16. Some Studies on Regeneration and Relative Growth of Limbs in Mayfly Larvæ.

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(Text-figures 1–6.)

Introduction.

Work has already been done which demonstrates that a region of the body showing strongly positive allometry (heterogony) influences neighbouring regions. This shows that a localized region of rapid growth cannot be regarded as completely isolated, but apparently operates within the main growth gradient of the body. In most cases studied the allometric organ has usually been an appendage (*e.g.*, the enlarged chela of the fiddler-crab) and the neighbouring parts, other appendages. In *Maia squinado* (Huxley, 1927), *Inachus dorsettensis* (Shaw, 1928), and *Palæmon carcinus* (Tazelaar, 1930) there is a slight increase in relative size in the appendages just posterior to the allometric appendage, but there is also a slight decrease in relative size in those immediately anterior to it.

A regenerating limb presumably forms a centre of very active growth, and the question arises as to whether it will have a similar effect on anterior and posterior appendages as a heterogonic organ. Przibram (1917) carried out a series of experiments on the effects produced by regenerating legs on neighbouring appendages in the mantid *Sphodromantis bioculata*. He found that after amputation of the fore or mid-leg the growth-rate of the leg posterior to it was first depressed during the period of most active regeneration, then accelerated, and finally dropped to or below normal. When the hind leg was amputated a similar effect was produced on the anterior legs, but the initial depression was greater and the later increase slighter.

In the following experiments, carried out on Mayfly larvæ, similar but cumulative effects on neighbouring appendages were expected, since the regenerating limb was removed repeatedly.

This work was done at King's College, London, and University College, Hull. I desire to express my appreciation to Dr. J. S. Huxley for his help and suggestions in this work.

Material and Methods.

The Mayfly larvæ were obtained chiefly from ponds in Richmond Park, and were nymphs of the species *Chlœon*, probably *C. dipterum*.

The right or the left middle leg was removed in young specimens and repeatedly removed after regeneration, so that it never attained its full size. The regenerated limb was removed when it had grown to about one-third of its original length. In some cases only one moult took place between successive amputations, but in others regeneration was much slower and two moults occurred before the limb was large enough to remove. The possible effect of this constant regenerating growth was then studied on the limbs anterior and posterior to it by measuring them and by comparing these values with those of the limbs of the opposite side. In addition the limbs of controls were measured and compared with them.

The number of times the regenerating limb was removed varied from

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one to eight and depended naturally upon the number of moults the nymph passed through. The limbs to be measured were removed at the breaking joint and mounted separately in glycerine. The magnified image of the limb was then projected on to graph paper, and always in the same region of the field, thus reducing to a minimum the error produced by the curvature of the field. For each segment the apparent length and the mean of a number of measurements of the breadth were obtained in millimetres. Since all limbs were measured at the same magnification they were comparable.

TABLE I.—Experimental Animals.

No.	No. of times operated.	Limb.	Femur.		Tibia.		Tarsus.		Total length.
			Length.	Average breadth.	Length.	Average breadth.	Length.	Average breadth.	
1..	8	1st unop.	108·8	20·1	72·0	10·6	73·0	8·9	253·8
		1st op.	107·8	20·9	71·5	11·8	72·5	10·2	251·8
		3rd unop.	139·0	23·2	89·3	12·1	79·7	11·0	308·0
		3rd op.	138.0	22·9	93·0	11·4	79·0	11·0	310·0
2..	3	1st unop.	127·8	23·6	86·7	13·9	91·0	9·9	305·5
		1st op.	127·3	23·7	85·0	14·4	91·0	10·4	303·3
		3rd unop.	152·5	25·7	94·0	13·6	84·7	10·8	331·2
		3rd op.	152·6	23·4	94·0	12·8	84·3	10·4	330·9
3..	7	1st unop.	102·2	18·7	63·4	9·5	67·6	7·8	233·2
		1st op.	100·0	18·9	63·0	10·2	68·0	8·3	231·0
		3rd unop.	125·2	21·3	78·5	10·9	72·0	9·1	275·7
		3rd op.	123·2	22·6	77·8	11·0	74·0	9·8	275·0
4..	4	1st unop.	150·4	27·3	99·3	14·6	98·0	12·3	347·7
		1st op.	153·0	25·1	98·8	13·1	98·4	11·9	350·2
		3rd unop.	185·2	27·5	114·5	13·4	98·5	13·2	398·2
		3rd op.	183·1	27·4	114·0	14·2	96·2	13·2	393·3
5..	6	1st unop.	127·0	24·7	82·8	15·1	85·7	12·0	295·5
		1st op.	128·8	22·2	85·0	14·0	87·4	12·0	301·2
		3rd unop.	152·5	23·8	96·5	12·7	87·5	12·1	336·5
		3rd op.	151·8	23·8	96·8	11·0	87·0	12·1	335·6
6..	5	1st unop.	126·2	22·1	76·0	14·0	84·6	11·2	286·8
		1st op.	129·1	21·6	78·7	15·7	84·5	12·3	292·3
		3rd unop.	149·1	20·9	92·5	12·7	84·5	11·6	326·1
		3rd op.	150·0	21·5	87·0	11·4	81·0	11·5	318·0
7..	5	1st unop.	131·3	23·5	81·0	12·3	87·0	9·6	299·3
		1st op.	132·5	23·1	81·5	12·2	86·0	9·6	300·0
		3rd unop.	163·8	25·8	101·0	13·2	93·0	10·9	357·8
		3rd op.	163·7	24·2	101·8	11·5	89·5	10·7	355·0
8..	1	1st unop.	142·7	..	96·4	..	90·4	..	329·5
		1st op.	144·2	..	94·0	..	94·9	..	333·1
		3rd unop.	163·5	..	105·8	..	88·0	..	357·3
		3rd op.	167·8	..	106·0	..	91·5	..	365·3
9..	4	1st unop.	140·2	..	91·0	..	91·4	..	322·6
		1st op.	140·2	..	90·7	..	90·4	..	321·3
		3rd unop.	165·7	..	106·0	..	91·5	..	363·2
		3rd op.	173·0	..	106·0	..	92·3	..	371·3

Results.

Table I. shows the lengths and breadths of the limb-segments anterior and posterior to the regenerating one, and those of corresponding segments of the opposite side. In the last column are the total lengths of limbs obtained by adding together the three measured segments. The terminal segment was too much curved to measure accurately.

When these figures are studied it is apparent that any differences which exist are not consistent. Thus in Mayfly 1 the first limb of the unoperated side is greater in total length than that of the operated side, and only in Mayfly 9

TABLE II.—Control Animals.

No.	Limb.	Femur.		Tibia.		Tarsus.		
		Length.	Average breadth.	Length.	Average breadth.	Length.	Average breadth.	Total length.
1 *c* ..	1st right.	105·2	22·6	67·0	12·4	74·2	9·2	246·4
	1st left.	106·0	24·9	68·7	13·9	67·0	11·1	241·7
	3rd right.	128·0	24·0	82·0	10·4	83·4	8·7	293·4
	3rd left.	130·6	26·1	84·6	12·7	84·0	10·5	299·2
2 *c* ..	1st right.	134·0	28·6	88·0	14·9	82·5	11·4	304·5
	1st left.	133·0	27·9	86·8	14·2	82·4	10·9	302·2
	3rd right.	173·3	32·3	108·0	15·3	94·7	12·1	376·0
	3rd left.	166·0	32·0	107·0	15·3	90·8	12·0	363·8
3 *c* ..	1st right.	104·3	23·2	72·7	12·9	73·0	10·2	250·0
	1st left.	108·0	23·8	71·6	12·6	74·2	10·4	253·8
	3rd right.	116·0	23·1	72·8	11·3	71·0	10·0	259·8
	3rd left.	126·0	25·9	91·7	11·1	80·6	11·3	298·3
4 *c* ..	1st right.	119·3	27·4	77·6	14·9	82·4	11·7	279·3
	1st left.	117·9	26·3	76·4	13·7	81·6	11·1	275·9
	3rd right.	152·5	25·6	87·3	12·0	89·0	10·5	328·8
	3rd left.	160·0	28·9	93·4	14·3	91·3	11·8	344·7
5 *c* ..	1st right.	107·0	24·6	68·0	13·1	75·8	10·5	250·8
	1st left.	107·8	25·6	67·4	13·7	75·7	10·6	250·9
	3rd right.	130·7	27·4	85·8	14·3	82·7	11·2	299·2
	3rd left.	132·0	25·7	84·0	13·2	83·9	9·9	299·9
6 *c* ..	1st right.	136·5	32·9	89·5	16·6	85·5	12·6	311·5
	1st left.	140·0	33·3	90·3	17·5	85·4	12·7	315·7
	3rd right.	173·8	32·4	111·2	14·1	93·0	11·1	378·0
	3rd left.	171·2	34·0	113·0	16·7	91·8	13·2	376·0
7 *c* ..	1st right.	113·0	28·1	72·0	15·5	74·0	12·8	259·0
	1st left.	116·5	26·7	72·5	15·4	76·7	12·3	265·7
	3rd right.	141·4	28·5	88·1	15·3	81·2	12·7	310·7
	3rd left.	139·0	27·6	87·5	14·2	81·5	12·3	308·0
8 *c* ..	1st right.	105·4	23·9	67·3	13·1	73·6	10·2	246·3
	1st left.	106·0	24·1	67·0	12·8	71·8	10·3	244·8
	3rd right.	134·0	26·5	78·5	13·5	81·0	10·6	293·5
	3rd left.	134·6	26·8	75·0	12·7	79·3	10·6	288·9
9 *c* ..	1st right.	121·5	27·8	79·3	14·5	79·2	12·1	280·0
	1st left.	116·4	26·9	81·2	14·3	76·0	12·6	273·6
	3rd right.	150·3	29·4	97·1	14·1	86·5	11·6	333·9
	3rd left.	152·0	30·1	100·0	15·0	86·7	12·5	338·7

is there a similar difference in length. In the other experimental animals the differences are again variable in both length and average breadth. Table II. gives corresponding data for the controls, and again the same type of inconsistent differences will be observed.

According to the results obtained in these experiments repeated regeneration of a limb does not have any apparent effect upon the growth of either the limb anterior or posterior to it, and therefore, does not compare with an organ showing strong positive allometry.

It should be emphasized that this does not necessarily contradict Przibram's results. He found a temporary change in growth-rate, settling down to normal or nearly normal. Our experiments were not designed to detect any such temporary effect, but were concerned only with any possible final (cumulative) effect.

The Relative Proportion of the Segments in Regenerating Limbs compared with those in Controls.

The proportion of the various segments to the limb as a whole was then studied in both regenerating and control limbs.

For this purpose a number of regenerating limbs were drawn, measured and compared with normal limbs of corresponding size (Tables III., III. *a*, IV., and IV. *a* respectively).

TABLE III.—Measurements of Regenerating Middle Limbs.

No.	Femur.			Tibia.			Tarsus.			Total length.
	Length.	Av. br.	Length / total l. per cent.	Length.	Av. br.	Length / total l. per cent.	Length.	Av. br.	Length / total l. per cent.	
8..	57·3	18·6	41·9	41·2	13·6	30·0	38·4	11·9	28.0	136·9
26..	86·3	25·9	43·1	57·0	17·1	28·5	57·7	14·6	28·8	200·1
5..	72·0	19·3	40·8	53·0	11·1	30·0	51·6	9·4	29·2	176·6
40..	89·8	20·3	41·8	65·8	12·3	30·6	59·4	11·3	27·6	215·0
42..	88·5	21·9	41·8	64·2	14·6	30·3	59·0	11·8	28·0	211·7
25..	77·0	24·4	43·0	49·4	16·5	27·6	52·7	12·8	29·4	179·1
11..	81·9	25·1	41·9	58·5	16·6	29·6	57·0	13·7	28·9	197·4
10..	56·6	25·9	50·1	30·8	13·9	27·6	25·0	12·1	22·4	111·8
45..	55·5	21·5	42·2	39·0	12·7	29·4	37·0	12·9	28·1	131·5
18..	63·8	16·5	44·5	39·7	10·9	27·7	39·6	10·2	27·7	143·1
28..	52·0	20·1	44·8	32·0	12·4	27·6	32·0	12·0	27·6	116·0
24..	49·0	19·5	41·7	38·4	12·3	32·8	29·8	10·8	25·4	117·2
19..	60·0	20·1	43·5	41·2	13·2	29·8	36·9	11·1	26·7	138·1
17..	60·0	25·8	40·9	44·6	17·4	30·4	42·0	16·1	28·6	146·6
27..	101·0	24·5	47·0	58·8	15·6	28·0	50·0	12·3	23·8	209·8
22..	82·0	23·5	38·6	67·4	14·4	31·8	62·8	11·4	29·6	212·2
20..	91·2	24·0	43·0	62·2	14·9	29·3	58·6	10·8	27·6	212·0
38..	86·0	21·3	44·6	54·7	13·3	28·3	52·3	10·7	27·1	193.0
41..	51·8	21·5	41·2	39·0	13·2	31·0	35·0	10·3	27·8	125·8
39..	69·9	26·5	41·9	53·0	16·8	31·8	44·0	14·4	26·4	166·8

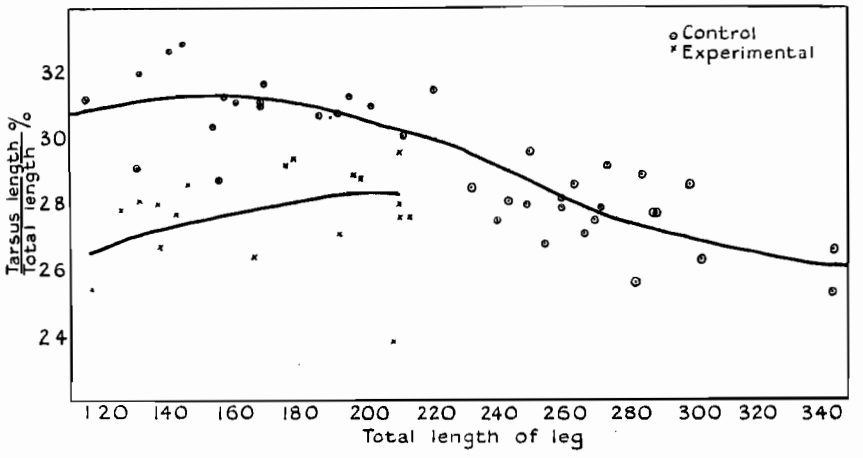
TABLE III. *a*.—Mean Measurements of Regenerating Middle Limbs.

Class. Length of limb.	No. in class.	Femur / total length per cent.	Tibia / total length per cent.	Tarsus / total length per cent.	Total average length.
110·0–135·0	5	43·88	29·76	26·37	120·46
135·0–160·0	4	42·70	29·51	27·72	141·18
160·0–185·0	3	41·89	29·74	28·38	174·17
185·0–210·0	4	44·38	28·60	27·11	200·08
210·0–235·0	4	41·31	30·31	28·19	212·73

TABLE IV. *a*.—Mean Measurements of Control Middle Limbs.

Class. Length of limb.	No. in class.	Femur / total length per cent.	Tibia / total length per cent.	Tarsus / total length per cent.	Total average length.
110–135	3	42·99	26·16	30·84	127·77
135–160	5	42·46	26·40	31·16	150·94
160–185	4	42·12	26·66	31·23	167·50
185–210	4	42·86	26·23	30·94	194·83
210–235	3	43·68	26·31	29·98	223·17
235–260	5	44·70	27·29	28·00	249·40
260–285	8	44·55	27·70	27·72	270·90
285–310	5	44·68	27·47	27·84	294·96
335–360	2	45·14	28·92	25·94	345·80

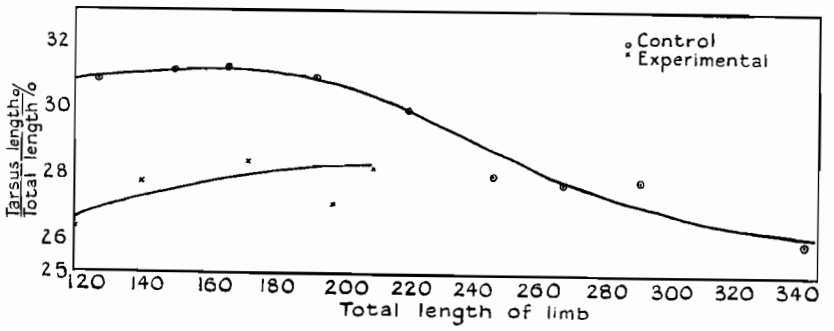
Text-figure 1.



Values for single individuals. See Tables III. and IV.

The values of $\frac{\text{length of tarsus}}{\text{total length}}$ per cent. (*i. e.*, the ratio of tarsus length to total leg length, expressed as a percentage) were plotted against the total length of the leg in regenerating limbs and controls (see text-figs. 1 & 2).

Text-figure 2.



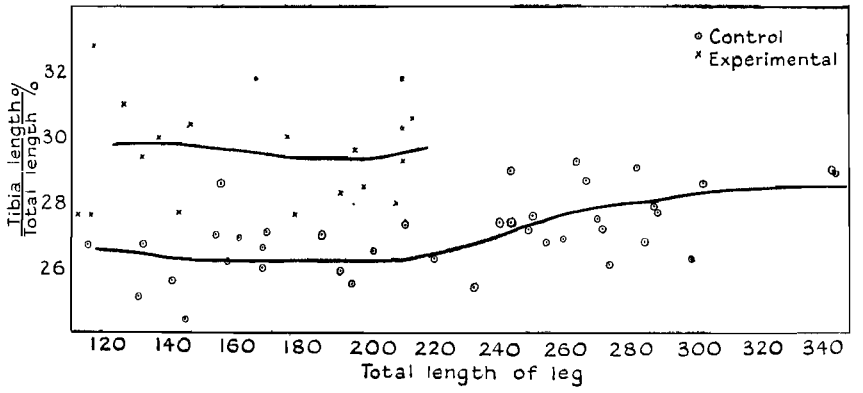
Mean class values. See Tables III. *a* and IV. *a*.

TABLE IV.—Small Control Mayflies : Middle Limb.

No.	Femur.			Tibia.			Tarsus.			Total. length.
	Length.	Av. br.	Length / total l. per cent.	Length.	Av. br.	Length / total l. per cent.	Length.	Av. br.	Length / total l. per cent.	
1..	58·8	12·4	41·8	36·0	6·2	25·6	46·0	6·2	32·7	140·8
2..	67·2	14·7	42·5	41·5	6·8	26·2	49·5	7·0	31·3	158·2
3..	59·4	11·6	45·5	32·8	6·6	25·1	38·4	5·1	29·1	130·6
4..	68·0	14·0	42·1	43·4	7·9	26·9	50·2	6·8	31·1	161·6
5..	72·7	16·0	43·0	44·0	9·0	26·0	52·5	7·5	31·0	169·2
6..	54·4	12·4	41·3	35·2	7·5	26·7	42·2	5·7	32·0	131·8
7..	48·4	11·4	42·1	30·7	7·1	26·7	35·8	5·9	31·2	114·9
8..	65·9	14·9	42·6	41·7	8·8	27·0	47·0	7·1	30·4	154·6
9..	62·0	13·2	42·8	35·4	7·8	24·4	47·8	6·2	32·9	145·2
10..	108·0	21·7	42·8	69·6	12·0	27·6	74·6	9·6	29·6	252·2
11..	71·4	13·4	42·3	45·0	6·7	26·6	52·5	6·3	31·1	168·9
12..	70·1	15·9	41·2	46·2	8·2	27·1	54·0	7·3	31·7	170·3
13..	90·7	19·9	42·6	58·2	11·3	27·3	64·0	8·9	30·1	212·9
14..	83·4	18·7	43·3	50·0	10·2	25·9	59·3	8·3	30·8	192·7
15..	66·5	15·8	42·7	44·6	9·9	28·6	44·8	8·3	28·7	155·9
16..	94·0	20·9	42·3	58·4	10·9	26·3	70·0	9·2	31·5	222·4
17..	85·0	17·3	43·3	50·0	8·5	25·5	61·4	7·6	31·3	196·4
18..	86·3	19·1	42·5	53·8	10·1	26·5	63·0	8·0	31·0	203·1
19..	79·3	17·4	42·4	50·6	10·2	27·1	57·4	8·2	30·7	187·1
20..	107·8	20·7	46·0	59·6	9·8	25·4	66·8	8·2	28·5	234·2
21..	129·8	23·0	44·6	80·5	11·4	27·7	80·7	9·7	27·7	291·0
22..	119·0	20·4	46·4	68·8	11·4	26·8	68·7	10·7	26·8	256·5
23..	109·0	20·3	45·1	66·3	10·5	27·4	66·6	8·3	27·5	241·9
24..	123·5	21·7	44·7	72·0	10·7	26·1	80·7	9·3	29·2	276·2
25..	123·0	24·3	44·9	74·5	11·9	27·2	76·3	9·5	27·9	273·8
26..	135·8	29·8	45·1	79·3	15·9	26·3	86·3	11·7	28·6	301·4
27..	118·6	25·1	45·3	70·4	13·3	26·9	73·2	11·3	27·9	262·1
28..	112·4	21·0	44·8	68·3	10·6	27·2	70·3	8·5	28·0	251·0
29..	117·5	21·9	44·8	70·6	10·9	26·9	74·0	9·0	28·2	262·1
30..	109·2	20·5	44·6	67·3	11·1	27·4	68·9	8·8	28·1	245·4
31..	129·0	22·4	45·3	82·7	11·6	29·1	73·0	11·1	25·6	284·7
32..	122·5	22·7	45·0	75·0	11·4	27·5	74·8	9·6	27·5	272·3
33..	154·2	27·5	44·5	100·0	14·4	28·9	92·0	12·4	26·6	346·2
34..	127·0	24·0	44·3	77·0	11·9	26·8	83·0	9·5	28·9	287·0
35..	129·0	26·4	44·4	81·0	12·2	27·9	80·3	11·0	27·7	290·3
36..	137·5	26·2	45·1	87·3	12·8	28·6	80·3	9·3	26·3	305·1
37..	112·2	22·3	42·2	77·8	11·7	29·3	76·0	9·4	28·6	266·0
38..	119·0	26·4	44·2	77·2	14·4	28·7	72·8	11·8	27·1	269·0
39..	158·0	29·8	45·8	100·0	14·9	29·0	87·4	11·4	25·3	345·4

In controls this percentage decreases with increase of size, which means that compared with the whole leg the rate of growth of the tarsus slowly decreases with increasing size. Over the range worked out this decrease is about 8 per cent.

Text-figure 3.

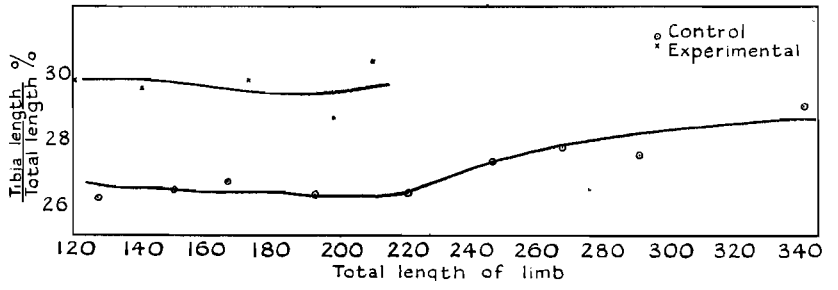


Values for single individuals. See Tables III. and IV.

In regenerating limbs the value $\frac{\text{length of tarsus}}{\text{total length}}$ per cent. over the range dealt with is lower than in the controls of the same size, but the value increases with size until it almost corresponds with the control value.

In the case of $\frac{\text{tibia length}}{\text{total length}}$ per cent. (see text-figs. 3 & 4) in controls the percentage increases with size to the value of 3 to 4 per cent. In the regenerating limb the percentage is definitely higher than in the controls and there is a somewhat irregular decrease with size.

Text-figure 4.



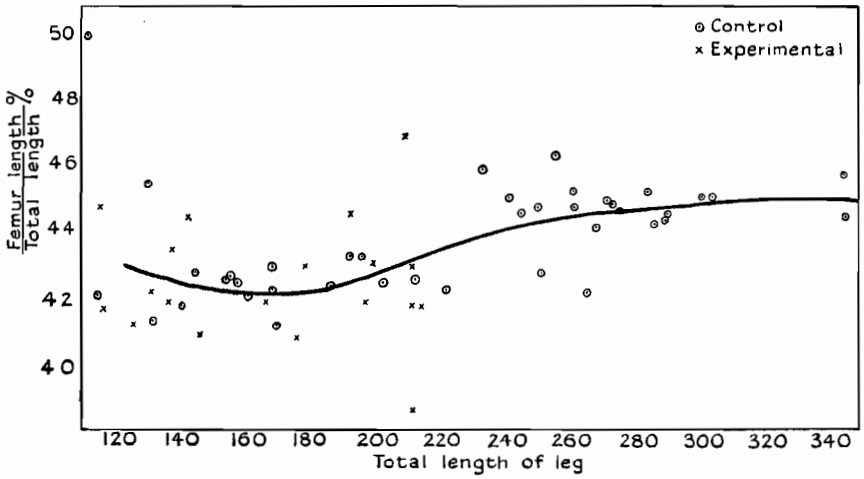
Mean class values. See Tables III. *a* and IV. *a*.

Lastly, text-figs. 5 & 6 show similar values plotted for the femur. Here again the percentage values increase with size in the controls, but in this case the values in the regenerating limbs appear to be approximately equal to the corresponding controls.

From these results it appears that in normal limbs the rate of growth of the tarsus decreases with increasing size but in both the tibia and the femur it increases.

In the regenerating limb, on the other hand, the tarsus is appreciably

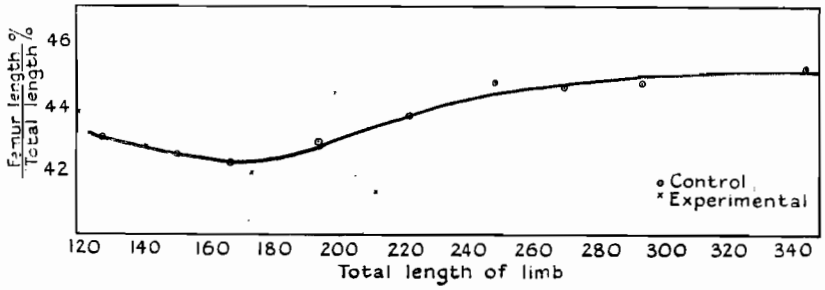
Text-figure 5.



Values for single individuals. See Tables III. and IV.

shorter in proportion to the rest of the limb and the tibia longer than in the control, but the control value is gradually attained by increased and decreased growth-rates respectively. The relative length of the femur in regenerating limbs is similar to that of the controls. Megusar (1910), in his experiments

Text-figure 6.



Mean class values. See Tables III. *a* and IV. *a*.

on regenerating limbs of various Orthoptera, measured the various segments of regenerating limbs and those of the corresponding limbs on the opposite side of the body. On working out the various percentages I found that the results varied and were not consistent. Moreover, the measurements taken were somewhat approximate.

It would thus seem that the distribution of growth-potential within the limb, as revealed by the relative sizes and the growth-rates of the separate joints, is different in normally growing and regenerating limbs. This would imply that the actual mechanism of growth is in some important respect different in the two cases. This would explain the failure of rapid regenerative growth to affect the normal growth of neighbouring limbs, as contrasted with the established effect of rapid normal growth. It might be that certain specific growth-promoting substances were concerned in producing normal allometric growth, and that their partition was disturbed by the excessive demands of high allometry ; if these substances were not involved in regenerative growth the failure of this latter to influence the growth of neighbouring limbs in the same way would be intelligible. However, this is purely speculative, and only further research can elucidate the problem.

Summary.

1. The middle walking-limb of one side in young Mayfly larvæ was kept in an actively regenerating condition by repeated removal, and the effect, if any, on the limbs anterior and posterior to it investigated.

2. Comparison with the limbs of the opposite side and with those of control animals showed no consistent difference in size.

3. The relative proportions of the segments of regenerating and control limbs were also studied.

4. In normal limbs the relative tarsus length decreases with increasing absolute size. In the regenerating limb this percentage is initially lower than in controls, but increases with increasing absolute size until the control value is reached. The relative tibia-length and femur-length in normal limbs increase slightly with increase in size. In regenerating limbs the relative tibia-length is at first higher than normal and then decreases irregularly with increase in size ; the relative femur-length remains about equal to that of controls.

5. This implies that the mechanisms of regenerative and normal growth are not identical, and may explain why rapid growth in regeneration does not affect the normal growth of neighbouring limbs, while that of normal growth in highly allometric limbs does do so.

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