Statistical evaluation was made by Student's *t*-test.

*Results.* As is shown in the Table, whole blood serotonin of normal blood amounted to 110–142 ng/ml. Negative ionization reduced these values to 84–89 ng/ml, whereas positive ionization raised them to 194–197 ng/ml. Blood fractionation into plasma, erythrocytes and thrombocytes showed a similar trend in all 3 blood constituents. Blood plasma contained 40–47 ng/ml serotonin; negative ionization reduced these values to 21–23 ng/ml, while positive ionization raised them to 70–83 ng/ml. The erythrocyte suspension contained 56–69 ng/ml serotonin; negative ionization reduced these values to 29–37 ng/ml, while positive ionization increased them to 103–110 ng/ml. The thrombocyte suspension contained only 10–20 ng/ml serotonin; negative ionization reduced these values to 5 ng/ml, whereas positive ionization increased them to 28–34 ng/ml.

*Discussion.* The experiments demonstrate the propensity of positive ions to cause serotonin release from the blood and that of negative ions to suppress such release in vitro. Thus the theory of the 'Krueger Effect' has been well proved. Krueger himself showed in vivo that big concentrations of positive ions raised serotonin blood levels in mice, while high concentrations of negative ions lowered them[7].

These results also indicate that during the separation of the thrombocytes from the erythrocytes part of them decayed and released their serotonin. In order to study the disposal of this serotonin, we carried out the experiments listed in the Table with siliconized blood fractions of plasma, erythrocytes and thrombocytes; furthermore with non-siliconized syringes and Petri dishes on full blood. In the former set-up, positive air ionization increased serotonin in the thrombocyte fraction only. In the latter set-up, microscopic inspection showed complete decay of the thrombocytes which had released their serotonin. This was taken up by the plasma (20–30%) and the erythrocytes (70–80%); in other words, in the absence of thrombocytes, serotonin transport is taken over by the erythrocytes, a phenomenon not duly appreciated until now. The decrease in serotonin levels after negative ionization in vitro is probably due to its normal breakdown by monoamine oxydase[12]. However, in vivo serotonin can also be converted to 5-hydroxyindole acetic acid (5-HIAA) by the cytochrome oxidase system, as shown by KRUEGER and SMITH[3]. The mechanisms of this breakdown are now studied by us in vivo and in vitro by applying special enzyme inhibitors.

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## Initial Transdetermination in the First Leg Discs of Different *Drosophila* Species[1]

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*Summary.* Initial transdetermination leads from lateral and medial halves of male first leg imaginal discs almost exclusively to structures of the base and the spread (blade) of the wing. Mesonotum never appeared. The frequency of transdetermination is species-specific and most probably cell-autonomous. Medial halves transdetermine more frequently than lateral halves. Under the influence of an equivalent amount of blastema growth *D. nigromelanica* transdetermine with a much higher frequency than *D. virilis*.

*Transdetermination* can be defined as a change of the determined state of cells to a different state from which they will initiate a pathway of differentiation that leads to structures that no longer correspond to the initial state of determination.

Transdetermination was discovered in blastemas of larval imaginal discs that were first cultured in the abdomen of adult females[4]. In this medium no differentiation but an extensive proliferation occurs. In order to find out whether the determined state remained stable, test pieces of the proliferated blastemas were transplanted back into metamorphosing larval hosts. With this method we found that there exists for each disc a given probability for the event and the direction of transdetermination[5–9]. After an initial transdetermination event, which would for example lead in *Drosophila melanogaster* from genital blastemas to leg structures, changes of second and third order could lead to wing or head and then to the notum of the mesothorax. Transdetermination of higher orders seems to occur only in cultures which have been transferred twice or many times from one adult host to another.

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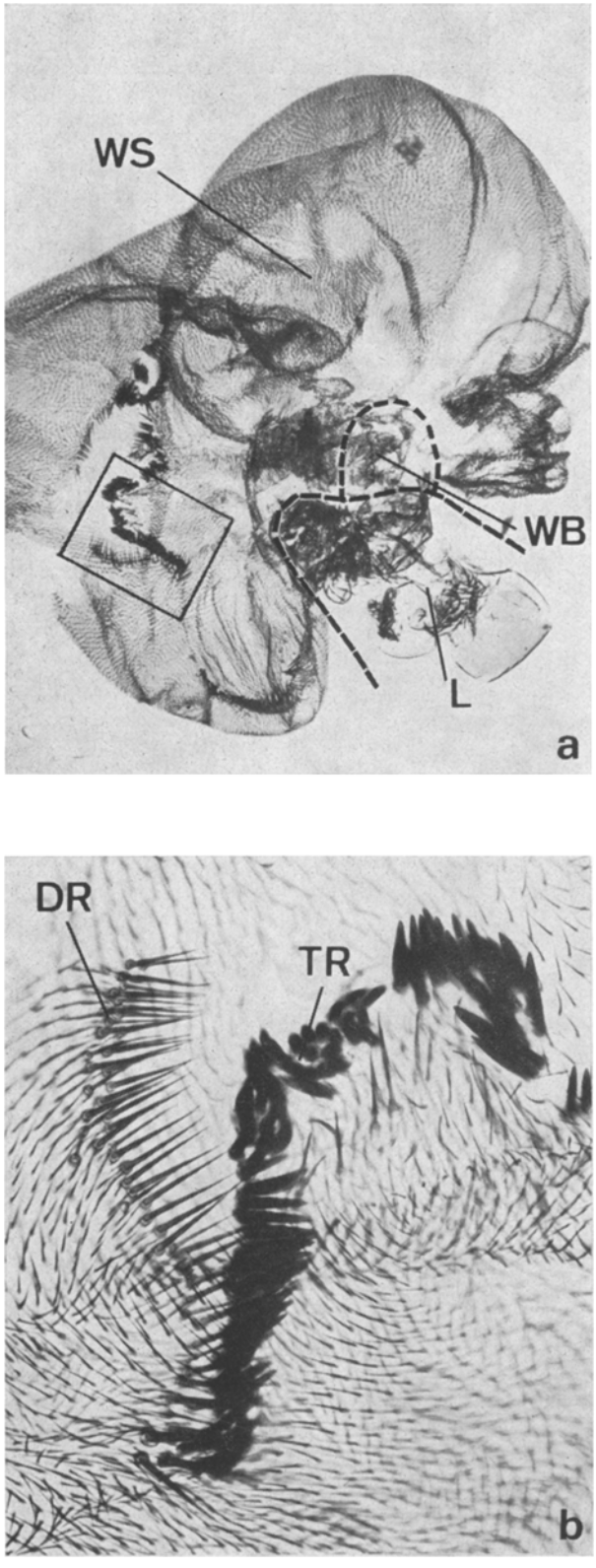


A case of initial transdetermination in a lateral half of a leg disc to wing (series 9 of Table I). a) shows the rather small autotypic leg part (L) in contact with a huge allotypic wing, which includes a small area of the wing base (WB) besides the much extended spread of the wing (WS). b) Details of wing area (indicated in Figure a) showing the bristles of a normal wing border with double row (DR) and triple row (TR).

Table I. Frequencies and directions of initial transdeterminations in lateral (L) and medial(M) halves of male first leg discs after 14 days of culturing in vivo

| Series Number | Tests N | tr | = % | Direction b | sp | b + sp | p |
|---|---|---|---|---|---|---|---|
| 1 n n L | 50 | 11 | 22% | 3 | 6 | 2 | – |
| 2 n n M | 48 | 16 | 33% | 6 | 8 | 2 | – |
| 1 + 2 | 98 | 27 | 27% | 9 | 14 | 4 | – |
| 3 n m L | 74 | 26 | 35% | 4 | 15 | 7 | – |
| 4 n m M | 70 | 29 | 41% | 4 | 10 | 10 | – |
| 3 + 4 | 144 | 55 | 38% | 13 | 25 | 17 | – |
| 1 + 2 + 3 + 4 | 242 | 82 | 34% | 22 | 39 | 21 | – |
| 5 v v L | 96 | 10 | 10% | 5 | 4 | 1 | – |
| 6 v v M | 114 | 16 | 14% | 9 | 6 | – | 1 |
| 5 + 6 | 210 | 26 | 12% | 14 | 10 | 1 | 1 |
| 7 v m L | 92 | 10 | 11% | 3 | 5 | 2 | – |
| 8 v m M | 91 | 15 | 17% | 9 | 3 | 3 | – |
| 7 + 8 | 183 | 25 | 14% | 12 | 8 | 5 | – |
| 5 + 6 + 7 + 8 | 393 | 51 | 13% | 26 | 18 | 6 | 1 |
| 9 n v L | 64 | 18 | 28% | 6 | 5 | 7 | 2 |
| 10 n v M | 72 | 27 | 38% | 9 | 6 | 12 | – |
| 9 + 10 | 136 | 45 | 33% | 15 | 11 | 19 | 2 |
| 11 v n L | 61 | 6 | 10% | 4 | 2 | – | – |
| 12 v n M | 70 | 13 | 19% | 11 | 2 | – | – |
| 11 + 12 | 131 | 19 | 14% | 15 | 4 | – | – |
| 13 ma ma L | 16 | 1 | 6% | – | 1 | – | – |
| 14 ma ma M | 27 | 7 | 26% | – | 4 | 3 | – |
| 13 + 14 | 43 | 8 | 19% | – | 5 | 3 | – |
| 15 ma m L | 25 | 3 | 12% | – | 2 | 1 | – |
| 16 ma m M | 26 | 5 | 19% | – | 3 | 2 | – |
| 15 + 16 | 51 | 8 | 16% | – | 5 | 3 | – |
| 13+14+15+16 | 94 | 16 | 17% | – | 10 | 6 | – |
| 17 f m L | 21 | 2 | 10% | 2 | – | – | – |
| 18 f m M | 15 | 5 | 33% | 4 | 1 | – | – |
| 17 + 18 | 36 | 7 | 19% | 6 | 1 | – | – |

N, number of test implants; tr and %, number and % of test implants in which transdetermination occurred. Direction to base (b), spread (sp), base and spread (b + sp) and palpus (p). Species used in the series 1–16: *D. nigromelanica* = n; *D. virilis* = v; *D. mauritiana* = ma; *D. funebris* = f; *D. melanogaster* = m (only as adult hosts). The first symbol refers to the species of the tested leg blastema (e.g. n in series 3); the second symbol stands for the adult host in which proliferation occurred (e.g. m in series 3).

Table II. Relation between amount of growth (G in % of original size) and frequency of transdetermination (Tr in %) in the series 1–8 of Table I.

| Series | N | | G (%) | Tr (%) |
|---|---|---|---|---|
| | C | T | | |
| 1 + 2 n n L + M | 109 | 99 | 70,5 | 27 |
| 3 + 4 n m L + M | 128 | 105 | 123 | 38 |
| 5 + 6 v v L + M | 133 | 60 | 68 | 12 |
| 7 + 8 v m L + M | 51 | 62 | 117 | 14 |

N, number of measured blastemas; C, controls without culturing; T, tests after culturing during 14 days.

We are however not certain whether such secondary changes are already possible in the first transfer generation, in which the blastemas grow for 2 weeks in the adult host. Moreover, we need more exact information about the initial transdetermination occurring in the first transfer generation. In the present experiments which deal with blastemas of the first leg only, we try to answer the following questions: a) Can cells of the leg disc transdetermine into wing as well as into antennal structures during the initial transdetermination event? Such a possibility has been suggested by the results of earlier experiments[5–9]. b) Do different parts of the disc transdetermine in different directions and with different frequencies? c) Is the frequency and direction of transdetermination in leg blastemas species-specific, as has been found for labial discs[10]?

*Materials and methods.* During the last few years we met serious difficulties bringing through metamorphosis larvae of *Drosophila melanogaster* into which a piece cultured in an adult host had been transplanted. It is not clear to what an extent a virus infection may be responsible for this problem. Since the transplantation of blastemas in other species did not suffer from this difficulty, we used for our studies male first leg discs of *Drosophila nigromelanica*, *D. virilis*, *D. funebris* and *D. mauritiana*.

The discs were dissected from mature larvae and longitudinally fragmented into a lateral and a medial half. These halves were implanted into adult hosts of the same or a different species, for which purpose, *D. melanogaster* could be used too. After a culture period of 14 days the enlarged blastemas were implanted into mature larval hosts of their own species. Some blastemas could be implanted in toto, others had become too large and were divided into several pieces. The carriers of these implants were opened shortly after hatching as adults. All metamorphosed implants were studied under a dissecting microscope.

Since we know that transdetermination is a function of the extent of proliferation[7], we measured the growth of the disc halves in different adult hosts (Table II). A method previously used in our laboratory[11] was applied. The living blastemas were compressed to a constant thickness between a slide and a coverslip. The area of the blastemas was projected onto paper and measured. In one series the blastemas were measured before culturing, in another series after culturing. Growth is given as a percentage of increase in area (Table II). All fly cultures were kept at 25° ± 1 °C on standard *Drosophila* food.

*Results and discussion.* 1. Longitudinal halves of first leg discs transdetermined *almost exclusively to wing* blastemas. Among the 1033 test implants of all species listed in Table I were 219 cases of transdetermination to *wing* (21.2%) and only 3 cases (0.3%) which showed structures of the palpus, i.e. derivatives of the antennal disc.

Earlier studies on leg discs of *D. melanogaster* had also revealed a high preference for the change from leg to wing[7–9]. But transdetermination to antennal structures was also found in about 10% of the cases. The much lower frequency observed in our present experiments may be due to technical differences in the experiments or to species differences. There is however another possibility which must be considered: It is possible that the antennal structures, though found in test pieces of the first transfer, had first passed through a wing stage before a transdetermination of second order had led to antenna or palpus. Indeed, in two of the three cases with palpus, mentioned above, the transplants contained wing parts as well as palpus structures; unfortunately, we were unable to examine the sister test piece of the third case since it was lost as a result of death of the host larva.

2. In all series the *medial halves* (M) transdetermined *more frequently than the lateral* (L) *halves:* even-numbered series versus add-numbered series in Table I. This difference had also been observed in experiments with *D. melanogaster*[12]. The higher frequency in medial halves may be related to the fact that this region of the disc has a distinct tendency for regeneration of the missing parts whereas the lateral halves preferentially form mirror image duplications[12].

3. The test implants very often contained only structures *of the wing base or only of the wing spread.* But in many implants (Figure) *both* wing areas were represented. In most of the series 1–12 (Table I) all three types were found. The lack of pure wing base in *D. mauritiana* (series 13–16) may not be significant. From the material as a whole we can conclude that all wing parts can result from transdetermined first leg blastemas regardless of whether they stem from lateral or medial halves; mesonotum (thorax) was never found.

4. *Each species has its characteristic frequency of transdetermination.* This was already indicated in experiments with labial discs[10]. In the present experiments the highest frequencies were found for *D. nigromelanica* (series 1–4, 9–10); much lower frequencies were typical for *D. virilis* (series 5–8, 11–12). *D. mauritiana* (series 13–16) and *D. funebris* (series 17–18) transdetermined with frequencies intermediate between those of *nigromelanica* and *virilis*. This last statement is based, however, on rather small numbers of test implants and is therefore preliminary. For first leg discs of *D. melanogaster* a transdetermination frequency of 34% has been reported[8]. This is of the same order of magnitude as the frequency in *D. nigromelanica*.

5. Within a given species the frequency of transdetermination is correlated with the *amount of growth*[7]. For *D. nigromelanica* and *D. virilis* proliferation was higher in adults of *D. melanogaster* than in their own hosts: Table II, series 3 + 4 and 7 + 8 versus 1 + 2 and 5 + 6. Correspondingly, we found higher frequencies of transdetermination after culturing in *D. melanogaster* than in a host belonging to the same species as the cultured blastema. But such an influence of the host seems to be of a trivial quantitative nature which does not interfere with the *species-specific autonomy for transdetermination* in the cultured blastemas. Such an autonomy is indicated when series 1 + 2 of table I are compared with series 9 + 10, or series 5 + 6 with series 11 + 12 (Table I). Moreover, from Table II follows that *nigromelanica* (series 3 + 4) transdetermine with a much higher frequency than *virilis* (series 7 + 8) although both blastemas enjoied the same amount of growth stimulation in *melanogaster*. A similar autonomy not dependent on the amount of growth was earlier found for labial discs[10].

6. In many cases the allotypic wing areas contain more cells than the autotypic leg structures (Figure). Since it seems unlikely that a *single* wing cell could have multiplied to such an extent, our new findings support the suggestion that transdetermination occurs simultaneously *in groups of cells*[5, 13].

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