found to be identical with the authentic 2-hydroxy-3-desoxyestradiol (III) (reported mp 222—224°[5]) by mixed melting point and chromatographic comparison. All these results together permitted the assignment of the sturcture 2-hydroxy-3-desoxyestrone (IV) (reported mp 202—204°[5]) to B. Indeed, comparison of the metabolite with the authentic sample showed identity in every respect, *i.e.* Zimmermann reaction and chromatographic constants (t_R 0.52; TL-I 0.76, II 0.52, III 0.38).

It should be now emphasized that hydroxylation *in vivo* takes place at C-2 and C-3 on aromatic A-ring. A problem whether these biotransformation products may have the lipid-shifting and/or estrogenic activities seems to be of particular interest. Further studies on the characterization of other metabolites including the conjugates are being conducted in this laboratory and the details will be reported in near future.

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Stimulation of Protein Synthesis in Mouse Liver by Insect-Moulting Steroids

Recently it has become generally recognized that insect-moulting steroids are widely distributed in the plant kingdom: isolation of the ponasterones, ecdysterone, inokosterone, pterosterone, and cyasterone, has been reported.[1]

In this communication, we will report that the administration of various moulting steroids to mice can elevate the protein synthetic activity in livers as well as does 4-chlorotestosterone, a potent anabolic steroid.

Male mice of *dd*-strain weighing 18—22 g were used throughout the experiments. Insect-moulting steroids dissolved in 0.9% saline solution were administered intraperitoneally or orally in a dose of 0.05 mg or 0.5 mg per 100 g of body weight. 4-Chlorotestostrone was also suspended in saline and injected in a dose of 1 mg per 100 g body weight. The mice were decapitated at the indicated time after treatment. Livers were removed rapidly and rinsed in an ice-cold 1.15% KCl, weighed and homogenized with 1.5 volumes of Medium K_1[2] by Potter-Elvehjem Teflon homogenizer. The homogenate was centrifuged at $20000 \times g$ for 15 minutes. The supernatant fluid (S-20 fluid) was used as enzyme source for the measurement of ^{14}C-amino acid incorporation *in vitro*. The protein synthetic activity *in vivo* was assayed as follows: 1 μC of ^{14}C-chlorella hydrolysate was injected 15 minutes before sacrifice to mice

1) *cf.* T. Takemoto, Y. Hikino, A. Arai, M. Kawahara, C. Konno, S. Arihara, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo), **15**, 1816 (1967).
2) K. Koike, T. Otaka, and S. Okui, *J. Biochem.*, **59**, 201 (1966).

which were pretreated with steroids. The livers were homogenized in 3 volumes of Medium K_1 and fractionated as described in the legend of Fig. 1. The incorporation of ^{14}C–amino acids was corrected for self–absorption effect.

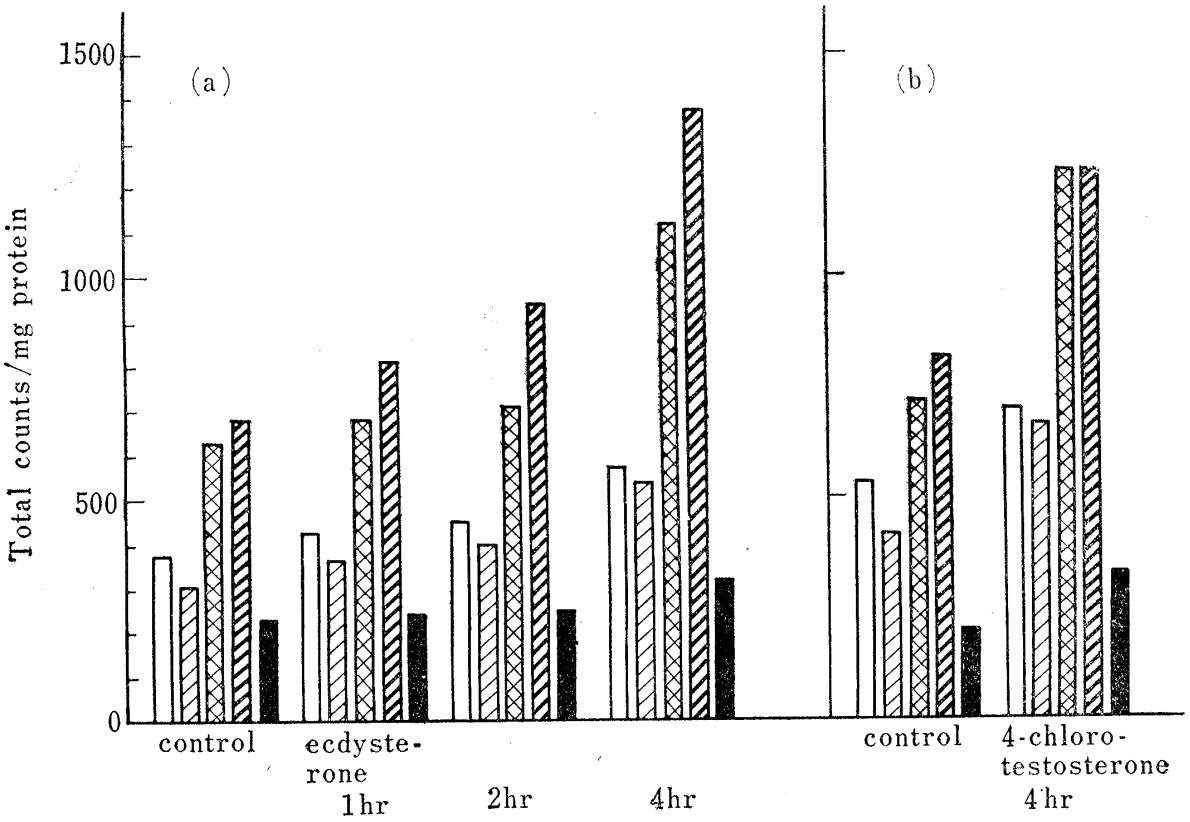

Fig. 1. Stimulatory Effect of Ecdysterone on Protein Synthetic Activity in Mouse Liver *in vivo*

Ecdysterone was injected intraperitoneally in a dose of 0.5 mg per 100 g body weight and 1 μC of ^{14}C-chlorella hydrolysate was also injected intraperitoneally 15 min before sacrifice. The livers were homogenized in 3 volumes of Medium K_1.[1] Nuclei and cell debris were sedimented at $600 \times g$ for 10 min, mitochondria at $9000 \times g$ for 10 min, microsomes at 85000 $\times g$ for 90 min, and supernatant was post-microsomal fluid.
The treatment of protein samples was the same as Table I.

Whole homogenate
Nuclei and cell debris
Mitochondria
Microsome
Supernatnat

The data in Table I indicate that the amino acid incorporation into S–20 fluid obtained from mice livers increased significantly at 2 and 4 hours after the treatment with various insect–moulting steroids. The magnitude of the increment was almost similar in all insect–moulting steroids used as comparable with 4–chlorotestosterone. When ^{14}C–alanine was used instead of ^{14}C–chlorella hydrolysate, the same results were obtained. Oral administration of 100 μg of ecdysterone produced the similar activation of amino acid incorporation in liver. Addition of ecdysterone into S–20 fluid *in vitro* could not alter the amino acid incorporation.

As shown in Fig. 1, incorporation of ^{14}C–amino acid *in vivo* was also obviously enhanced by treatment of the steroids in all subcelullar fractions especially in microsomes.

Fig. 2 shows the time course of activation of amino acid incorporation *in vitro*. The elevated incorporation returned to the normal level after 8 hours of treatment with 0.05 mg

Table I. Stimulatory Effect of Various Insect-Moulting Steroids on the Incorporation of ^{14}C-Chlorella Hydrolysate into Acid Insoluble Protein Fraction *in vitro*

Treatment	Time after injection (hr)	cpm/mg protein	Stimulation (%)
Control		307± 4	100
Ecdysterone	1	400±62	130
	2	553±31	180
	4	603±12	196
Inokosterone	1	327± 4	107
	2	522±10	170
	4	625±13	206
Ponasterone A	1	291±10	95
	2	582±15	190
	4	602± 8	196
Cyasterone	1	350±10	114
	2	540± 9	176
	4	632±21	206
Pterosterone	1	328±20	107
	2	571±16	186
	4	603±23	196
4-Chlorotestosterone	1	398± 8	130
	2	580±16	189
	4	610±20	199

The system consisted of: 0.2 ml S-20 fluid, 50 μmoles sucrose, 25 μmoles Tris (hydroxymethyl)-aminoethane (pH 7.6), 5 μmoles $MgSO_4$, 12.5 μmoles KCl, 2.0 μmoles ATP, 0.2 μmole GTP, 10 μmoles Phosphocreatine, 10 μg creatinephosphokinase (E.C. 2. 7. 3. 2), and 0.1 μC of uniformly labeled ^{14}C-chlorella hydrolysate (sp. act. 4.2 mC/mg) in 0.5 ml. Incubation was carried out under air for 25 min at 37°. The methods for washing and measurement of radioactivity in protein samples were described previously.[3]

Each result is the mean ± standard error for ten mice used.

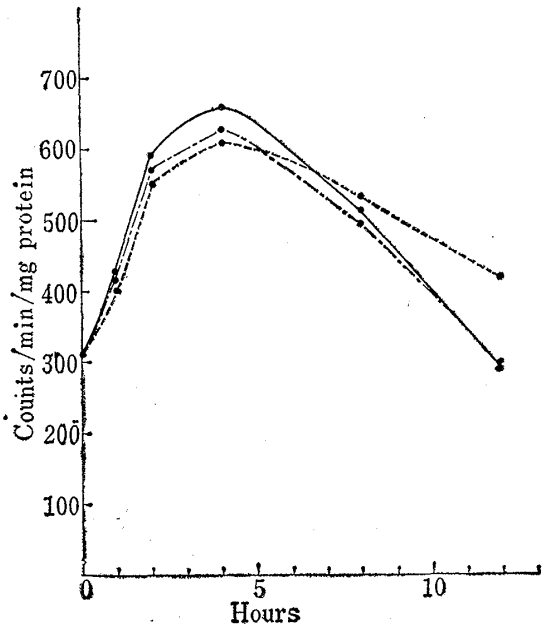

Fig. 2. The Time Course of the Stimulation of Amino Acid Incorporation after the Injection of Ecdysterone and 4-Chlorotestosterone

The methods were the same as Table I.

——— Ecdysterone 0.05 mg/100 g body weight

- - - - - - Ecdysterone 0.5 mg/100 g body weight

——— 4-Chlorotestosterone 1 mg/100 g body weight

3) K. Koike, T. Otaka, and S. Okui, *J. Biochem.*, **61**, 679 (1967).

ecdysterone per 100 g body weight. But in ten-fold dose the effect was still remaining for at least 12 hours after treatment. Duration of stimulating effect seemed to be dependent on doses used, but the absolute increment was about the same in these two doses. The activity of 4-chlorotestosterone returned to control level within 12 hours after treatment even in a dose of 1 mg per 100 g body weight.

The precise mechanism of the activation of protein synthesis by the insect-moulting steroids is now under investigation and will be reported in detail elsewhere.

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