ed to severe hemorrhage and suppression in sera from rats treated with DOCA.

Summary. Bilateral nephrectomy followed by immediate exsanguination allows the collection of blood from rats for the estimation of PRA in a manner that preserves the expected alterations to varying stimuli. This procedure can be used where relatively large samples of blood are required for treatment to inactivate angiotensinase and for the generation of angiotensin.

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Response of Chromosomal Puffs to Crystalline Hormones *in Vivo** (33842)

WALTER J. BURDETTE AND MASATOSHI KOBAYASHI

The University of Texas M. D. Anderson Hospital, and Tumor Institute at Houston, Texas 77025 and The Sericultural Experimental Station, Tokyo, Japan

Since the isolation and identification of alpha-ecdysone and ecdysterone by Butenandt, Karlson, Huber, Hoffmeister, and their collaborators (10, 11, 13), additional compounds differing in hydroxylation have been isolated not only from *Bombyx* but also from crustaceans and even from plant sources such as bracken and fir (9, 15, 16, 18, 20–22). In previous studies, Burdette and Bullock (5) extracted five active fractions from *Bombyx,* and Burdette and Anderson (3) found that the puffing pattern in Drosophila responded to ecdysones just as Clever and Karlson reported for Chironomus (13), although the chromosomes seemed refractory at times. Since the present pattern of sensitivity for puffing is activated by ecdysones and more than one ecdysone probably occurs naturally, it seemed appropriate to expose Drosophila larvae to several crystalline ecdysones and to determine the visible response of chromosomes to these hormones. In addition, proteinic brain hormone derived from *Bombyx* was also inoculated into larvae and the size of puffs was determined subsequently.

Procedures. A single test consisted of 20 late fifth-instar Drosophila larvae inoculated with 10 μl of hormone as a 10% solution in Ringer's saline. Stock proteinic brain hormone was used in two concentrations, one *Bombyx* unit in 100 μg (designated A) and one *Bombyx* unit in 50μg (designated B). Injections were made with a microsyringe and needle into anterior segments which were undisturbed until the time designated for the particular test had elapsed. [The method has been described previously (1).] In respective tests observations were made 20, 40, 60, and 90 min after injection.

Salivary glands were removed from the an-

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TABLE I. Comparison of Results of Treating Oregon R Larvae with Ecdysterone (Iso-inokosterone).[a]

	Exposure (min): 20		40		60		90	
Puff	Mean	Median	Mean	Median	Mean	Median	Mean	Median
a Hormone								
2B	1.7	1.7	1.9	1.7	1.8	1.7	1.6	1.6
71	1.6	1.5	1.5	1.5	1.6	1.6	1.7	1.5
72	1.6	1.5	1.4	1.3	1.6	1.6	1.5	1.5
74	1.4	1.4	1.4	1.5	1.4	1.4	1.5	1.4
75	1.5	1.4	1.5	1.5	1.4	1.4	1.4	1.3
b Control								
2B	1.5	1.5	1.7	1.45	1.9	2.0	1.6	1.6
71	1.5	1.5	1.4	1.35	1.6	1.55	1.7	1.6
72	1.4	1.5	1.3	1.3	1.3	1.3	1.6	1.55
74	1.3	1.1	1.4	1.3	1.5	1.5	1.5	1.5
75	1.3	1.2	1.4	1.35	1.6	1.45	1.6	1.55

[a] Mean and median ratios of diameters of puffs and adjacent bands; 18 observations per puff.

terior segment at the time specified in each experiment, compressed, and stained with acetoorcein. Preparations designated as controls received 10 μl of Ringer's solution without hormone. Since it was impractical to measure all puffs, a group was selected for study consisting of puffs at 2B on chromosome 1 and 71, 72 and 74, 75 on the left arm of the third chromosome. Both the breadth of these puffs and the diameter of adjacent bands were measured.

The problem arising from inability to standardize the amount of pressure when the chromosomes are compressed is minimized when ratios of diameters of puffs to adjacent bands are compared. Therefore these ratios have been computed, and the mean and median of the ratio between puff and neighboring band are presented on Tables I–IV. Results for each hormone are paired with corresponding control observations in each table. Each mean and median is calculated

TABLE II. Comparison of Results of Treating Oregon R Larvae with Inokosterone.[a]

	Exposure (min): 20		40		60		90	
Puff	Mean	Median	Mean	Median	Mean	Median	Mean	Median
a Hormone								
2B	1.9	1.9	2.2	2.25	1.8	1.65	1.7	1.45
71	1.5	1.5	1.6	1.65	1.6	1.6	1.4	1.35
72	1.5	1.6	1.5	1.6	1.5	1.3	1.4	1.3
74	1.4	1.4	1.5	1.45	1.4	1.35	1.4	1.3
75	1.5	1.45	1.5	1.5	1.3	1.35	1.4	1.4
b Control								
2B	1.5	1.5	1.7	1.45	1.9	2.0	1.6	1.6
71	1.5	1.5	1.4	1.35	1.6	1.55	1.7	1.6
72	1.4	1.5	1.3	1.3	1.3	1.3	1.6	1.55
74	1.3	1.1	1.4	1.3	1.5	1.5	1.5	1.5
75	1.3	1.2	1.4	1.35	1.6	1.45	1.6	1.55

[a] Mean and median ratios of diameters of puffs and adjacent bands; 18 observations per puff.

TABLE III. Comparison of Results of Treating Oregon R Larvae with Ponasterone A.[a]

	Exposure							
(min):	20		40		60		90	
Puff	Mean	Median	Mean	Median	Mean	Median	Mean	Median
a Hormone								
2B	1.8	1.7	1.9	1.7	1.9	1.9	1.6	1.55
71	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.4
72	1.5	1.5	1.6	1.5	1.5	1.4	1.4	1.4
74	1.5	1.4	1.4	1.5	1.4	1.3	1.5	1.45
75	1.5	1.45	1.5	1.5	1.6	1.55	1.5	1.5
b Control								
2B	1.5	1.5	1.7	1.45	1.9	2.0	1.6	1.6
71	1.5	1.5	1.4	1.35	1.6	1.55	1.7	1.6
72	1.4	1.5	1.3	1.3	1.3	1.3	1.6	1.55
74	1.3	1.1	1.4	1.3	1.5	1.5	1.5	1.5
75	1.3	1.2	1.4	1.35	1.6	1.45	1.6	1.55

[a] Mean and median ratios of diameters of puffs and adjacent bands; 18 observations per puff.

for 18 measurements. The percentage difference when ratios for preparations with and without hormone are compared appears in Table V. An antecedent positive sign indicates a relative increase following inoculation with hormone; a negative sign indicates that the control preparations had larger puffs than those receiving hormone.

Although extremes in size are responsible for differences in mean and median values, increments observed were generally sufficiently uniform to be convincing. Significant differences emerging from the *t* test are given in Table V. Puffs I-2B, III-72, and III-75 show a greater immediate response to ecdysterone than puffs III-71 and III-74, and the increment is sustained best for puff III-72. When inokosterone is injected, the response of puff III-72 and possibly III-71 is delayed to the 40-min interval. Also the latter puff is much smaller when compared to its control after 90 min than when ecdysterone is the hormone used. The immediate response of puff III-74 is greater after ponasterone A than after either inokosterone or iso-inokosterone. On the other hand, the relative size of puff I-2B is greater following exposure to ponasterone A and inokosterone than after administration of ecdysterone and is better sustained when inokosterone is used.

The lesser concentration of proteinic brain hormone produced a large increase in relative size of puffs after 40 min, which either was not initiated in the case of puff I-2B or was only a third to one half as great at the end of 20 min. When the concentration of proteinic brain hormone was increased, a relatively greater response occurred after 20 min than after 20 min for puffs III-71 and possibly III-72; whereas the impressive response within 20 min was sustained for puffs I-2B and III-75 and increased for puff III-74 after 40 min.

Discussion. The information obtained clearly demonstrates earlier increase in the size of salivary chromosome puffs chosen as indices to measure response after treatment with three crystalline ecdysones and proteinic brain hormones than in control preparation not exposed to additional hormone. However, neither inokosterone nor ponasterone A provoke exactly the same pattern of changes in size of puffs that follow administration of ecdysterone (iso-inokosterone). When the lower concentration of proteinic brain hormone was administered, the relative mean ratios were several fold greater for all puffs at the 40-min interval than at the 20-min interval. This fits in well with a tropic action on ring gland which in turn produces ecdysones

TABLE IV. Comparison of Results of Treating Oregon R Larvae with Proteinic Brain Hormone.[a]

Puff	Exposure (20 min)						Exposure (40 min)			
	Control		Brain hormone (A)		Brain hormone (B)		Control		Brain hormone (A)		Brain hormone (B)	
	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median	Mean	Median
2B	1.66	1.65	1.70	1.5	2.03	2.0	1.51	1.5	1.94	2.0	1.84	1.9
71	1.47	1.4	1.6	1.7	1.73	1.7	1.54	1.45	1.78	1.85	1.64	1.45
72	1.23	1.2	1.34	1.3	1.36	1.3	1.28	1.25	1.56	1.6	1.38	1.25
74	1.28	1.3	1.42	1.4	1.62	1.5	1.23	1.2	1.51	1.45	1.61	1.6
75	1.33	1.3	1.47	1.4	1.77	1.7	1.24	1.25	1.62	1.6	1.60	1.6

[a] Mean and median ratios of diameters of puffs and adjacent bands; 18 observations per puff; (A), activity of one Bombyx unit/100 μg; and (B), activity of one Bombyx unit/50 μg.

responsible for the puffing. The response was moved forward to 20 min when the concentration of proteinic brain hormone was doubled. This could also be explained by direct effect of this hormone on the chromosome or the presence of ecdysone in the preparation, but these possibilities seem less likely to apply.

Previously Burdette (2) demonstrated that events in metamorphosis are correlated with titer of ecdysones in *Bombyx*. Also previous work of Burdette and Coda (6) demonstrating enhancement of the rate of protein synthesis in mammalian tissues by ecdysone has been confirmed recently by Okui *et al.* (18) who tested ecdysterone, inokosterone, ponasterone A, cyasterone, pterosterone, and 4-chlorotestosterone.

It is therefore interesting to begin a more detailed analysis of action of different ecdysones at the chromosomal level. However, caution is advisable in interpretation of results. Our earlier studies on the group of puffs used in this investigation (3) showed that puffs in Drosophila may be refractory to stimulation at times and that standardization of a control model is difficult to achieve. The use of size of puffs to infer production of messenger RNA is not altogether a safe conjecture, since size may depend on a number of conditions such as alteration of ionic and gaseous milieu and exposure to virus (4, 7, 8, 16). Despite these restrictions, however, it does seem apparent that hormones differing in hydroxylation and puffs at different loci exhibit characteristic stimuli and effects respectively. Reasons for the behavior of the puffs described must be sought for each puff and each hormone.

Summary. Exposure of salivary-gland chromosomes *in vivo* to ecdysterone (isoinokosterone), inokosterone, ponasterone A, and proteinic brain hormone results in increased mean ratio of diameter of five index puffs in relation to adjacent bands. The rate and pattern of response differ characteristically for each hormone and each puff studied. In lower concentration the response to proteinic brain hormone is slower than that to ecdysterone.

TABLE V. Percentage Change in Mean Ratio of Breadth of Puffs to Diameter of Adjacent Bands; Action of Hormones on the Rate of Puffing.[a]

Hormone	(min)	Puff: 2B	71	72	74	75
Ecdysterone (Iso-inokosterone)	20	13[b]	7	14[b]	8	15[b]
	40	12	7	8	0	7
	60	—5	0	23[b]	—7	—12[b]
	90	0	0	—6	0	—12[c]
Inokosterone	20	27[b]	0	7	8	15[b]
	40	30[b]	14[c]	15[b]	7	7
	60	—5	0	15[c]	—7	—19[b]
	90	6	—18[b]	—12[c]	—7	—12[c]
Ponasterone A	20	20[b]	0	7	15[b]	15[b]
	40	12	7	23[b]	0	7
	60	0	—6	15[b]	—7	0
	90	0	—18[b]	—12[c]	0	—6
Proteinic brain hormone (A)	20	0	7[c]	8	8[b]	15[b]
	40	27[b]	20[b]	23[b]	25[b]	33[b]
Proteinic brain hormone (B)	20	18[b]	13[b]	17	23[b]	38[b]
	40	20[b]	7	8	33[b]	33[b]

[a] (A), 1 *Bombyx* unit/100 μg; (B), 1 *Bombyx* unit/50 μg.
[b] $.0001 < p < .05$.
[c] $.05 < p < .1$.

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