A comparative study of the frequency of the formation of microchaetae without sockets on the head, thorax and abdomen revealed the interesting fact that it is higher on the head than on the thorax (Figure 3). The microchaetae of the abdomen (third abdominal tergite) are only affected to a slight degree at any time of the injection. But unlike the bristles of the head and thorax, they form bristle organs without sockets at a relatively uniform level of 1.7–2.5%, except for the late third larval instar, where only 0.3% of all bristles lack sockets. In contrast to the situation on the head and thorax, the abdominal bristle-forming cells do not exhibit a distinct sensitive phase for the formation of bristles without sockets. It is possible that Mitomycin C does not reach all the bristle forming cells of the abdomen, or that these cells are relatively insensitive to the drug. It is known that during metamorphosis the abdomen is formed in a different way and later than the other integumental structures. Unlike all other bristles of the adult fly, the bristles of the abdomen do not derive from cells of imaginal disks, but from hypodermal histoblasts, which are located in 4 small cell nests in each abdominal segment except the last one.

Finally, the injection of Mitomycin C into larvae and pupae leads to the occurrence of the following modifications in bristles with sockets, some of which are phenocopies of bristle mutants[4]: 1. Formation of double sockets with varying phenotypes, whereby some resemble the mutant *Hairless* (H; 3–69.5). 2. Sockets with tiny bristles, or sometimes only protuberances are formed. These modifications occur predominantly on the thorax. 3. Incomplete bristle and/or socket secretion, which were found especially on the thorax and head. 4. Differentiation of twin bristles, which sometimes are phenocopies of the mutants *split* (*spl*; 1–3.0) or *Dichaete* (*D*; 3–40.7). Those aberrations occurred predominantly on the abdomen. 5. The formation of T-shaped bristles which were described earlier was observed[5]. Some of those modifications can be considered as phenocopies of the mutant shaven-naked (sv^n ; 4–3.0).

Unlike the formation of bristles without sockets, the above listed bristle modifications occurred relatively seldom; the highest frequency of any modification was only 1.3% of the bristles formed. Therefore, the effect of Mitomycin C on the suppression of the formation of the socket is considered to be highly specific.

Zusammenfassung. Durch Injektion von Mitomycin C in verschieden alten Larven, Vorpuppen und Puppen von *Drosophila melanogaster* wurden die sensiblen Phasen für die Bildung sockelloser Borsten und den Ausfall ganzer Borstenorgane auf dem Kopf, Thorax und Abdomen bestimmt. Makrochaeten weisen ein Sensibilitätsmaximum im späten dritten Larvenstadium, Mikrochaeten im Vorpuppenstadium auf. Am häufigsten treten sockellose Borsten auf dem Kopf, am seltensten auf dem Abdomen auf. Ausser sockellosen Borsten werden in geringen Frequenzen noch andere Borstenveränderungen, unter anderem Phänokopien von borstenmodifizierenden Mutanten erzeugt.

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Detection of Antigens on Filarial Larvae by Means of Immune Adherence

There are few simple and convenient methods for the detection of antibodies to, or antigens of, small metazoan parasites. The sensitive technique of immune adherence should lend itself readily to the detection of immunological reactions at the surface of such parasites. Immune adherence involves the attachment of complexes of antigen, antibody and the first four components of complement to a receptor present on the erythrocytes of most normal humans and some primates. In the case of particulate antigens the attachment is visible microscopically[1–3]. The technique has been applied by Soulsby[4] to the detection of antigens on the surface of the larvae of *Ascaris suum*. We wished to see whether it could also be applied to the detection of surface antigens of *Breinlia sergenti*, which is a filarial parasite of a primate, the slow loris (*Nycticebus coucang*) and has as intermediate host the mosquito *Armigeres subalbatus*[5]. This was found to be possible and further experiments were carried out to see whether the antigens detected on the filarial larvae were present on adults or microfilariae, or on larvae of other filarial species; and whether the slow loris made a detectable immune response to the surface antigens of the larvae.

Third stage larvae were dissected from the heads of *Armigeres* mosquitoes which had taken a blood meal from infected lorises 14–16 days earlier. For immunization of rabbits the larvae from heavily infected mosquitoes were collected in 0.9% saline. Because of clumping the total number of larvae making up the original suspension could not be counted but it was estimated to be of the order of 50/ml. They were frozen and thawed 4 times and homogenized in a Potter-Elvehjem homogenizer. One rabbit was injected i.p. with 1 ml of suspension; another rabbit was injected s.c. in 2 sites with a total of 2 ml of an emulsion of equal volumes of the suspension and Freund's complete adjuvant. 2, 3 and 4 weeks later each rabbit received an i.p. injection of 1 ml of the suspension. They were bled after another week and the pooled antiserum was stored at −25 °C. Before use aliquots of the antiserum were diluted 1:5 and heated to 56 °C for 20 min. Serial dilutions were made from this.

For immune adherence tests the diluent used was isotonic veronal buffer containing Ca^{2+} and Mg^{2+} (ref. [6]) and 0.1% gelatin (Isogever). Wax rings about 1 cm diameter were made on glass microscope slides and a 0.025 ml drop of Isogever placed within each ring. Filarial larvae were dissected from mosquitoes into saline, then 1–3 larvae, depending on the number available, placed in each drop. One drop of rabbit antiserum diluted in Isogever was added to each drop and the slides were left in a moist box at room temperature for 15 min. As a source of complement one drop of diluted guinea-pig serum (lyophilized guinea-pig serum, Com-

Reaction of antiserum to *Breinlia* larvae with other filarial larvae

Larvae of	Intensity of immune adherence with antiserum dilution									
	5	10	20	40	80	160	320	640	1280	None
Breinlia	0	+++	+++	n.d.[a]	n.d.[a]	++++	++	+	0	0
D. immitis	±	+	+++	+++	++	?[b]	0	0	0	0
B. pahangi	+[c]	+[c]	++[c]	+[c]	+[c]	+[c]	0	0	0	0
B. malayi	0	0	0	0	0	0	0	0	0	0

[a] n.d., not done. [b] A few erythrocytes attached to the head end only. [c] Erythrocytes mostly attached to the head of *B. pahangi*.

monwealth Serum Laboratories, Melbourne) was added; a 1:100 dilution was found to provide adequate complement activity. After 30 min incubation in a moist box at 37 °C a drop of 0.25% thrice washed human erythrocytes (group O) was added and the final mixtures were incubated at 37 °C for a further 30 min. A coverslip was then placed over the drop, which was examined by phase contrast or light microscopy. The results were scored from O (no erythrocytes adherent) to ++++ (the larvae almost completely covered with erythrocytes). Controls in each experiment included mixtures containing normal rabbit serum or diluent instead of antiserum, and mixtures containing antiserum or normal rabbit serum but no complement.

Antiserum, but not normal rabbit serum, was found to induce adherence of human erythrocytes to *Breinlia* third stage larvae in the presence of normal guinea-pig serum (Figure). There was a strong prozone: no adherence occurred with antiserum diluted 1:5 and adherence was stronger with antiserum diluted 1:125 than 1:25. The following additional controls were all negative: use of heated (56 °C, 20 min) guinea-pig serum instead of normal guinea-pig serum; omission of guinea-pig serum; addition of 0.1*M* EDTA; use of sheep erythrocytes or trypsin treated human erythrocytes instead of normal human erythrocytes. Thus, in the requirements for antibody, for active complement and for indicator erythrocytes known to possess the immune adherence receptor the reaction fulfilled the criteria for immune adherence[1,2]. No erythrocytes adhered to debris from the mosquito tissues (when this was present) indicating that the antiserum was directed against larval antigens, not mosquito antigens adsorbed to the larvae.

No immune adherence occurred when adult *Breinlia* worms from the peritoneal cavity of the loris, or microfilariae isolated from infected loris blood by the method of WONG[7] were used instead of larvae.

Lorises were infected subcutaneously with 37 to 65 larvae injected s.c. (2 animals) or i.p. (2 animals). They were bled by cardiac puncture at approximately 2 weekly intervals for 2 to 5 months. The sera were absorbed twice with packed human erythrocytes to remove a powerful haemagglutinin. None of the sera reacted with the larvae as detected by immune adherence.

The antiserum was titrated by immune adherence against larvae of *Dirofilaria immitis* (from *Aedes togoi* mosquitoes), *Brugia pahangi* (from *Armigeres subalbatus* and *Aedes togoi* mosquitoes) and *Brugia malayi* (from *Aedes togoi* mosquitoes). The results are shown in the Table. The antiserum reacted with *D. immitis* larvae, though less strongly than with those of *Breinlia*. It also reacted with *B. pahangi* larvae, but weakly and then mainly with antigens apparently concentrated at the head end. It failed to react with *B. malayi* larvae.

Immune adherence is thus a useful and simple technique for detecting immunological reactions to filarial larvae. By means of immune adherence antigens on the surfaces of larvae can be detected. In both these respects we agree with SOULSBY[4] who used another nematode. The antigens appear to be absent from other stages of *Breinlia*. The point of potentially the most practical importance is that the antiserum used could distinguish between *B. pahangi* and *B. malayi* which are morphologically indistinguishable. Further studies using antisera to *B. pahangi* and *B. malayi* and possibly *D. immitis* larvae may be useful.

Résumé. On a décelé des antigènes à la surface des larves de *Breinlia sergenti* par la réaction d'adhérence immune. Ils n'ont pas été décelés sur les adultes ni sur les microfilaires, avec un sérum de lapin anti-larves. L'antisérum a réagi avec les larves de *Dirofilaria immitis* et *Brugia pahangi*, mais non pas avec les larves de *Brugia malayi*.

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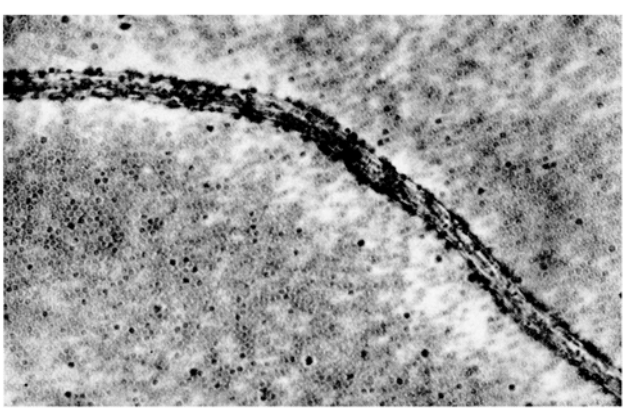

Immune adherence reaction with third-stage larva of *Breinlia sergenti*. Human erythrocytes attached to surface of larva.

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