NOTIZEN

Requirement of Cellular RNA-synthesis for the Inhibition of *Semliki Forest* Virus by poly (rI)·poly (rC)

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Synthetic polynucleotides are able to stimulate the synthesis of interferon and confer a virus-refractive state to animal cells [1]. Previous results by SCHAFER and LOCKART have presented evidence supporting the notion that the virus resistant state of monkey cells treated with polynucleotides is due to the interferon producing capacity of polyinosinicpolycytidylicacid (poly (rI)·poly(rC)) [2]. These results are consistent with findings of TAYLOR and MATSUYAMA et al., who have shown that the inhibition of *Sindbis* Virus by poly(rI)·poly (rC) similarly to the inhibition by interferon can be counteracted if the preincubation of the cells with the doublestranded polynucleotide is carried out under conditions of inhibited cellular RNA-synthesis [3, 4]. On the other hand, direct effects of the polynucleotides on virus specific RNA-synthesis have been observed [5]. Results are presented showing that the inhibition of *Semliki Forest* Virus replication by poly(rI)·poly(rC) is reversed under conditions of reduced RNA-synthesis which suggest that the inhibitory action of the polymer is not a direct effect on viral macromolecule synthesis but is mediated by substances, which are induced during the exposure of chick embryo cells to poly (rI)·poly(rC).

All experiments were carried out in confluent chick embryo fibroblast cultures. Inhibitors were added 4 hours before infection to 5 ml of Eagles medium plus 10% calfserum. Before infection with *Semliki Forest* Virus medium containing the inhibitors was removed, the cells washed once with medium and the virus inoculum added in 0.5 ml of medium (m.o.i. 0.01 PFU/cell). After one hour of adsorption the virus was removed and the cells were incubated for 16 hours in normal growth medium. Cells were stored at $-60°$ until further used. For determination of virus yield the cells were frozen and thawed three times and sonicated for 3 minutes. Virus yield was determined by a plaquetest on established human amnion cells (U-cells). By addition of cell homogenates from uninfected cells treated with inhibitors or DEAE-dextrane to the plaquetest it was assured that the virus titers in U-cells were not influenced by traces of these compounds.

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Actinomycin D (Serva, Heidelberg) inhibited RNA-synthesis as measured by ^{3}H-uridine incorporation under the experimental conditions by 95 percent. Inhibition of RNA-synthesis by Actinomycin D was not affected by the simultaneous presence of poly(rI)·poly (rC) or DEAE-dextrane. Poly(rI)·poly(rC) was obtained from Microbiological Associates. Hypochromic shift at 260 nm (50 μg/ml): 0.34. pH at which 50% of the doublestranded polynucleotide was denatured: 10.5 [6]. DEAE-dextrane was obtained from Pharmacia, Upsala.

Table 1 shows the growth of *Semliki Forest* Virus in chick embryo fibroblasts pretreated with poly(rI)·poly (rC) under various conditions. The same preparation of *Semliki Forest* Virus ($5 \cdot 10^4$ PFU/ml in U-cells) was used in both experiments.

line	poly-IC μg/ml	Actinomycin D μg/ml	DEAE-dx μg/ml	Expt. 1 SFV-Titer in PFU/ml	Expt. 2 SFV-Titer in PFU/ml
1	—	—	—	$1{,}6 \times 10^7$	n. t. *
2	—	—	50	$1{,}8 \times 10^7$	$2{,}4 \times 10^7$
3	—	1	—	$2{,}0 \times 10^7$	$2{,}4 \times 10^7$
4	—	1	50	$2{,}1 \times 10^7$	$2{,}0 \times 10^7$
5	2	—	—	n. t. *	$2{,}4 \times 10^7$
6	5	—	—	n. t.	$1{,}8 \times 10^7$
7	10	—	—	$0{,}9 \times 10^7$	$1{,}6 \times 10^7$
8	20	—	—	$0{,}8 \times 10^7$	$1{,}9 \times 10^7$
9	2	1	—	n. t.	$3{,}1 \times 10^7$
10	5	1	—	n. t.	$3{,}3 \times 10^7$
11	10	1	—	$1{,}4 \times 10^7$	$3{,}9 \times 10^7$
12	20	1	—	$1{,}6 \times 10^7$	$4{,}5 \times 10^7$
13	2	—	50	n. t.	$5{,}4 \times 10^5$
14	5	—	50	n.t.	$3{,}2 \times 10^5$
15	10	—	50	$2{,}0 \times 10^5$	$5{,}9 \times 10^5$
16	20	—	50	n. t.	$5{,}1 \times 10^5$
17	2	1	50	n. t.	$2{,}9 \times 10^7$
18	5	1	50	n. t.	$2{,}8 \times 10^7$
19	10	1	50	$1{,}2 \times 10^7$	$3{,}3 \times 10^7$
20	20	1	50	$2{,}3 \times 10^7$	$4{,}0 \times 10^7$

* n.t. = not tested.

Table 1. Growth of *Semliki Forest* Virus in chick-embryo fibroblasts pretreated with various inhibitors. For experimental conditions see text.

As has been shown in other virus-cell systems pretreatment of chick embryo fibroblasts with poly(rI)·poly(rC) alone has only a slight inhibitory effect on the growth of *Semliki Forest* Virus (line 1 – 12). The inhibition is expressed after simultaneous addition of DEAE-

dextrane. Virus yield was reduced to about 2% of the controls (line 2, 13–16). If RNA-synthesis is inhibited by the addition of Actinomycin D, the inhibition of virus growth by poly(rI)·poly(rC) is prevented and the virus yield is the same or even enhanced compared to control cultures (line 4, 17–20). These findings are similar to those obtained previously with *Sindbis* Virus and suggest that poly(rI)·poly(rC) exerts its effect by inducing the formation of one or more intermediate substances, which are responsible for the growth inhibition of *Semliki Fores* Virus [3, 4]. One possibility which cannot be excluded though is that poly(rI)·poly(rC) inhibits virus growth not by inducing specifically an antiviral substance but rather because of an unspecific toxic effect on the host cell, which is not appearing in Actinomycin D treated cells. Since it has been shown that doublestranded polynucleotides can induce interferon this type of protein is one of the possible candidates for an induced intermediate substance. Besides the possibility of additional sofar not detected antiviral substances in poly(rI)·poly(rC) treated cells the presence of interferon could secondarily result in the induction of other cellular antiviral proteins.

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Biosynthesis of Juvenile Hormone from 10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoic Acid in the Adult Cecropia Moth

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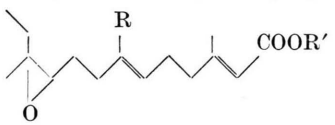
The biosynthesis of juvenile hormone (JH, **1**) and its lower homologue (JH-II, **2**) in the adult silkmoth *Hyalophora cecropia* (L.) involves L-methionine, which provides the ester methyl group for both compounds [1]. The substrate for the methylation reaction, however, has not yet been identified. We now wish to report that adult male *H. cecropia* are able to synthesize JH from *dl-trans,trans,cis*-10-epoxy-7-ethyl-3,11-dimethyl-2-6-tridecadienoic acid (**3**). For the preparation of labelled **3** we took advantage of the fact that JH, when injected into late fifth instar larvae of the tobacco hornworm, *Manduca sexta* (JOHANNSON), is rapidly inactivated by enzymatic hydrolysis to **3** [2]. Labelled **3** was most conveniently prepared by *in vitro* incubation of 2-^{14}C-*dl*-JH with *Manduca* blood.



cis *trans* *trans*

1	$R = -C_2H_5$	$R' = -CH_3$
2	$R = -CH_3$	$R' = -CH_3$
3	$R = -C_2H_5$	$R' = -H$

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Materials and Methods

2-^{14}C-*dl*-JH [3] ($5.0 \cdot 10^6$ dpm, spec. act. 25 mCi/mmole) was incubated at 37° with 2 ml hemolymph of 13–19 day-old last instar larvae of *M. sexta*. After 2 hrs. the mixture was extracted four times with 2 ml ether–ethanol (2:1) and the extract resolved by thin layer chromatography on silica gel with hexane–ethyl acetate (7 : 3). The epoxy acid on the TLC-plate (R_f ~0.3) was located with a radio scanner and eluted with peroxide free tetrahydrofuran (**3**; $4.1 \cdot 10^6$ dpm, 82%). From a second zone (R_f ~0.8) unchanged JH was recovered (**1**; $0.87 \cdot 10^6$ dpm, 17%).

The epoxy acid **3** was dissolved in TRIS/HCL buffer pH 8.5 just prior to application and injected (3 male Cecropia moths, 2 days old, 50 µl/moth) through the membrane between the 6th and 7th abdominal segments. In a second experiment [methyl-^{3}H]-L-methionine (2.6 Ci/mmole, Schwarz Bioresearch Inc. N. Y., in Insect-Ringer solution, 30 µl/moth) was administered together with **3**. The other experimental conditions and the isolation procedure for JH and JH-II were the same as previously described [1, 4].

Results and Discussion

Five hours after injection of the racemic 2-^{14}C-epoxy acid **3** into 2 day-old male Cecropia moths the JH was isolated. It contained 3.9% of the administered label (Table I, experiment 1). In a second experiment [methyl-^{3}H]-L-methionine was injected simultaneously with 2-^{14}C-**3** and the incubation period extended to 15 hours (Table I, experiment 2). In this case 5.8% of the ^{14}C and 0.11% of the ^{3}H was recovered with the JH. The purity of the isolated JH was checked through catalytic hydrogenation: the reaction product methyl 3,11-dimethyl-7-ethyl-tridecanoate [5] after gas chromatographic