toepad (0.4 ml per rabbit); 2 of them were boosted with the same amount of antigen-twice at 1 week intervals and twice at 1 month intervals. Monthly bleedings of the rabbits were monitored by Ouchterlongy gel diffusion[5] against a rabbit serum albumin-β-ecdysone conjugate (E-RSA). 1 month after the primary injection, all 3 rabbits showed a precipitin band against E-RSA but not against RSA alone. Monthly bleedings were also tested against $^3H$-β-ecdysone (New England Nuclear) by radioimmunoassay. Antiserum from each rabbit was diluted in saline and 0.1 ml was mixed with 0.1 ml of $^3H$-β-ecdysone (about 2000 cpm, 160 picograms, 6 Ci/mmole) in test tubes. After incubation at 37 °C for 1 h, 0.1 ml of normal rabbit or sheep serum was added as a carrier, followed by 7 ml of half-saturated ammonium sulfate. After precipitation and washing with ammonium sulfate solution (twice) the precipitates were dissolved in 1 ml Soluene 100 (Packard) to which 10 ml of toluene-omnifluor (Packard) was added and counted in an Ansitron II liquid scientillation counter. Controls were run with preimmunization bleedings.

The specificity of the antibodies was studied by radioimmunoassay competition experiment. Saline solutions containing 2000 cpm of $^3H$-β-ecdysone and 0.05 ml of serial dilutions of unlabelled β-ecdysone and related compounds were incubated for 1 h at 37 °C, diluted with carrier, precipitated with ammonium sulfate, and counted as above.

*Results and discussion.* The results of competition experiments in which radioactive $^3H$-β-ecdysone was displaced with increasing molar concentrations of unlabelled ecdysone and its analogs are shown in the Table. The assay was found to be both sensitive and specific. As little as 80 picograms of β-ecdysone could be detected. With regard to specificity, modifications at C-20 were most critical. Indeed, compounds lacking hydroxyl substituent in this position were either weakly competitive or non-competitive in the assay.

After this work was begun, another radioimmunoassay for β-ecdysone was reported[6] in which attachment to the protein carrier was via a derivative of the 6-keto group. Cross reactions with β-ecdysone and inokosterone were also observed. No quantitative data were given for the latter; 50% displacement of $^3H$-β-ecdysone by α-ecdysone occurred at about a 5-fold higher concentration than required for displacement by cold β-ecdysone. This compares with a factor of 19.6 in our assay (Table).

It is interesting to note that rabbit $R_3$, which received only 1 injection of the immunogen, produced the highest titer of specific antibody. Although one should not draw a general conclusion from these results, we have observed this phenomonen at times with other immunogens as well[7].

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*Résumé.* Nous avons préparé des anticorps contre l'hormone de mue $\beta$-ecdysone par immunisation avec un conjugué haptène-protéine. Les antisérums ont pu détecter des quantités de $\beta$-ecdysone aussi petites que 80 picogrammes. Les affinités relatives pour le $\beta$-ecdysone et l'$\alpha$-ecdysone des antisérums sont de 20 contre 1.

R. C. LAUER[8], P. H. SOLOMON[9], K. NAKANISHI[9], and B. F. ERLANGER[8]

*Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York (New York 10032, USA), and Department of Chemistry, Columbia University, New York (N.Y. 10027, USA), 17 December 1973.*

[8] Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York (New York 10032, USA).
[9] Department of Chemistry, Columbia University, New York (New York 10027, USA).

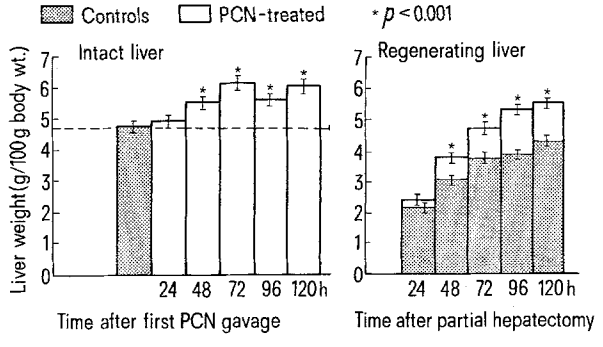
## Effect of Pregnenolone-16 $\alpha$-Carbonitrile on Mitotic Activity in the Intact and Regenerating Rat Liver

A number of investigators have noted mitotic activation of liver cells in animals treated with a variety of hepatic microsomal enzyme inducers[1–6]. But generally, the mitotic response was smaller than in partially hepatectomized rats. Because of this, the hepatomegaly following administration of some drugs was rather considered as hypertrophy of the parenchymal liver cells provoked by increased cell proteins, RNA and lipid content[7,8]. Nevertheless, the proliferative effect of some inducers under certain experimental conditions was high enough to be considered as a liver growth promoting factor[1,3,4,6]. However, because of the limited number of observations, the relationship between the proliferative effcet of the drugs and the induction of drug metabolizing enzymes is poorly understood.

Several groups have examined the response of regenerating liver to a variety of drug-metabolizing enzyme inducers during different stages of liver regeneration[9–14]. The general conclusion was that the metabolic adjustment of the liver imposed by partial hepatectomy exerts suppressive influence on the initial activation of the drug-metabolizing enzymes[9]. A recent report from this laboratory indicates that pregnenolone-16$\alpha$-carbonitrile (PCN) represents an exception. The administration of a single or repeated doses of PCN, at time of partial hepatectomy or 20 h later, resulted in a 3- to 5-fold increase in ethylmorphine demethylase (EMD) and aniline hydroxylase (AH) activity in regenerating and sham-operated rat's livers[15]. Otherwise PCN, like other drug-metabolizing enzyme inducers, brings about a complex biochemical and structural reorganisation of hepatocytes accompanied by marked hepatomegaly[16–18]. In view of these data, it seemed of interest to examine the liver growth promoting influence of PCN in intact and partialy hepatectomized rats with special reference to mitotic activation of parenchymal liver cells.

*Materials and methods.* 2 series of experiments were performed on female ARS Sprague-Dawley rats (Madison, Wisconsin, USA), averaging 100 g (90–110 g) and maintained ad libitum on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water. In the 1st series, we used 36 animals, of which 6 served as controls. In the 2nd, 60 partially hepatectomized rats[19] were divided into 2 equal groups. Here, the first group served as controls.

PCN (3$\beta$-Hydroxy-20-oxopregn-5-ene-16$\alpha$-carbonitrile; Upjohn-U.14.975) was given to each remaining group in both series at the dose of 10 mg in 1 ml water (homogenized with a trace of Tween 80) twice daily by stomach tube for 1, 2, 3, 4, or 5 days. Treatment and partial hepatectomy were started at the same time. The animals were operated on and killed between 09.00 and 10.00 h. Liver sections were fixed in alcohol formol, embedded in paraffin and stained with hematoxylin-phloxine. The number of mitoses and parenchymal liver cells were determined by examining 115 visual fields at $\times 560$. The mitotic index




The effect of PCN on liver weight in intact and partially hepatectomized rats.

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