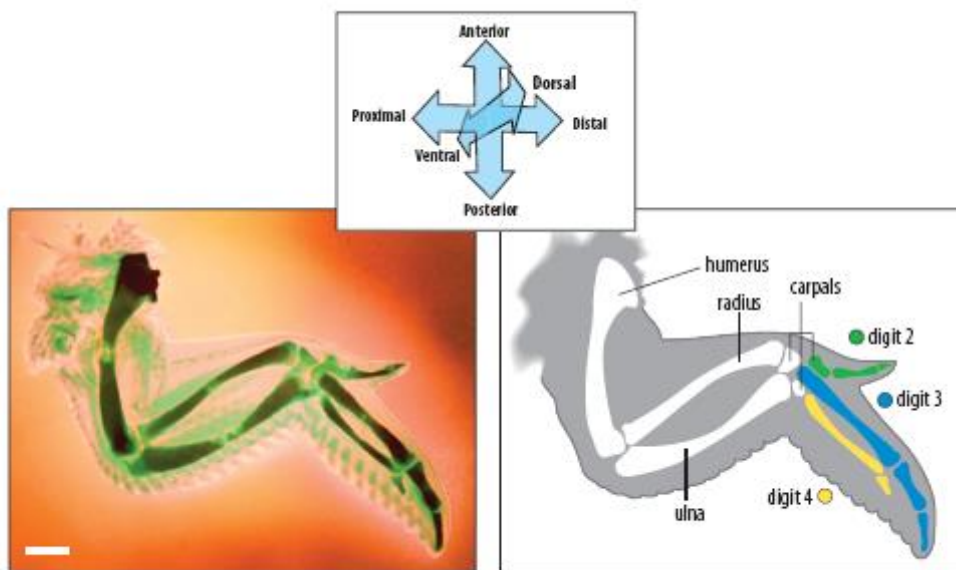


IN SILICO PRACTICAL - CHICK WING BUDS

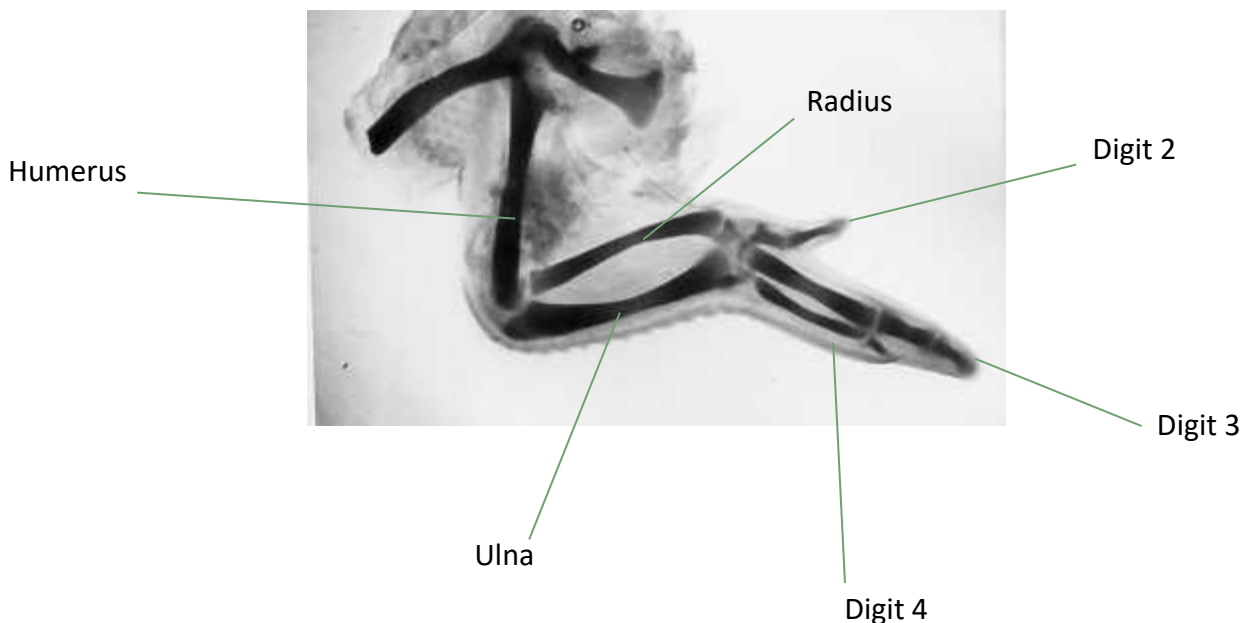
PATTERN FORMATION OF THE DIGITS IN THE DEVELOPING CHICK WING

You are provided with the data from 5 different embryological experiments investigating how the pattern of digits is specified in the chick wing. Each experiment was carried out 5 times and pictures are provided showing you the skeleton of the 10 day wing that was obtained in each case in the 5 different experiments. To help you to identify the skeletal elements, you are provided with Figure 11.2 and another labelled skeleton of normal chick wing. You are also provided with pictures of whole mounts of chick wing buds at different stages of development on which *in situ* hybridization has been carried out to show the distribution of transcripts of the *Sonic hedgehog (Shh)* gene (purple staining).

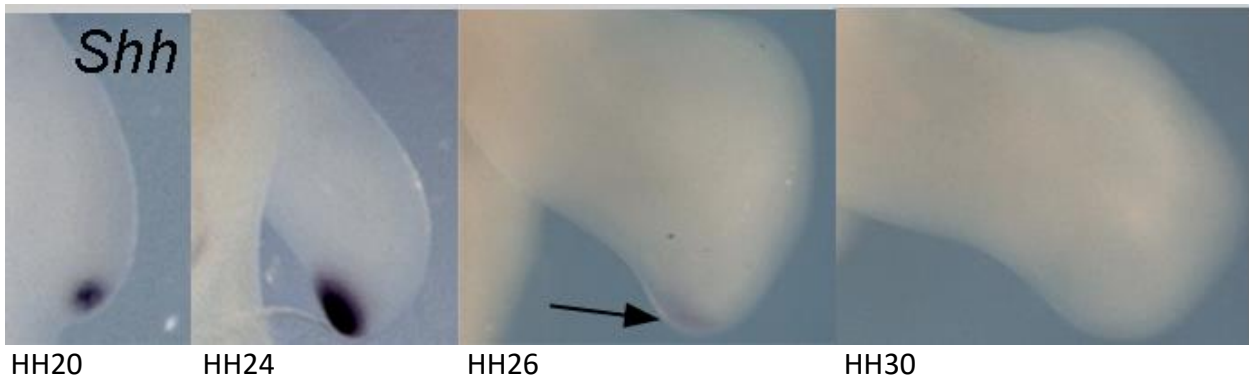
CHICK WING SKELETON



10 DAY OLD CHICK WING SKELETON



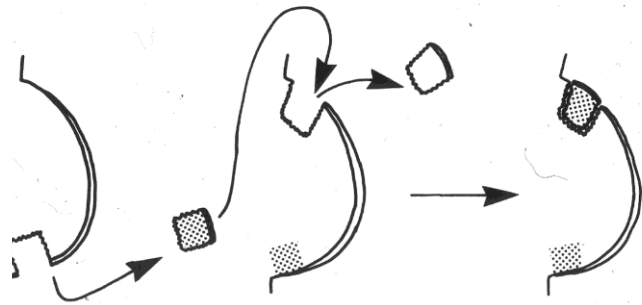
SHH EXPRESSION IN CHICK WING BUD AT DIFFERENT STAGES OF DEVELOPMENT



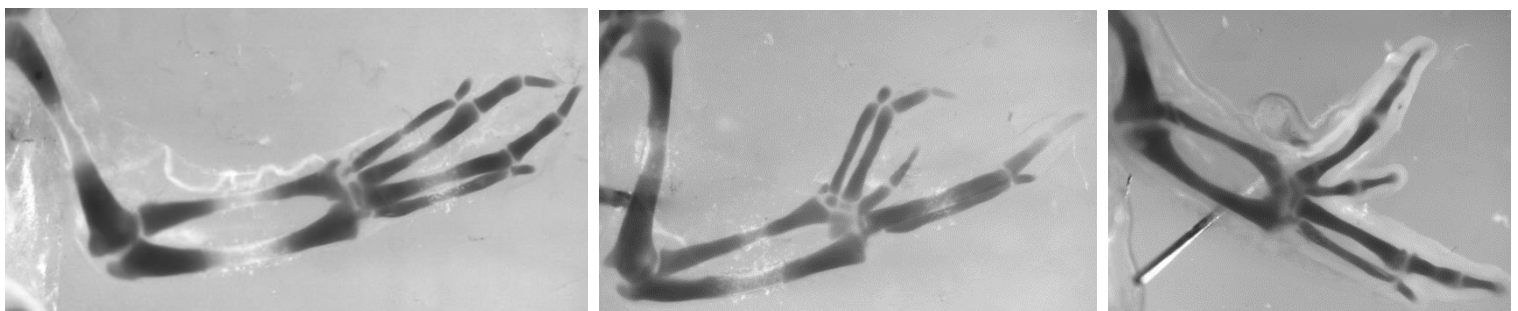
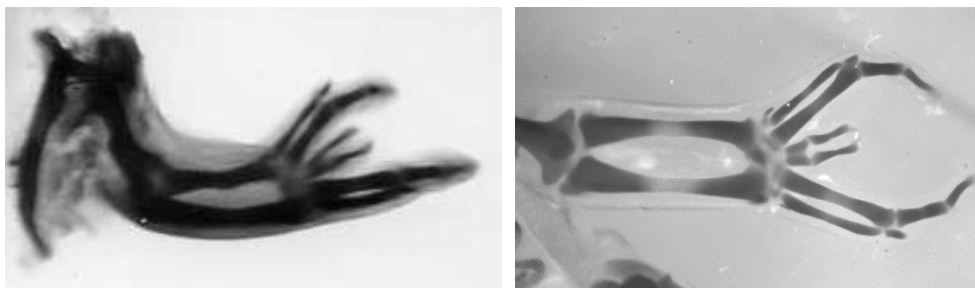
HH= HAMBURGER AND HAMILTON STAGES; ARROW INDICATES WEAK PURPLE STAINING

EXPERIMENT 1

Posterior margin of early stage 20 wing bud grafted to anterior margin of a stage 20 host wing bud. Operated embryo with wing bud with graft re-incubated for 7 days.

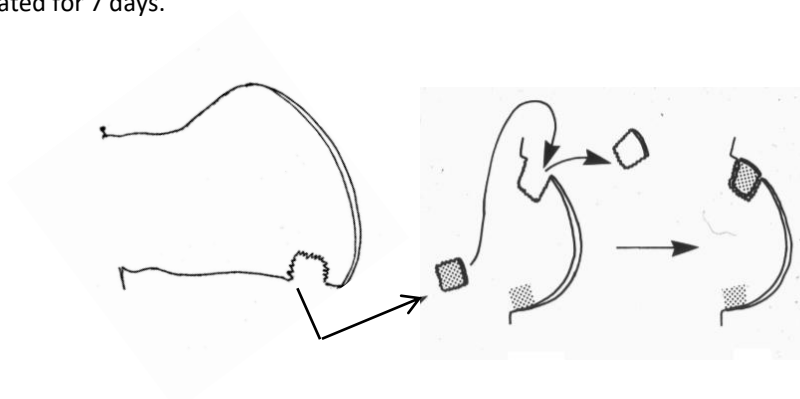


EXAMPLES OF 10 DAY WINGS STAINED TO SHOW CARTILAGE SKELETON:

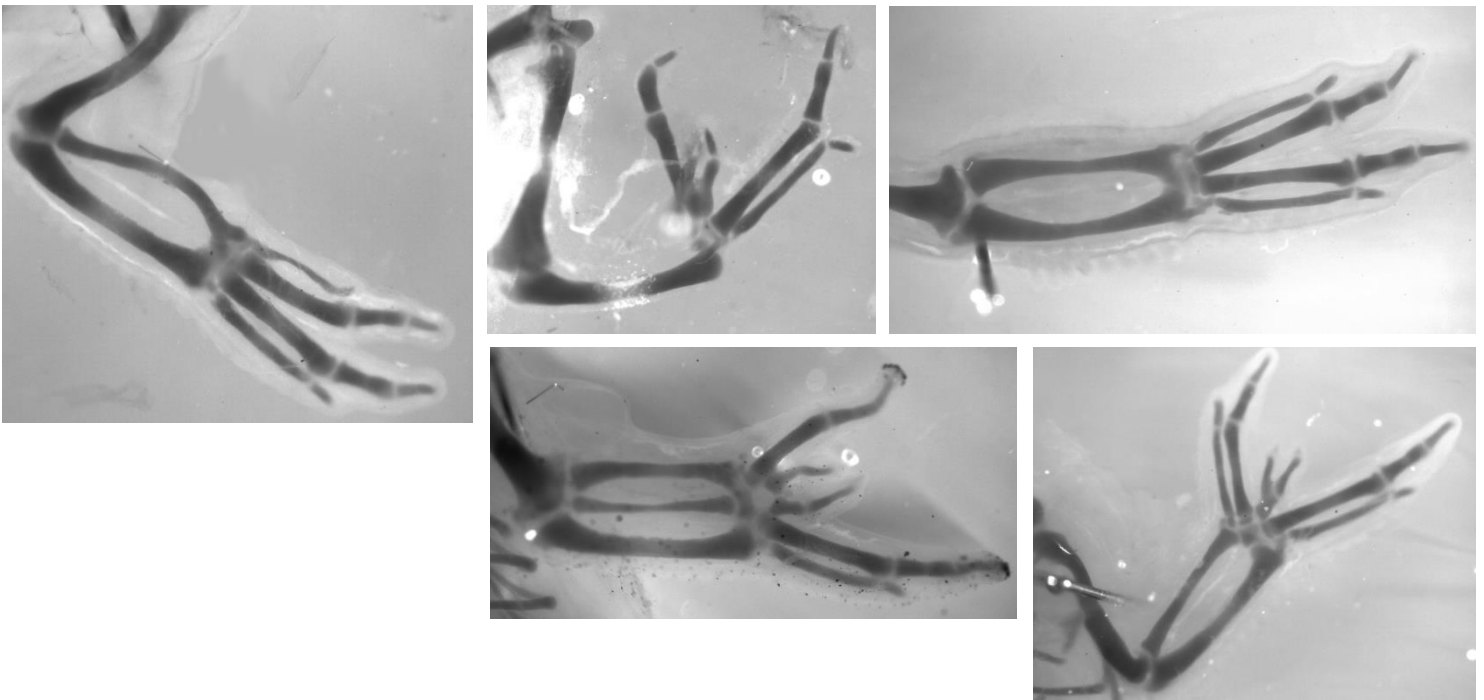


EXPERIMENT 2

Posterior margin of stage 26 wing bud grafted to anterior margin of a stage 20 host wing bud. Operated embryo with wing bud with graft re-incubated for 7 days.

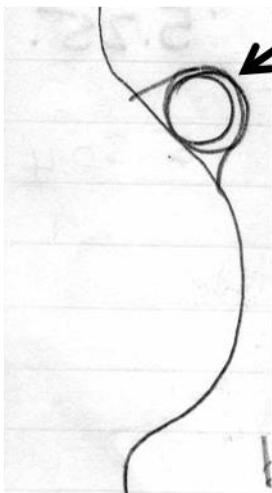


EXAMPLES OF 10 DAY WINGS STAINED TO SHOW CARTILAGE SKELETON:



EXPERIMENT 3

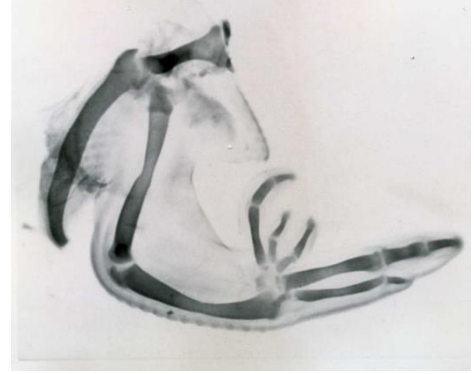
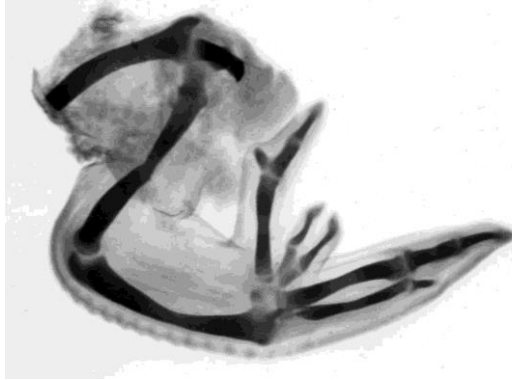
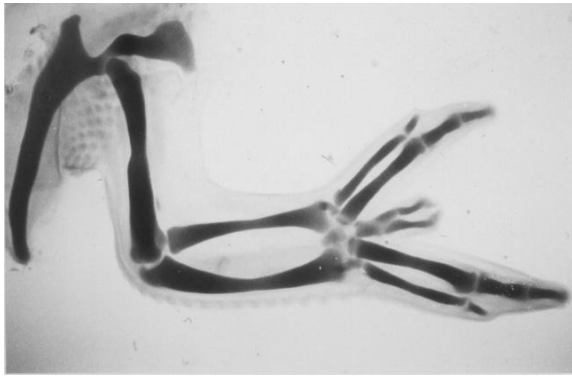
anterior



posterior

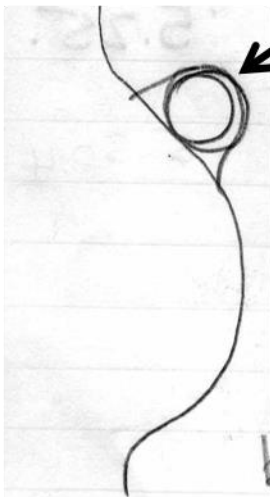
Bead soaked in retinoic acid implanted at anterior margin of early host wing bud. 7 days later, wings stained to show cartilage skeleton. Operated embryo with wing bud with bead re-incubated for 7 days.

EXAMPLES OF 10 DAY WINGS STAINED TO SHOW CARTILAGE SKELETON:



EXPERIMENT 4

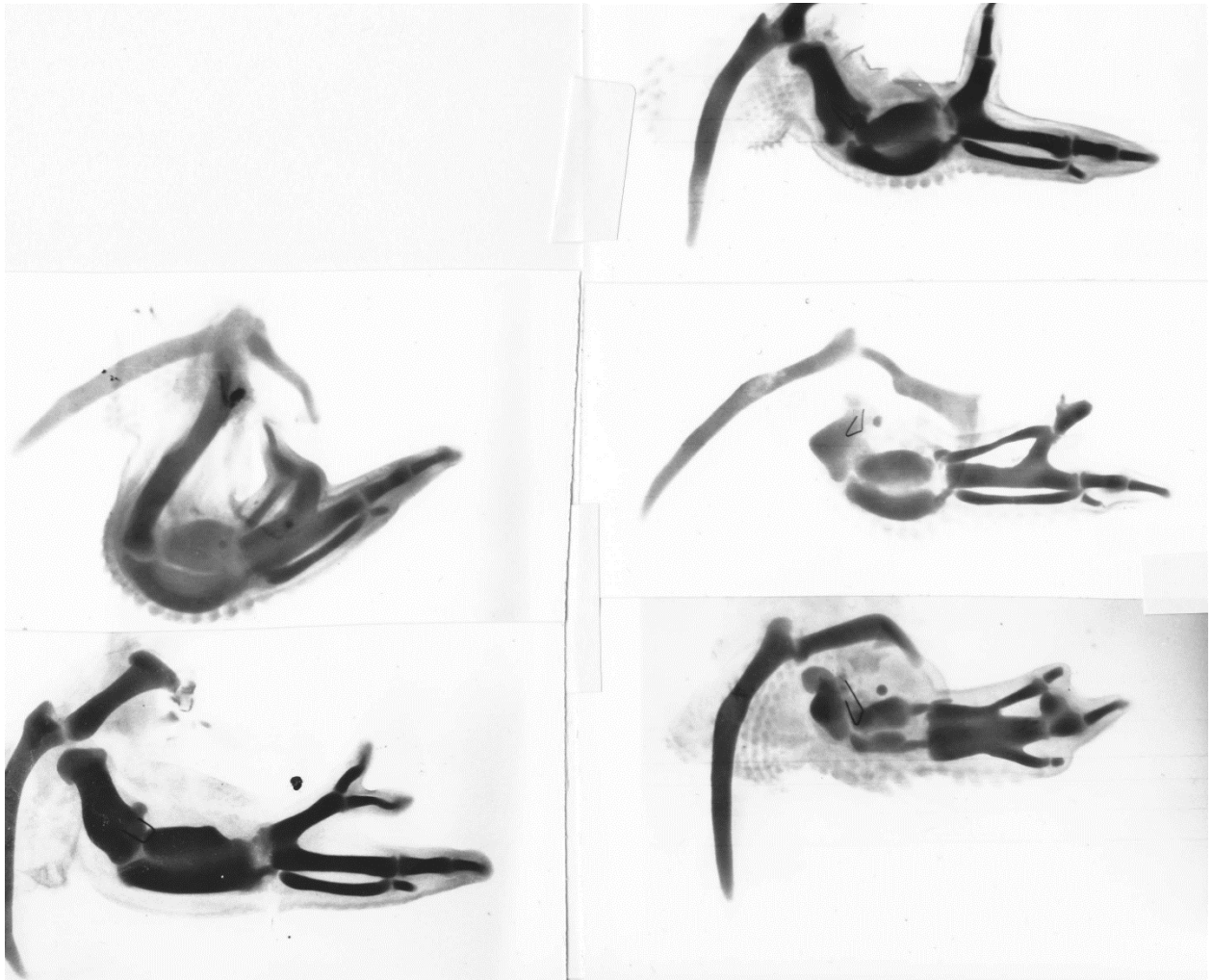
anterior



posterior

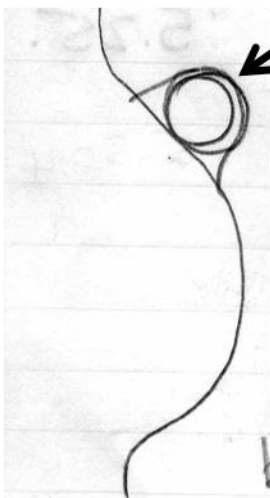
Bead soaked in high concentration of Shh protein implanted at anterior margin of early host wing bud. Operated embryo with wing bud with bead re-incubated for 7 days.

EXAMPLES OF 10 DAY WINGS STAINED TO SHOW CARTILAGE SKELETON:



EXPERIMENT 5

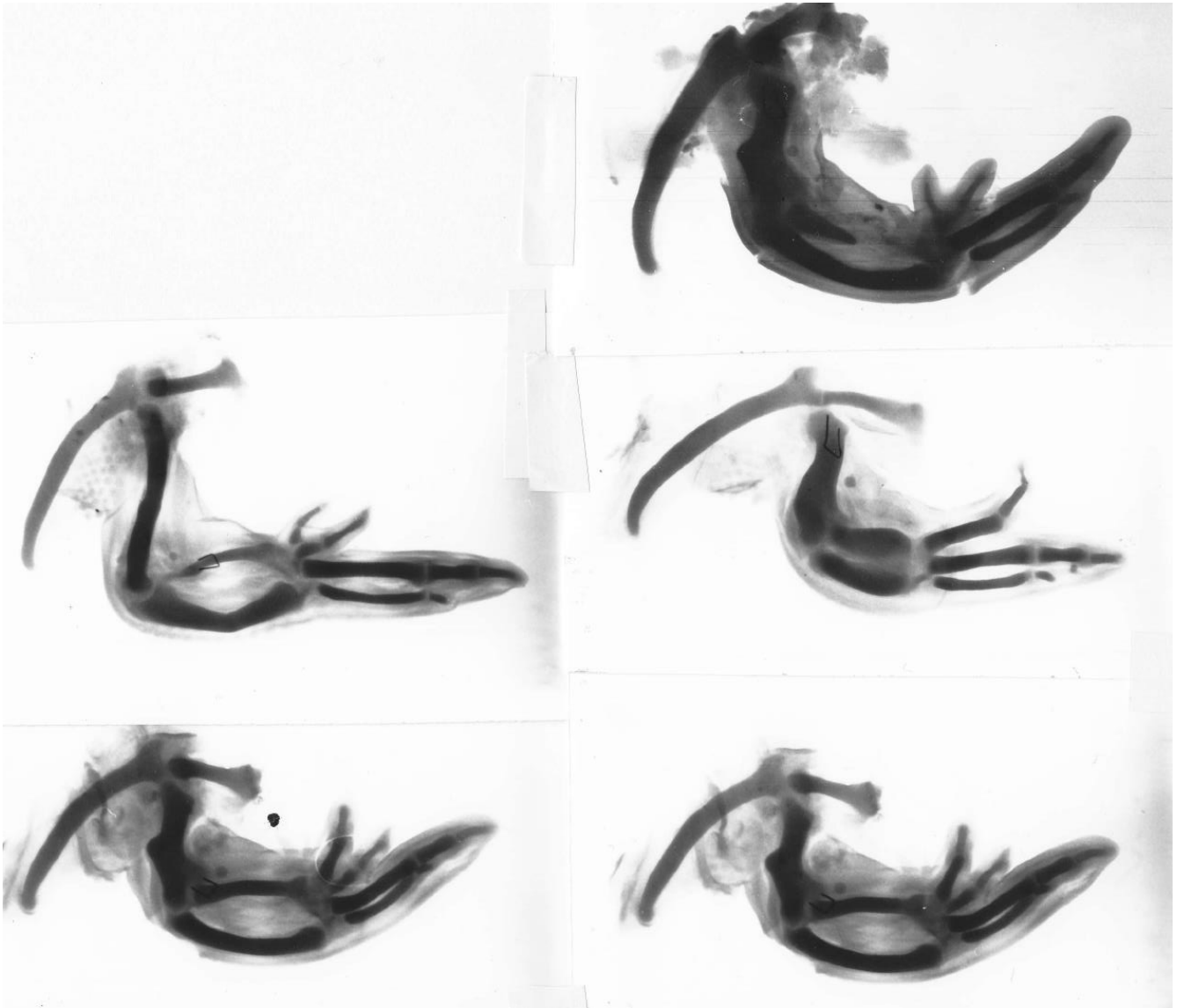
anterior



Bead soaked in low concentration of Shh protein implanted at anterior margin of early host wing bud. Operated embryo with wing bud with bead re-incubated for 7 days.

posterior

EXAMPLES OF 10 DAY WINGS STAINED TO SHOW CARTILAGE SKELETON:



EXERCISE

- 1) Identify each digit in the experimental wings to define the pattern of digits across the antero-posterior axis (the normal pattern of digits is 2, 3, 4). Analyse the patterns of digits and draw up a table showing results for each experiment. How could you quantitate the digit patterns? Why would having quantitative measures be useful?
- 2) Experiment 1 is the classical experiment that first identified the polarizing region in early chick wing buds. In experiment 2, tissue is grafted from the posterior margin of a later wing bud. How do the results of experiment 2 compare with the results of experiment 1? What do these results imply?
- 3) How do the effects of local application of retinoic acid (experiment 3) compare with the effects of the grafts in experiments 1 and 2? How could you interpret these effects of retinoic acid? What further experiments would you carry out?
- 4) How do the effects of local application of Shh protein (experiments 4 and 5) compare with the effects of the grafts in experiments 1 and 2? Relate the effects of applying Shh to the anterior margin to the pattern of *Shh* expression of at different stages in wing development? The posterior margin of the mouse limb bud also expresses *Shh*. Predict what would happen if you grafted the posterior margin of a mouse limb bud to the anterior margin of a chick wing bud?

- 5) Compare the effects of beads soaked in a low concentration of Shh protein with those of beads soaked in a high concentration of Shh protein? What is the significance of these results in terms of models for digit patterning?

ACKNOWLEDGEMENTS AND REFERENCES

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