tent of the infection. Of 29 mice, 9 rats, 10 guinea pigs, 5 chickens, and 2 rabbits held in the rooms for not less than 20 days, 23 mice, 1 guinea pig, and all 9 rats tested were positive for HI and CF antibody to Sendai virus. The chickens and rabbits were negative. The infection is apparently being maintained by the introduction of uninfected mice from mouse breeder colonies which in turn become infected at a high rate within 1 to 4 weeks. Only mice held in filter-topped jars (*10*) were provided with a barrier sufficient to prevent infection.

In a study reported elsewhere (*5*), many of the serums of mice from the breeder colonies reported here were also tested for antibody to reovirus type 3, pneumonia virus of mice, K virus, polyoma, Theiler's encephalomyelitis (GDVII), mouse adenovirus, and mouse hepatitis. The Sendai virus was the fifth most prevalent indigenous mouse virus of the eight viruses for which we tested, with 6 of 21 colonies examined being found positive. Sendai infection differed from other indigenous murine virus infections in that the mean incidence of infection within infected colonies was 76 percent (Table 1) whereas the mean incidence of each of the other examined murine virus infections was less than 50 percent.

The results reported here indicate that Sendai virus exists within mouse breeder colonies in the United States as a widespread enzootic infection. The disease is highly prevalent within infected colonies and appears to be continuous rather than cyclic.

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Control of Synthesis of RNA and Protein in Diapausing and Injured Cecropia Pupae

Abstract. *Injury to diapausing pupae of the cecropia silkworm moth stimulates the synthesis of RNA in all pupal tissues and the synthesis of several blood proteins; such injury also induces the precocious synthesis of a protein, termed "injury protein," which normally appears in the blood during adult development. Actinomycin D, injected in concentrations of 2 micrograms per gram of body weight, blocks the injury-stimulated increase in blood protein synthesis and the injury-induced synthesis of injury protein. However, at concentrations of 0.5 micrograms per gram it prevents the induction of injury-protein synthesis but does not prevent the increased synthesis of other blood proteins. These results suggest that low concentrations of actinomycin may inhibit the synthesis of new kinds of messenger RNA but still permit the continued synthesis of messenger RNA's already in production at the time the actinomycin is injected.*

Injury to diapausing cecropia pupae induces the synthesis of a new blood protein and increases the rates of synthesis of preexisting blood proteins. In this report we show that threshold concentrations of actinomycin D prevent the synthesis of the new blood protein but do not prevent the injury-induced stimulation of synthesis of proteins already in production.

Previous experiments have established that within a week of pupation the giant silkworm moth *Hyalophora cecropia* enters a period of pupal diapause which commonly lasts for 6 to 10 months. Except for the hemocytes and gonads, its cells neither divide (*1*) nor engage in DNA synthesis (*2*) during this period. Even extensive injury does not stimulate cell division (*1*) or DNA synthesis in tissues not directly involved in closing the wound (*3*). Because the metabolic processes associated with DNA synthesis and cell division are absent, other metabolic changes stand out in sharp contrast.

For example, the diapausing pupa is capable of considerable synthesis of RNA and protein, and the degree of this synthetic activity can be altered by several means. If the pupa is wounded by removing a portion of the integument, its rate of respiration increases manyfold (*4*), and this is accompanied by a generalized increase in the rates of synthesis of RNA (*5*) and protein (*6*, *7*). Blood proteins, among others, are synthesized rapidly in injured pupae (*6*).

In the experiments described here, cecropia pupae were injured by excising the legs. To determine which tissues showed an increased synthesis of RNA in response to injury, conventional autoradiographic methods were employed with the liquid emulsion technique (*8*). Tritiated uridine (10 μc/g wet weight) was injected at various times after injury. Autoradiographs revealed that RNA synthesis increased in almost all pupal tissues within 24 hours of injury. Similar results were obtained with diapausing pupae of a number of species. The results of a careful analysis of autoradiographs of 20 diapausing and injured pupae of the closely related cynthia moth, *Samia cynthia*, are summarized in Table 1. Some humoral agent released at the site of injury (*9*) stimulated an increased synthetic activity throughout the pupa. Similar studies with tritiated thymidine revealed that injury caused no general stimulation of DNA synthesis in any tissue but the hemocytes.

Increased synthesis of certain blood proteins by cecropia pupae was demonstrated by electrophoresis on horizontal starch gels (*10*). Blood (10 μl) was taken for electrophoresis from a small slit in the wings at the time of the injury and at intervals thereafter. The changes in protein concentration were so great as to be immediately evident in visual comparisons of stained gels.

About eight pupal blood proteins were easily identified on starch with the amido-schwartz stain, and six esterases were detected with 1-naphthyl acetate as substrate. In favorable preparations more bands were identifiable. By disc electrophoresis on acrylamide gels (*11*), a minimum of 11 proteins in diapausing pupal blood were separated.

Starch gel electrophoretic patterns revealed that most of the pupal blood proteins began to increase in concentration within a day of injury. Injury also led to the appearance in the blood of a protein normally absent from the blood of uninjured pupae, hereafter called "injury protein." Injury proteins have also been detected electrophoretically by Laufer (*12*). The injury protein discussed here has the same electrophoretic mobility as a protein which normally appears during adult development and appears to be identical with it. Thus, by wounding a pupa, one induces the precocious synthesis of a protein which normally appears later in development.

In a starch gel prepared in a 0.03*M* borate buffer (*p*H 8.6) the injury protein migrated toward the negative pole, and was the fastest of four negatively-migrating esterases. The injury band contained relatively little protein in comparison with the amount of esterase activity, indicating that the esterase activity is not incidental. When a pupa was injured by excising the leg region, the injury protein first became detectable in the blood 12 hours after the injury and increased in amount for at least 3 days. In blood samples taken 5 months after injury, the injury band was weak or absent, but reappeared when the pupa was again injured.

To determine whether injury stimulates synthesis or merely causes the release of injury protein, pupae were injected with puromycin, which inhibits protein synthesis apparently by functioning as an analogue of aminoacyl sRNA (soluble RNA) (*13*). Injection of 0.36 mg/g of puromycin at the time of injury prevented the appearance of injury protein and also the increase in concentration of the other blood proteins. This suggests that injury stimulates the synthesis of injury protein. This was confirmed by injecting injured pupae 1 day after injury with 5 μc (per gram live weight) of C^{14}-labeled algal protein hydrolysate (1.48 mc/mg); a sample of blood was collected from each pupa 12 hours after the injection

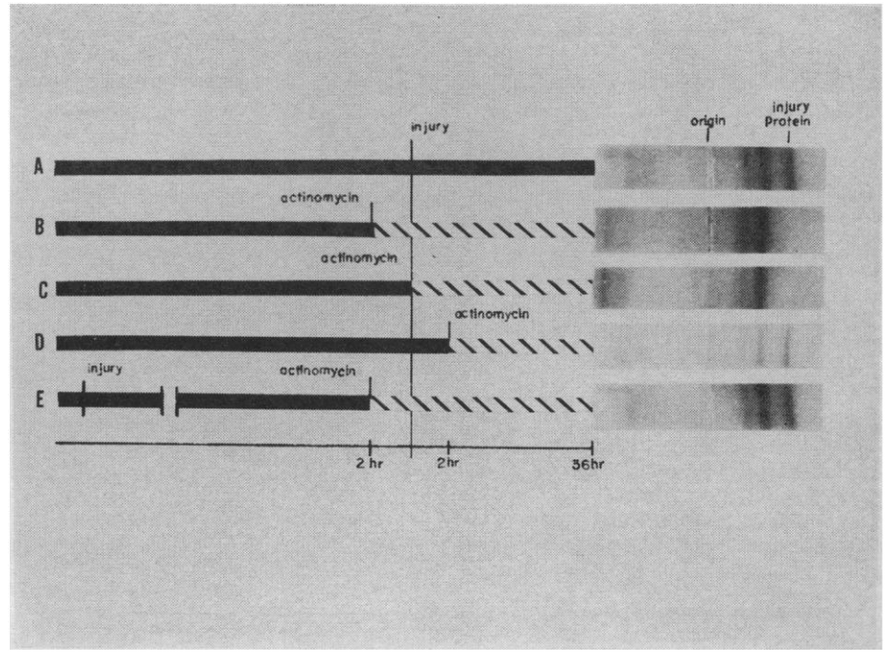

Fig. 1. Starch gel electrophoretic patterns showing effect of actinomycin D (0.5 μg/g) on the injury-induced synthesis of injury protein (stained for esterases). Blood samples taken 36 hours after injury. Starch gels prepared in 0.03*M* borate buffer, *p*H 8.6, and run in 0.3*M* borate for 2.5 hours at 6 v/cm. *A*, Injury only; no actinomycin injected. *B*, Actinomycin injected 2 hours before injury. This pattern resembles the pattern of uninjured blood except for the increased intensity of the bands. *C*, Actinomycin injected at the time of injury. *D*, Actinomycin injected 2 hours after injury. *E*, Actinomycin injected 2 hours before second injury to a pupa injured 5 months previously.

of isotope, and the proteins in 0.1 ml of this blood were separated electrophoretically on a starch gel. The gel was lightly stained for esterases, and the injury band cut out and homogenized in 0.6-percent NaCl after repeated freezing and thawing. The homogenate was dialysed overnight against distilled water and was centrifuged, and samples of the supernatant fluid were taken for counting and for estimation of the protein content by the Folin method. The results, summarized in row 1 of Table 2, revealed that the injury band contained a heavily labeled protein, indicating that injury protein had been synthesized and not merely released in response to the injury. Other proteins were also labeled, indicating that they too were synthesized in response to injury. Data for the slowest of the four negatively-migrating esterases (designated esterase "−1") are recorded in Table 2, row 1.

The synthesis of a new protein in response to injury led us to investigate whether (i) a gene is "turned on" by the injury factor to make a new mRNA (messenger RNA), or whether (ii) a preexisting but inactive mRNA is activated. Pupae were injected at various times before and after injury with 0.5 to 4 μg/g of actinomycin D, which inhibits DNA-dependent RNA synthesis in many systems (*14*). One hour later, 10 μc of tritiated uridine was injected. Two hours after injection of the isotope, the insects were fixed and autoradiographs prepared. In uninjured diapausing pupae, 0.5 μg/g effectively blocked RNA synthesis and no labeling of the nuclei was detected.

In injured pupae, 0.5 μg/g of actinomycin suppressed most, but not all, of the RNA synthesis and a small amount of nuclear labeling was detected in autoradiographs. When 2 or 4 μg/g of actinomycin was injected into pupae 2 hours before injury, no injury protein was formed and there was no increase in the concentration of other blood proteins during the 8 to 10 days these pupae survived. Injection of 1 μg/g 2 hours before injury prevented both the synthesis of injury protein and the stimulation of synthesis of other blood proteins for 7 days. On the 8th day one of three pupae began to synthesize other blood proteins (but not injury protein) at an increased rate.

Pupae which received 0.5 μg/g of actinomycin behaved differently. When

Table 1. Effect of injury to diapausing pupae on the incorporation of tritiated uridine. The insects were killed 2 hours after receiving isotope. After this short period, labeling in uninjured pupae was restricted to the nucleus, but in injured pupae some of the isotope appeared in the cytoplasm as well. The data are based upon average grain density. Each "+" represents approximately 15 grains/100 μ^2; "±" indicates variable number of cells taking up less than 15 grains/100 μ^2.

Tissue	Before injury	24 hours after injury
Epidermis	+	+++
Trachea	±	+++
Hemocytes	++	*
Fat body (general)	+	++
Perigonadal fat body	+	+++
Ventral muscle	+	++
Heart	++	++++
Pericardial cells	+++	+++
Nerve	+	++
Oenocytes	+	++
Oviduct	+	+

* Because hemocytes are mobile cells which increase in number in response to injury, it is difficult to determine the effect of injury on their rate of uridine incorporation.

injected at the time of injury (Fig. 1*c*) or 2 hours before injury (Fig. 1*b*), the injury protein did not appear in the blood, even in samples taken 2 weeks after the wound was inflicted. One group of pupae were given a second injury 8 days after the injection with actinomycin and the initial injury. Blood samples taken 2 days after the second injury contained no injury protein, showing that the synthesis of this protein was still blocked. In contrast, when 0.5 μg/g of actinomycin was administered 2 hours after wounding, injury protein appeared within 18 hours and was in high concentration at 36 hours (Fig. 1*d*). When 0.5 μg/g of actinomycin was injected into a pupa which already had some injury protein in its blood (for example, from an injury received some months earlier), and the pupa was wounded 2 hours later, the amount of injury protein in the blood increased (Fig. 1*e*). When C^{14}-labeled algal protein hydrolysate was injected into actinomycin-treated injured pupae that had no injury protein in their blood at the time of actinomycin injection, then no labeled or unlabeled protein was detected in the starch gels at the position corresponding to the wound band. However, when the same experiment was repeated on an actinomycin-treated pupa which had some injury protein in its blood, then injury stimulated the further synthesis of this injury protein (Table 2, rows 2 and 3). At this concentration of actinomycin, injured pupae were able to increase the rate of synthesis of proteins that they had been making, but were unable to synthesize a new protein. One explanation of these results is that mRNA's which were being made at the time of actinomycin treatment continued to be made at an increased rate, but a new kind of mRNA was not.

Table 2. Effect of actinomycin D on the incorporation of C^{14}-labeled amino acids into blood proteins of injured cecropia pupae. Samples were counted to a preset count of 10^4 in a Packard Tricarb scintillation counter, with a scintillation fluid made up of dioxane, 75 ml; anisole, 12.5 ml; dimethoxy ethane, 12.5 ml; 2,5-diphenyloxazole, 0.7 g; and 1,4-di(5-phenyl-2-oxazolyl)benzene, 50 mg (*15*). Results are expressed as averages of determinations made in duplicate on each of two pupae.

Presence of injury band before injury	Injury protein (count/min)		Esterase "−1" (count/min)	
	Total	Per μg protein*	Total	Per μg protein*
No actinomycin injected				
Absent	76	19	38	5.5
Pupae injected with actinomycin (0.5 μg/g)				
Absent	0	0		
Faint	19	4.5	11	2.7

* All samples of injury protein contained less than 4 μg of protein.

The inhibitory action of actinomycin provided us with an opportunity to determine where the injury protein is made. Pupae were injected with 0.5 μg/g of actinomycin D to inhibit the synthesis of injury protein. Into such pupae, blood-free tissues of previously injured pupae were implanted, and blood samples were taken at intervals and checked for injury protein. This experiment revealed that the hemocytes of injured pupae are one source of injury protein, and that these hemocytes synthesize and release injury protein into the blood of actinomycin-treated hosts, presumably using the mRNA of the injury protein which they had made before they were exposed to the actinomycin in the blood of the host.

We conclude from these experiments that (i) all pupal tissues are affected by the injury factor, and (ii) the injury factor induces in blood cells and perhaps in some other tissues the synthesis of a new mRNA for injury protein, and also increases the rate of synthesis of other mRNA's, some of which are involved in the increased rate of synthesis of blood proteins. A possible explanation of the results is that at appropriate concentrations of actinomycin, the synthesis of new kinds of mRNA is blocked, but the pupa continues to synthesize and increase the rate of synthesis of other kinds of mRNA which were being made at the time of application of actinomycin. Apparently in this system an inactive gene is more sensitive to actinomycin than are certain active genes. Quite apart from this hypothesis, the fact remains that in our experiments actinomycin D prevented the synthesis of one protein but not others. It may prove worth while for students of development to examine not only the effects of blocking nuclear RNA synthesis completely with actinomycin, but also the effects of subthreshold concentrations of actinomycin on the rates of synthesis of a series of proteins in developing organisms. The synthesis of some proteins may be affected more than others, enabling the experimenter to alter the protein composition of cells and tissues.

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