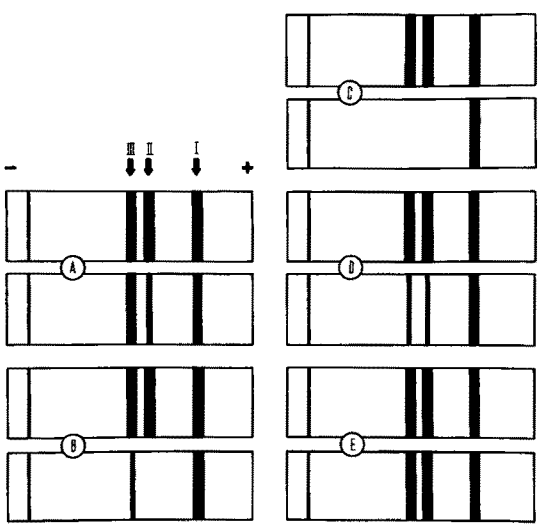
Role of Intracellular Symbionts in the Fatbody of Cockroaches: Influence on Hemolymph Proteins

The peculiar bacteria-like bodies occurring intracellularly in the fatbody of the cockroach are symbionts which reside in specialized cells within the fatbody[1]. They have been eliminated from their hosts by heat and various antibiotics[1-3] in an effort to determine their function, but their exact chemical role with regard to the physiology of the host has remained obscure. Elimination of the symbionts from the host has resulted in poorly-developed and moribund offspring[1]. Their intimate association with the fatbody, along with findings implicating fatbody in the synthesis of proteins in other insects[4-8], suggests their possible connection with synthetic processes relative to the proteins. In this study, cockroaches were treated to reduce the number of symbionts in the fatbody and samples of hemolymph subjected to electrophoretic analysis to determine what influence the symbionts have on the hemolymph proteins.

All cockroaches used were adult *Blaberus craniifer* reared under identical conditions in the laboratory and chosen on the basis of weight. 3 experimental runs were carried out, each involving 4 groups of animals kept in separate containers, male and female experimentals and male and female controls. Experimentals were injected in the abdomen with daily doses of 1000 U of penicillin G (potassium) in 0.1 ml of saline, and controls were injected in a similar manner with saline alone. Injections were made during the first 15 days of an experimental period of 40 to 50 days. The relative number of bacteroids was estimated through microscopic examination of fatbody smears stained to show them. Hemolymph samples were collected by removing a limb and draining the sample into a tube of saline. 4 applications of the sample were made on cellulose polyacetate strips[9] which were run for 1 h at 200 V and pH 8.8[10]. Standard procedures were followed in subsequent operations[11].

Hemolymph from control animals consistently showed 3 bands, here designated I, II and III. Both sexes showed the same pattern with band II less intense in the males (Figure A). No detectable changes occurred in the patterns from experimental animals during the first 8 days post treatment. After this time definite changes became evident. Bands II and III began to diminish, and finally by 16 days post treatment disappeared entirely (Figure B and C). In all cases the decrease in intensity of bands II and III was accompanied by an increase in the intensity of band I. The absence of bands II and III continued for approximately 2 weeks, after which there was a gradual reappearance of the proteins (Figure D and E). The original pattern became re-established by 40 days post treatment.



Electrophoretograms of hemolymph. A) Comparison of normal male (lower) and female (upper). B–E) Comparison of hemolymph from control (upper) and experimental (lower) females. B) Decline of bands II and III at 11 days post treatment. C) Disappearance of bands II and III by 16 days post treatment. D) Return of bands at 23 days post treatment. E) Complete return of bands by 31 days post treatment.

Although both sexes were treated and sampled, emphasis was placed on females once it was found that both showed the same pattern. This was done in an effort to determine the relationship of changes in hemolymph proteins to the development of oocytes. Work on other insects has indicated that these proteins are incorporated into developing oocytes[6-8,12]. Band II, which is always more intense in females, corresponds to a fraction that has been called the female protein[6] or yolk protein[12] and represents the protein fraction which is critical to development of oocytes and egg capsules in *B. craniifer*. The decline and disappearance of this band in the treated females is attended by a striking effect upon eggs and capsules. The capsules from treated females were distorted and abnormally dark; many of them were expelled prematurely in a shrunken and wrinkled condition. The eggs were smaller than usual and most did not hatch. A few nymphs emerged from the eggs but showed abnormal coloration and died almost immediately.

The findings suggest that sufficient amounts of required proteins are not available for incorporation into the developing egg when the number of bacteroids in the fatbody is reduced. The decline and disappearance of the hemolymph proteins constituting bands II and III in treated animals indicates that the bacteroids are necessary for the synthesis of these proteins in the fatbody of *B. craniifer* and shows a definite correlation between the number of symbionts and the quantity of the proteins. In addition, the obvious impairment of reproductive processes in experimental females indicates that the same proteins are critical to the maturation of eggs.

Zusammenfassung. Zur Herabsetzung der Anzahl von Symbionten in einem Fettkörper der Küchenschabe *Blaberus craniifer* wurde eine Penicillin-G-Behandlung durchgeführt, was eine Reduktion bei 2 Hämolymphe-Proteinen verursachte. Diese mit der Symbionten-Aktivität und Fortpflanzung verknüpften Proteine treten am Ende der Behandlung wieder in Erscheinung.

W. A. Garthe and M. W. Elliott

Department of Biological Sciences,
Northern Illinois University,
DeKalb (Illinois 60115, USA), 9 November 1970.

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