Chen, P.S. and H. Weideli. Zoologisches Institut der Universität, Zürich, Switzerland. Free ninhydrin-positive components and proteins in the hemolymph of Drosophila larvae infected by Pseudeucoila bochei Weld.

The relationship of the parasitic wasp Pseudeucoila bochei to D. melanogaster has been extensively investigated in this laboratory (Jenni 1951, Schlegel-Oprecht 1953, Walker 1959, Meier-Grassmann 1962). It was found that there are Drosophila strains which are able to form a pigmented capsule around the parasitic embryo within the larval body cavity, and the encapsuled parasite subsequently dies. On the other hand, there are Pseudeucoila strains which can inhibit the process of capsule formation of the host larva. Detailed analyses indicated that the defensive reaction is strain-specific with respect to both the host and the endoparasite and appears to be under genic control. In order to get further information about this phenomenon we carried out a preliminary investigation of the free ninhydrin-positive components and proteins in the hemolymph of Drosophila larvae infected by Pseudeucoila. For comparison the hemolymph composition of non-infected larvae from parallel cultures was also analyzed. Drosophila larvae of the strain Hindelbank were raised on standard medium at 25°C and subjected to infection by Pseudeucoila (strain Erlenbach) at the age of 56-60 hrs. after oviposition. Hemolymph samples were taken at 10 hr. intervals until the host larvae reached puparium formation. The technique of 2-dimensional paper chromatography was used for the analysis of amino acids and peptides, and disc electrophoresis for the separation of proteins. We found that the total concentration of free ninhydrin-positive components of the infected larvae at 72 hrs. of age amounts to 10% higher than that of non-infected controls. According to Walker (1959) this corresponds to the time of the beginning of capsule formation through the aggregation of lamellocytes around the parasitic embryo. At 88 hrs., when the deposition of melanin in the aggregated lamellocytes takes place, the value of the infected larvae is even 25% higher. A total of 26 ninhydrin-positive substances was identified on 2-dimensional paper chromatograms. Among these phosphoethanolamine maintains a consistently higher concentration in the infected larvae than in the controls during the period from 60 to 80 hrs. The same is true for ornithine at 70 hrs. of age. On the other hand, the content of -alanine and one acidic peptide appears to be significantly reduced. Using disc electrophoresis at least 10 protein fractions migrating in the anodal direction could be separated. Significant differences between infected and normal larvae were detected in the gel region about 8 mm from the origin. Based on the staining intensity of the protein bands, one fraction (No.5) appears to be reduced in the infected larvae aged 60 to 80 hrs., whereas the opposite is true for another fraction (No.8).

In order to test to what extent the hemolymph of the host larva is related to the process of capsule formation, the following blood transfusion experiment was designed. As mentioned above, Drosophila larvae of the strain Hindelbank were infected by Pseudeucoila at 56-60 hrs. after egg-laying. According to Walker (1959) in this strain 97.6% of the infected larvae form a capsule around the parasitic embryo. About 10 hrs. after infection hemolymph was collected, and 0.1 to 0.2 µl of this was injected into infected Drosophila larvae of the strain Luxor which, in contrast to the donor strain, has an extremely low rate of capsule formation (2.9%). After injection the larvae were kept under normal culture conditions until the time of pupation, and the number of individuals which formed a pigmented capsule was determined. In two series of experiments we found that the rates of capsule formation were 11% and 29% higher in the injected larvae compared to those which received no hemolymph of the strain Hindelbank. It seems that some components in the larval hemolymph are probably essential to initiate the defensive reaction. Since we have so far carried out only a limited number of experiments, this result can not yet be considered as conclusive. It would also be of interest to see if the opposite is true by injecting hemolymph from the strain Luxor into infected larvae of the strain Hindelbank.

Up to this time we do not know how the observations reported here are causally related to the infection process. There is, however, no doubt that during the period of capsule formation significant quantitative changes in the chemical composition of the larval hemolymph take place.