James, A., M. Bownes* and S. Glenn**.
Sidney Farber Cancer Institute, Boston, Massachusetts; *Edinburgh University, Edinburgh; **Center for Pathobiology, University of California, Irvine. The re-establishment of pattern elements in regenerating imaginal wing discs of D. melanogaster.

We have attempted to analyze how regeneration occurs in imaginal discs by determining the sequence in which pattern elements reappear during regeneration of a fragment of the wing disc.

Late third instar wing discs were cut into a small 02 fragment corresponding to presumptive notum and a large 28 fragment corresponding to the wing hinge and blade (Bryant 1975). The 02 pieces were metamorphosed immediately as controls. The 28 pieces were injected into females and allowed to grow for 1 to 5 days before metamorphosis in a larval host was induced. The metamorphosed implants were scored for the regeneration of the bristles found in the notum.

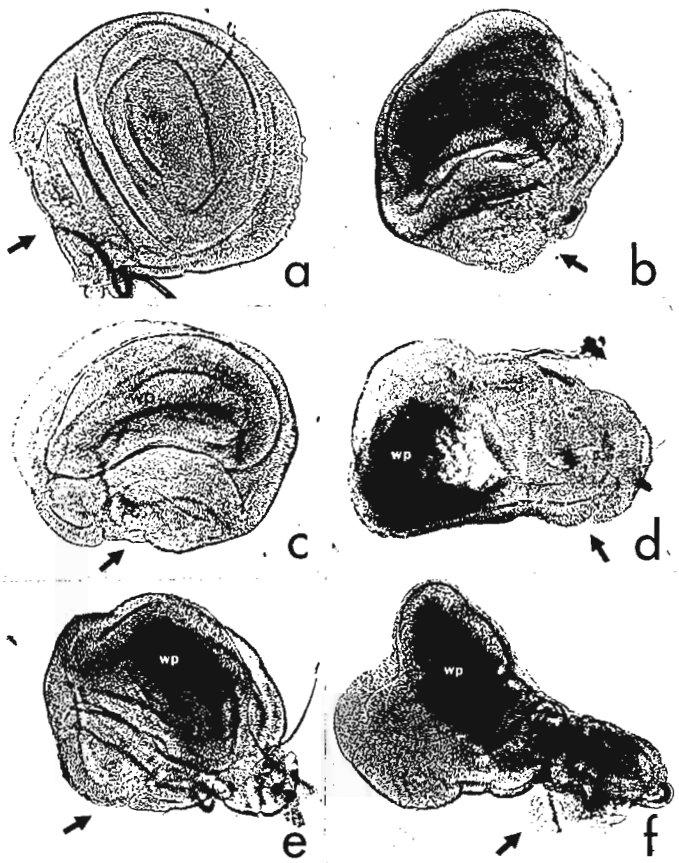

Fig. 1. Wound healing and growth during culture. (a) 28 fragment immediately after cutting and after (b) 1 day, (c) 2 days, (d) 3 days, (e) 4 days, (f) 5 days of culture in an adult female abdomen. → marks the original wound line.

The process of wound healing and growth observed in the majority of discs can be seen in Fig. 1. Before culture in the adult abdomen the 28 fragment has as exposed surface of cells. After culture for 1 to 2 days these cells have healed together. Maximum growth occurs between 2 and 4 days of culture.

The results of the sequence of regeneration are shown in Table 1. Regeneration in the 28 pieces was measured as an increase in the frequency of those elements expected from the immediate metamorphosis of the 02 controls. Fig. 2a shows the wing disc fate map and the location of the cutting line. The bristles scored were the presutural bristles, the anterior and posterior notopleural bristles, the anterior and posterior supraalar bristles, the anterior and posterior postalar bristles, the anterior and posterior dorsocentral bristles and the scutellar bristles. All of these bristles, with the exception of the presutural bristle, were present at least 75% of the time in 02 control implants.

28 fragments metamorphosed immediately in larvae produced no thoracic bristles. Implants cultured for one day showed very little regeneration. The pattern elements which were regenerated at a low frequency were those structures near to the original 02 cutting line, notopleural bristles, presutural bristles, supraalar bristles, and structures furthest from the cut edge, the scutellar bristles. Fig. 2b shows the frequency of regenerated elements and three outside markers. By the second day all of the pattern elements of the notum were present at a low frequency except the postalar bristles, which were missing completely. As seen in Fig 2c, the notopleural, supraalar, and scutellar bristles were present in the highest frequencies. Implants cultured for three days had regenerated the notopleural and scutellar bristles more than 20% of the time (Fig. 2d). The dorsocentral and presutural bristles were regenerated with lower frequencies. The notopleural, supraalar, postalar, and scutellar bristles were regenerated at least 70% of the time in pieces cultured for four days (Fig. 2e). The remaining bristle elements were present more than 50% of the time, except for the presutural bristle which was present 20% of the time. However, the presutural bristle is often absent in control implants, being present in only 62% of the 02 control fragments. In

TABLE 1. Structures identified in regenerating fragments of wing discs

Element scored	control 0 Day 02	control 0 Day 28	experimental 1 Day 28	experimental 2 Day 28	experimental 3 Day 28	experimental 4 Day 28	experimental 5 Day 28
Presutural Bristles	62		5	4	13	20	26
Notopleural Bristles	81		10	15	57	80	56
Supraalar Bristles	93		5	15	25	85	67
Post alar Bristles	86				30	70	59
Dorsocentral Bristles	86			4	17	55	56
Scutellar Bristles	94		5	8	52	70	63
Anterior notal wing process		46	52	54	96	95	74
Tegula	8	88	81	85	100	100	81
Humeral plate		79	67	92	100	90	81
Unnamed plate		75	48	88	100	100	74
Axillary sclerites							
first	6	96	76	96	100	100	93
second	1	100	95	100	100	100	85
third		92	90	100	78	100	81
fourth	69	33	52	23	35	60	44
Proximal costa		96	67	88	83	95	81
Medial costa		96	86	88	87	100	81
Distal costa		92	71	85	78	90	81
Triple row		100	76	92	96	85	74
Double row		96	52	81	74	60	74
Posterior row		25	38	69	52	95	70
Sc4d		92	52	92	65	80	59
Sc25		100	90	96	87	95	85
Prealar apophysis		21	90	73	74	65	67
Yellow club		88	90	85	91	85	89
Proximal ventral radius		92	67	73	83	90	78
Pleural wing process		71	90	92	96	90	93
Axillary pouch		75	76	77	96	75	89
Total implants	84	24	21	26	23	20	27

All figures refer to the percentage occurrence of each element in the total implants obtained for that series.

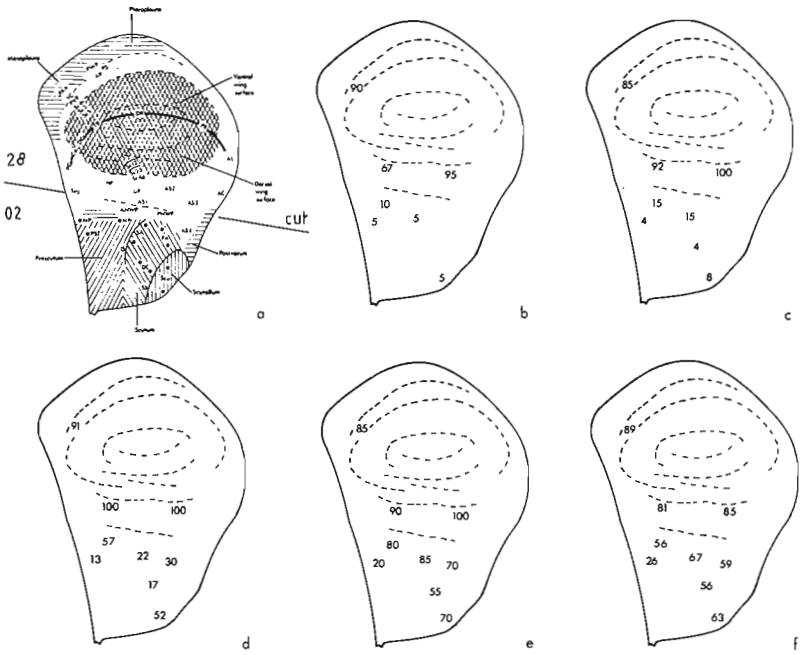

Fig. 2. (a) Fate map of wing disc (after Bryant 1975). Markers used in this study: ANWP, anterior notal wing process; AP, axillary pouch; AS, axillary sclerites, first, second and third; PCO, MCO, and DCO, proximal, medial and distal costa; DC, dorsocentral bristles; HP, humeral plate; NP, notopleural bristles; PS, pleural sclerite; PWP, pleural wing process; PA, postalar bristles; PAA, prealar apophysis; PST, presutural bristles; PVR, proximal ventral radius; TR, OR, and PR, triple row, double row and posterior row of wing margin hairs; Scu, scutellar bristles; Sc4d and Sc25, group of 4 and group of 25 sensilla campaniformia on the dorsal radius; SA, supraalar bristles; Reg, tegula; UP, unnamed plate; YC, yellow club. For rest of abbreviations see Bryant 1975. (b) Frequency of presence of pattern elements in disc fragments cultured for one day. The frequencies of 3 unregenerated markers are given in the 28 piece to contrast with the frequency of markers in the regenerating 02 region. (c) Two-day culture periods. (d) Three-day culture periods. (e) Four-day culture periods. (f) Five-day culture periods.

the five day implants all pattern elements of the notum appeared less frequently than in the four day pieces, with the exception of the presutural bristle which now appeared in 26% of the implants.

Using statistics we were able to conclude that the sequence with which the bristles reappeared was: (1) notopleurals, (2) supraalars and scutellar bristles, (3) presuturals, postalars and dorsocentral bristles. It should be noted that presutural bristles are not included in the figures since they are often not differentiated in the controls (Table 1).

During regeneration the cells respond to positional cues which are set up in the growing tissue mass and these in turn define which part of the regenerate the cells will make. Initially there are not enough cells to regenerate the entire thorax and cells must decide which pattern elements to differentiate first. One might have expected a simple sequence beginning close to the cut surface and moving towards the edge of the fate map of the disc until the pattern of the thorax is complete. It appears, however, that regions close to the cut edge, notopleural and supraalar bristles, and those furthest from it, scutellar bristles, are re-established first in the regenerating disc and the remaining structures are then intercalated.

Reference: Bryant, P.J. 1975, J. exp. Zool. 193: 49-78.

Jenkins, J.B. Swarthmore College, Swarthmore, Pennsylvania. Paternal age and mutagen sensitivity.

This study was undertaken to ascertain whether the chronological age of Drosophila males was a factor in the sensitivity of germ cells to ethyl methane-sulfonate (EMS) mutagenesis. Ore-R males of different ages were fed EMS (40 mM for 8 hours) by the Lewis technique, then mated individually to 2 day old ed $dp^{ov'}cl$ virgin females. The F_1 from post-meiotic male germ cells only (first 6 days of mating) was scored for dp mutations. As can be seen in this preliminary analysis, 27 day old males are substantially more susceptible to EMS mutagenic action than 2 day old males. The basis for