tion accelerates the loss of the aldehyde component required for the luciferase reaction. The enzyme thus escapes inactivation from radiation as well as from its catalysis of light production. Since the latter is an oxidative reaction, it is indeed curious that the enzyme can withstand oxidative destruction from radiation but not from the oxidative chemical steps it catalyzes (*6*).

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Changes in Titer of Ecdysone in Bombyx mori during Metamorphosis

Abstract. The amount of ecdysone extracted from *Bombyx mori* rose precipitously immediately before, and fell immediately after, transition from the larval to the pupal stage, and thereafter a secondary rise in titer was observed. These observations correlate very well with physiologic sequences of metamorphosis, critical period, and oxygen uptake.

Ablation and transplantation experiments have demonstrated clearly that metamorphosis in insects is under hormonal control. Both Fukuda (*1*) and Williams (*2*) were able to demonstrate that the growth and differentiation hormone is secreted by the prothoracic gland and that this gland is activated by neurosecretion from the brain (*3*). Recently, Kobayashi and Burdette (*4*) have demonstrated synergism between ecdysone and brain hormone, a finding which indicates a direct action of the latter in addition to its tropic function. By means of a modification of the method of Butenandt and Karlson (*5*) to isolate ecdysone, the level of this hormone in the tissues has been followed before and after pupation in samples of *Bombyx mori* (*6*).

Samples (*7*) of full-grown larvae, silkworms in the prepupal stage, and silkworms at 1, 2, 3 to 4, 5 to 6, and 6 to 7 days of age (*8*) were procured in large enough quantities to assure a yield of active hormone. The *Calliphora* test was used for bioassaying the material, and different concentrations were tested until activity was detected or until the amount of crude extract was so great as to preclude additional injection because of the discrepancy in size between the *Calliphora* larvae and the volume to be injected.

Activity was obtained in the bioassays in five of the seven samples. The results are indicated in Table 1. No hormone was detected in full-grown larvae, and the greatest amount was found during the prepupal stage. The amount of hormone then declined until the pupae were 2 days of age, when the sample was not active. Activity was found again in subsequent samples.

When the activity is calculated on the basis of the number of *Calliphora* units per gram (wet weight) of tissue, it is found that approximately 6 *Calliphora* units per gram were present immediately before pupal moulting. When the titer is calculated in terms of the mean wet weight of *Bombyx* at this stage, it is found that 8 units were extracted from each silkworm of this strain. For an accurate expression of actual level of hormone at a given stage, the number of units should be multiplied by a constant, *K*, which takes into consideration the efficiency of the extraction procedure.

When one considers the titer of hormone in relation to the life cycle of the insect, it is apparent that metamorphosis occurs shortly after a large amount of hormone is secreted, and that afterward the level of hormone falls rapidly. A more gradual increase in the secretion of hormone then occurs, presumably preparatory to emergence. The critical period in *Bombyx* and the oxygen uptake also fall into a logical sequence with respect to the observed changes in titer of ecdysone (*9*).

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Table 1. Titer of ecdysone during metamorphosis of *Bombyx mori*.

Stage (25°C equivalent)	Wet weight (g)		Yield of crude hormone (g)	Bioassays (No.)	Maximum pupation (%)	C.U.*	
	Total sample	Mean				Per gram	Per silkworm
Full-grown larvae	4589.4	2.589	0.1760	8	25	I†	I†
Prepupae	7061.0	1.359	0.0655	3	56	5.8	7.9
1 day	6865.3	1.113	0.0628	3	67	1.9	2.1
2 days	7498.8	1.118	0.0628	6	35	I	I
3–4 days	7026.7	1.243	0.1230	4	80	3.7	4.6
5–6 days	7164.2	1.142	0.1339	6	59	1.6	1.8
6–7 days	7111.2	1.213	0.1064	4	80	3.1	3.7

**Calliphora* units. †Inactive.

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27 October 1961

Prezygotic Selection in ABO Blood Groups

Abstract. A statistical method was devised to test whether prezygotic selection was operating in ABO blood groups, and it was demonstrated, with data from Japanese families, that heterozygous AO and BO fathers transmitted more than 50 percent O-bearing sperm (approximately 55 percent) to their children. Neither sperm incompatibility nor reproductive compensation could account for the results.

Selective mechanisms operating on ABO blood groups have so far been found to act only at postzygotic stages (*1*), while little attention has been paid to prezygotic selection, for which evidence is reported here.

Three different mechanisms of prezygotic selection are possible: (i) meiotic drive (*2*) or unequal production of gametes carrying different alleles in heterozygous parents; (ii) sperm competition, occurring independently of female genotype; (iii) sperm competition as a result of serological incompatibility between sperm carrying the A or B gene and the anti-A or anti-B antibody in the uterine secretion (*3*). The first two possible mechanisms occur in animals and plants (*4*).

Although by statistical methods a distinction cannot be made between the first and second mechanisms, it is possible to determine whether or not prezygotic selection is operating to an appreciable extent. For this purpose, we introduce the concept which may be called "family size equivalent" for children of a given genotype. The family size equivalent for O children of O × O matings is, of course, the mean number of children from such matings. The corresponding value for ♀O × ♂A matings is given by the following formula:

$$O_{eq}(♀O \times ♂A) = m \times \frac{1}{n} \times \frac{1}{P_O}$$

where m is the number of O children from ♀O × ♂A matings, n is the number of such matings, and P_O is the probability that an O child will be produced from such matings. This value represents the mean number of children if all AO sperm carrying the A gene were eliminated and only sperm carrying the O gene participated in the fertilization. If there were no sperm selection, the difference

$$\Delta = O_{eq}(♀O \times ♂A) - O_{eq}(O \times O)$$

would be equal to zero, but if there were selection, say for O-sperm and against A-sperm, then the sign of Δ would be positive. Zygotic selection would not affect the sign of Δ, since the two corresponding figures are calculated for O children of O mothers. A comparison between O_{eq}(♀O × ♂B) and $O_{eq}(O \times O)$ will serve as a test for prezygotic selection in BO fathers. Many other instances in which the genotypes of mothers and children are constant but only fathers' genotypes differ can be used to test the hypothesis of prezygotic selection in males, the null hypothesis being the expectation that Δ is equal to zero.

In order to check this hypothesis, data on Japanese families published between 1932 and 1944 were used. The data were obtained from eight papers dealing with 2243 families with 5336 children. The data of each paper were pooled together, since there was no heterogeneity in the distribution of blood groups among the parents. For the values of gene frequencies, we have used the summary table of Kobayashi (*5*), based on the data of more than a half million Japanese, which give $p = 0.2773$, $q = 0.1705$, and $r = 0.5522$ for A, B, and O, respectively. There were no significant differences in the distribution of blood groups between our pooled data and those given by Kobayashi ($\chi_3^2 = 5.02$, $.50 > p > .10$). The bias, which obviously derives from the fact that the family data did not include any childless marriages, was removed by adjusting the number of matings; this correction was based on other data (*6*) which provided the proportion of childless marriages.

Table 1. Comparisons of family size equivalents for children of given genotypes. Each pair is made up of two mating types in which the mothers' genotypes are identical but the fathers' genotypes differ.

Pair No.	Family size equivalents for children of given genotypes		Difference between two equivalents (Δ)
	Part A		
1	O_{eq}(♀O × ♂A) : 2.152	O_{eq}(O × O) : 2.074	+0.078 ± 0.147
2	O_{eq}(♀O × ♂B) : 2.306	O_{eq}(O × O) : 2.074	+0.232 ± 0.183
3	O_{eq}(♀A × ♂B) : 2.246	O_{eq}(♀A × ♂O) : 2.025	+0.221 ± 0.255
4	O_{eq}(♀B × ♂A) : 2.240	O_{eq}(♀B × ♂O) : 1.715	+0.525 ± 0.274
5	A_{eq}(♀A × ♂B) : 2.313	A_{eq}(♀A × ♂O) : 2.119	+0.194 ± 0.208
6	A_{eq}(♀O × ♂AB) : 1.983	A_{eq}(♀O × ♂A) : 1.664	+0.319 ± 0.247
7	B_{eq}(♀B × ♂A) : 2.342	B_{eq}(♀B × ♂O) : 2.131	+0.211 ± 0.239
8	B_{eq}(♀O × ♂AB) : 1.813	B_{eq}(♀O × ♂B) : 1.881	−0.068 ± 0.267
	Total		+0.214 ± 0.082*
	Part B		
9	O_{eq}(A × A) : 2.220	O_{eq}(♀A × ♂O) : 2.025	+0.195 ± 0.216
10	O_{eq}(B × B) : 1.848	O_{eq}(♀B × ♂O) : 1.715	+0.133 ± 0.294
11	A_{eq}(♀AB × ♂B) : 2.816	A_{eq}(♀AB × ♂O) : 2.078	+0.737 ± 0.481
12	A_{eq}(♀B × ♂AB) : 3.125	A_{eq}(♀B × ♂A) : 2.735	+0.390 ± 0.382
13	B_{eq}(♀AB × ♂A) : 2.249	B_{eq}(♀AB × ♂O) : 2.590	−0.342 ± 0.403
14	B_{eq}(♀A × ♂AB) : 2.353	B_{eq}(♀A × ♂B) : 1.571	+0.782 ± 0.379
15	AB_{eq}(AB × AB) : 2.880	AB_{eq}(♀AB × ♂A) : 1.937	+0.943 ± 0.466
16	AB_{eq}(AB × AB) : 2.880	AB_{eq}(♀AB × ♂B) : 2.761	+0.119 ± 0.530
17	AB_{eq}(♀A × ♂AB) : 1.882	AB_{eq}(♀A × ♂B) : 1.442	+0.440 ± 0.310
18	AB_{eq}(♀B × ♂AB) : 2.762	AB_{eq}(♀B × ♂A) : 1.574	+1.188 ± 0.333
	Total		+0.527 ± 0.123†

*$0.5 > p > .01$. † $p < .01$.

The results are summarized in Table 1. For testing our hypothesis, there are 18 pairs of mating types within which the family size equivalents can be compared respectively. For reasons mentioned below, the 18 pairs were arranged in two groups, one containing eight pairs (Table 1, part A) and the other ten pairs (Table 1, part B). The sign of Δ is positive in 16 cases. The mean of the Δ's differs significantly from zero for the eight-pair group ($\Delta = +0.214 \pm 0.082$; $.05 > p > .01$); this difference is highly significant for the ten-pair group ($\Delta = +0.527 \pm 0.123$, $p < .01$) and for both groups together ($\Delta = +0.350 \pm 0.078$, $p < .01$). The results strongly support our hypothesis that there is prezygotic selection for O sperm. The proportion of O sperm in AO and BO fathers was estimated to be 0.545 and 0.546, respectively. It is interesting that the two figures are almost identical.

The aforementioned view might be opposed by the argument that reproductive compensation combined with the effect of maternal-fetal incompatibility could produce similar results. Such compensation, if any, would give rise to the bias only when the family size equivalents of incompatible and compatible matings were compared. Since the deviations remain significant when comparisons are made between compatible versus compatible or incompatible versus incompatible matings (Table 1, part B), such a possibility is excluded.

Sperm incompatibility could also contribute to the deviations in the eight-pair group, but not to those found in the ten-pair group. Although this possibility cannot be excluded, our data strongly indicate that prezygotic selection in males is due mostly to either meiotic drive or to sperm competition which occurs independently of the environment in the female body.

A question may arise as to whether or not prezygotic selection occurs in females. To answer this question, a method similar to the one described in this report was applied, but the results were negative; there was no consistent evidence of prezygotic selection in females.

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26 September 1961

Intergeneric Relationships among Various Scorpion Venoms and Antivenins

Abstract. Several species of scorpions separated by geographical barriers have certain venom components in common. A study of the comparative venom-antivenin reactions by gel diffusion indicates an approach to the preparation of a polyvalent antivenin against scorpion stings.

The First International Conference on Venoms in 1954 emphasized the importance and complexity of certain harmful substances secreted or ejected by animals (*1*). As indicated by Balozet (*2*), in French North Africa deaths due to scorpion stings are more frequent than deaths due to venomous snakes.

The dissemination of U.S. military personnel over worldwide areas engenders the necessity of investigating antivenin preparations. The final goal is the development of a satisfactory polyvalent scorpion antivenin for therapeutic use.

As a first approach to the problem, this study indicates the prominent comparative venomologic precipitin reactions among 12 scorpion venoms (representing four genera) and five commercially prepared horse antivenins from widely separated world areas.

The venoms were air-dried droplets collected either by electrical stimulation of the living specimens (*2*) or saline extracts of the telsons. Venoms analyzed were from the following areas: São Paulo, Brazil (*3*); Johannesburg, South Africa (*4*); Ankara, Turkey (*5*); Algiers, Algeria (*6*); Camp Bullis, Texas; Tepic and San Blas, Nayarit State, Mexico; and Manzanillo, Colima State, Mexico. The Texas and the Mexican areas provided several species (*7*). Commercially prepared horse antivenins were from São Paulo, Brazil (*8*); Algeria (*9*); Ankara, Turkey (*10*); Mexico City, Mexico (*11*); and Johannesburg, South Africa (*12*).

Immunodiffusion in agar columns was the method of choice for this study, since the venom concentrations were insufficient to give satisfactory resolution by paper electrophoresis or multi-well agar plates. In the agar column method used for these analyses, venom and antivenin-agar mixtures were separated by a clear layer of 0.3-percent saline agar. This clear area formed the reaction arena in which the precipitin systems appeared. Other details of this double diffusion method have been described by Oakley and Fulthorpe (*13*). All of the 60 homologous or heterologous reactions were prepared in duplicate. Where required, the venoms were dissolved in saline at concentrations of 10 mg/ml or 5 mg/ml. The reactions of equal concentrations were compared by making cathetometric measurements of their respective precipitin systems after 68 hours' diffusion at 30° ± .01°C and subsequently computing the diffusivity ratios (*P* values) as described by Preer (*14*). Such values represented the relative diffusion rates of various venom components when reacted with a reference antivenin, or of antivenins when a venom was used as the reference standard.

Since the quantities of venoms were not sufficient for identification of the comparable antigen-antibody systems by the classical absorption method, the identity of comparable precipitin zones was established mathematically by the methods of Oudin (*15*). In brief, finding equal pairs of the 1567 mean *P* value differences permitted simultaneous identification of comparable precipitin systems when one venom was reacted with two or more antivenins and when two or more antivenins were reacted with one venom. The principle of this mathematical identity is based on the diffusion differences of two or more venom mixture components when each will react separately with two or more antivenins (*15*).

Figure 1 diagrammatically shows the only comparable precipitin systems among the various venom-antivenin reactions when all venoms were reacted with the same antivenin (upper row) and when each venom was reacted with the various antivenins (lower row). The results (upper row) show that the venom from *Androctonus australis*, an Algerian scorpion, shares at least one precipitin system in common with two species of *Centruroides* (Mexican) when each venom was reacted with anti-*Tityus spp.* serum from Brazil; two species of Mexican *Centruroides* show comparable systems when reacted with antivenins prepared against venoms of *Parabuthis spp.* from Johannesburg, South Africa; and *Androctonus australis* (Algiers) venom has a precipitin system comparable to that of venom from *Centruroides suffusus* (Mexican) when each was diffused into *Androctonus* antivenin. The situation was more complex when each venom was reacted separately with each antivenin. As shown in the lower row of Fig. 1,

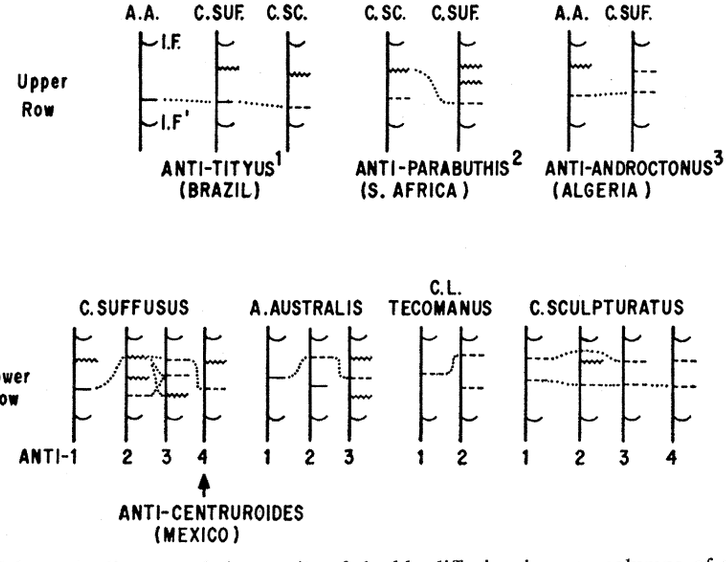

Fig. 1. Schematic diagram of the results of double diffusion in agar columns of various homologous and heterologous scorpion venom–horse antivenin reactions. Dotted lines connect related precipitin systems. IF and IF′ represent the interfaces of venom and clear agar and of clear agar and antivenin-agar mixture, respectively. Precipitin systems are shown as lines between the IF's. Solid lines indicate dense zones; wavy lines, medium density; dashed lines, faint zones. A.A., *Androctonus australis*; C.SUF., *Centruroides suffusus*; C.SC., *C. sculpturatus*. Reaction time, 68 hours at 30° ± .01°C. See text for method of zonal identification.