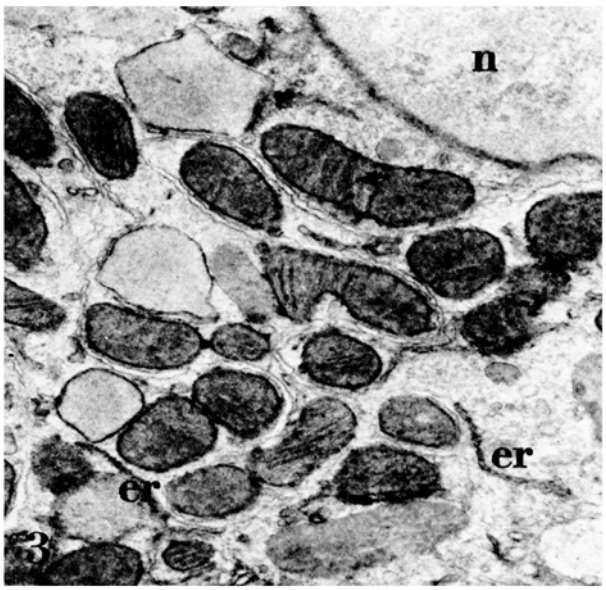

Fig. 3. The nuclear (N) envelope and the sacs of the endoplasmic reticulum (er) also contain reaction product in some cells. The reaction in the intracristal space of mitochondria (arrow) is not visible in some cells. ×20,000.

strate only lysosomal location of reaction product[7-11]. The reason for this discrepancy is not yet obvious but it may be due, in part, to the use of a minimal perfusion time for fixation and the addition of DMSO to the fixative. HOPSU et al.[9] report that prolonged fixation cut down about 22% of the activity in the kidney, 12% in the liver, 18% in the spleen and 8% in the brain.

The addition of DMSO has been reported to activate the acid phosphatase[19]. The solution of DMSO-glutaraldehyde, which was employed here, might result in either acceleration of the flow of medium into the mitochondria or activation of the enzyme. Denaturation of the membrane of the mitochondria as well as the limiting membrane of the lysosome by DMSO might play a role in these effects.

The cytochemical finding of arylsulfatase activity in mitochondria encourages study on the similar localization of acid phosphatase, the intracellular location of which has been restricted to lysosomes and related structures such as part of the Golgi complex. The different location of arylsulfatases in lysosomes and the dual incubations[11] for arylsulfatase and acid phosphatase indicate the heterogeneity of lysosomes in regard to their enzyme content[7]. Such heterogeneous distribution of arylsulfatase in the lysosome is in contrast to that of the mitochondrion.

Arylsulfatases A and B (type II) in the mammalian liver cells can be distinguished from C (type I) by their substrate specificity, by strong phosphate and sulfate inhibition, and by their optimal pH. Arylsulfatase A, B and C have their optimal pH range about 4.2, 5.5 and 8.0 respectively[1].

At the electron microscope level, there was no noticeable difference between arylsulfatase of A and B over a wide range of pH (4.5–7.4)[9]. As for arylsulfatase C, which is presumed to be located mainly in the microsomal fraction in contrast to arylsulfatase A and B which are located both in the microsomal and the mitochondrial fractions, an attempt was made to replace lead nitrate with lead citrate as the capturing reagent, which resulted in a clear incubation medium in the higher pH range for alkaline phosphatase[20]. This is still in progress in our laboratory but so far we need further study on the substrate. However the occasional localization of arylsulfatase in the sacs of the endoplasmic reticulum is noticed[21-23].

Résumé. Des tissus de rats fixés par perfusion avec une solution de glutaraldéhyde et de DMSO ont permis de localiser le produit de réaction de l'arylsulfatase B dans les mitochondries ainsi que dans d'autres organites. Dans les mitochondries, l'arylsulfatase a été décelée entre les membranes externe et interne de même qu'à l'intérieur des crêtes. La matrice mitochondriale n'a donné aucune réaction.

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[19] D. W. MISCH and S. M. MARGARET, Proc. 3rd Int. Cong. Histochem. Cytochem. New York (1968), p. 179.

[20] H. MAYAHARA, H. HIRANO, T. SAITO and K. OGAWA, Histochemie *11*, 88 (1967).

[21] Supported by grants from Medical Research Council of Canada and the Muscular Dystrophy Association of Canada.

[22] Dr. T. MAKITA is a recipient of post-doctoral fellowship from the Muscular Dystrophy Association of Canada.

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Determination of the Sensitive Phase for Bristle Organ Modifications upon Injection of Mitomycin C into Larvae and Pupae of *Drosophila melanogaster*

The injection of Mitomycin C into the body cavity of third instar larvae of *Drosophila melanogaster* during cellular differentiation results in the formation of bristles without sockets and bracts on the head, thorax, wing, leg, and external genital organ of the adult fly[1]. However, no altered bristle organs could be found on the abdomen. Furthermore, it has been shown[2] that the treatment of foreleg imaginal disks of *D. melanogaster* with Mitomycin C in vitro and subsequent implantation into larval hosts results not only in the formation of bristles without sockets, but considerably reduces the number of bristle organs that differentiate. Therefore, the questions arose 1. whether or not the abdominal bristle organs have a different sensitive phase for the Mitomycin C effect than do all other bristle organs, and 2. whether the sensitive phase for reducing the number of bristle organs and for the loss of sockets on the integument are the same. The easily distinguishable large and small bristles of the thorax and head facilitated comparison between the sensitive phase for macrochaetae and microchaetae formation. The investigation of thousands of bristles after Mitomycin C injection also revealed structural modifica-

tions of the bristle organ itself other than the loss of the socket.

Larvae, prepupae and pupae of the mutant strain *yellow* (*y*, 1–0.0) of *D. melanogaster* were used in all experiments. The age of the larvae at the time of injection of Mitomycin C is given in hours after egg deposition. 5–8-day-old fertilized females were allowed to lay eggs for 10 min in a dish of freshly made food. The embryos and larvae were kept in an incubator at 25 °C. The age of the prepupae and pupae is based on the time of formation of the puparium. Mitomycin C was dissolved in insect Ringer solution (Conc. 0.025–0.05 mg/ml) and 0.05 or 0.1 µl was injected into larvae and/or pupae of different ages by the use of a micropipette. In control experiments, insect Ringer solution alone was injected.

The first experiment demonstrates the sensitive phase at which the inhibition of bristle organ formation takes place after injection of Mitomycin C. The differences in the effect of the inhibitor agent on the number of macro- and microchaetae of the thorax is shown graphically in Figure 1. The highest sensitivity of the macrochaetae is reached after injection of the drug into 96 h old larvae. The microchaetae, however, attain their maximum sensitivity during the prepupal stage. Macro- and microchaetae of the head show exactly the same difference in their maximum sensitivity as do the thoracic bristles. The difference in maximum sensitivity for macro- and microchaetae formation does not necessarily reflect a time difference in the formation of the two bristle types. Differential permeability might well account for the observed difference in response of the macro- and microchaetae forming cells.

In comparable experiments, WADDINGTON[3] treated *Drosophila* larvae and pupae with heavy doses of X-rays and found that irradiation of late third instar larvae affected predominantly the formation of the macrochaetae, whereas X-ray treatment of young prepupae and pupae resulted in a more severe damage of the microchaetae. The agreement between the results obtained with X-rays and Mitomycin C on the sensitive phase of macro- and microbristle forming cells indicates that the differential uptake of Mitomycin C may not account for its differential effect.

In a second experiment we determined the sensitive period at which bristles are formed without sockets. Figure 2 shows that the macrochaetae of the thorax have a maximum sensitive phase in the late third larval stage, whereas the microchaetae reach the highest sensitivity in the prepupal stage. Therefore, the phase of maximum sensitivity for the formation of bristle organs without sockets coincides with the phase of maximum sensitivity for the inhibition of bristle formation.

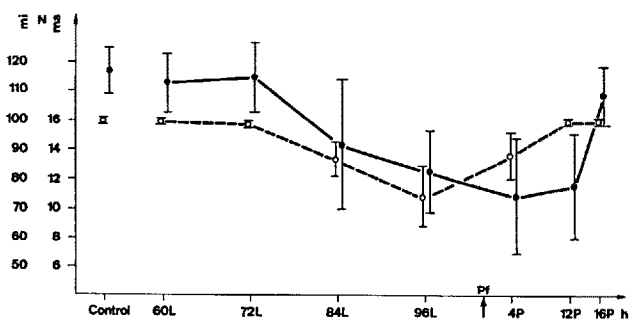

Fig. 1. The sensitive phases for bristle organ differentiation on the thorax after injection of Mitomycin C into larvae and pupae of different ages. The age of larvae (L) is given in h after egg deposition, and that of pupae (P) in h after puparium formation (Pf). ○——○, macrochaetae (ma); ●—●, microchaetae (mi). Standard deviations are indicated as vertical lines. N, number of bristles formed. Injected dose of Mitomycin C: 0.0025 µg, except for 16-hour-old pupae: 0.005 µg.

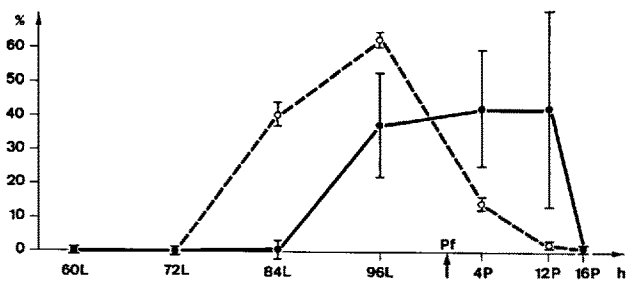

Fig. 2. The effect of injection of Mitomycin C into larvae and pupae of different ages on the formation of macrochaetae (○——○) and microchaetae (●—●) without sockets on the thorax (Ordinate in %). The ages of larvae (L) are given in h after egg deposition, that of pupae (P) after puparium formation (Pf). Standard deviations are indicated as vertical lines. Injected dose of Mitomycin C: 0.0025 µg, except for 16-hour-old pupae: 0.005 µg.

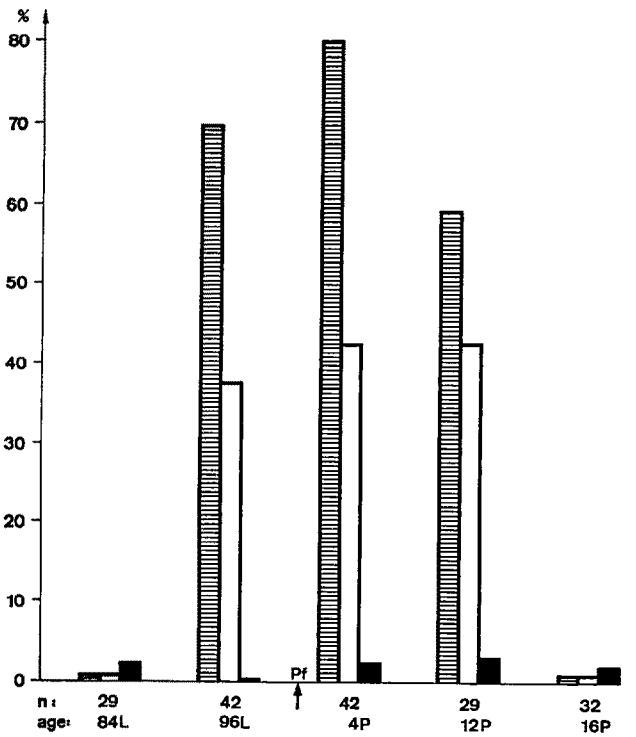

Fig. 3. The effect of injection of Mitomycin C into larvae and pupae of different ages on the formation of microchaetae without sockets (Ordinate in %) on the head (hatched column), thorax (white column) and abdomen (black column). Ages of the larvae (L) are given in h after egg deposition and of the pupae (P) in h after puparium formation (Pf). *n* equals the number of treated larvae and pupae in each experiment. Injected dose of Mitomycin C: 0.0025 µg, except for 16-hour-old pupae: 0.005 µg.

[1] H. TOBLER, Experientia *25*, 213 (1969).
[2] H. TOBLER and M. PFLUGER, Wilh. Roux' Arch. EntwMech. Org. *164*, 293 (1970).
[3] C. H. WADDINGTON, J. exp. Biol. *19*, 101 (1942).

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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[4] D. H. Lindsley and E. H. Grell, Carnegie Inst. Wash. Publ. *627* (1968).
[5] A. Lees and C. H. Waddington, Proc. R. Soc., B. *131*, 87 (1942).
[6] Present address: Department of Biology, The Johns Hopkins University, Baltimore, (Md. 21218, USA).

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-