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THE GROWTH STIMULATION OF BLOW-FLY LARVAE FED ON FATIGUED FROG MUSCLE. II

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I. INTRODUCTION

FOX AND SMITH (1933) showed that fly larvae fed on frog muscle previously fatigued by electrical stimulation through the nerve grow to a larger size than controls fed on unstimulated muscle. Their Table I records eighteen experiments in which the larvae fed on fatigued muscle had, at the peak of their growth curve, a mean percentage excess weight over those fed on resting muscle of $9{\cdot}6 \pm 1{\cdot}8$. This excess, being more than five times its standard error, is significant.

Since these results were published I have continued the investigation and have found that heat destroys the growth stimulating property of fatigued muscle, and that the blood of frogs whose muscles have been artificially fatigued also acts as a growth stimulant. This apparently indicates that muscular contraction in frogs produces a substance which can stimulate the growth of fly larvae, that the substance is thermolabile; and that it passes from the muscle into the blood stream of the frog. But during each period of 24 hours that the larvae remain on their successive meals of muscle, bacteria grow on the muscle. It is conceivable, then, that a more numerous, or a different, bacterial flora grows on the fatigued than on the non-fatigued muscle, and that these bacteria are really responsible for the differential growth of the larvae. This question must be settled before it is certain that a growth-stimulating substance has been secreted by the contracting muscle.

I have therefore made counts of the numbers of bacteria on the two kinds of muscle after fly larvae have lived on them, and I have found that there is no significant difference in the bacterial population densities. This settles the quantitative possibility: no more bacteria grow on the fatigued than on the non-fatigued muscle. But the qualitative question still remains open: the bacteria may be different in kind. Unfortunately I have no opportunity for continuing the work, and the results up to date are reported below in the hope that another worker will repeat the experiments under aseptic conditions. Only when this has been done will it be certain that the fatigued muscle has really produced a growth-stimulating substance.

As before, the larvae of the blow-fly, *Calliphora erythrocephala*, were used in the present investigation. The technique of feeding and weighing the larvae, and of preparing the resting and fatigued frog muscle, was the same as that described by Fox and Smith (1933).

II. GROWTH ON HEATED MUSCLE

Resting and fatigued muscle of *Rana temporaria* were prepared in the usual way and were then exposed to steam for 5 min. The results of feeding larvae with muscle so treated are given in Table I: there was no difference between the weights of the two lots of larvae.

Table I. *Average weights, at the peak of the growth curve, of larvae fed on heated resting and heated fatigued muscle*

Exp. no.	Resting muscle		Fatigued muscle		Increase in weight %
	No. of larvae*	Mean weight of 1 larva mg.	No. of larvae*	Mean weight of 1 larva mg.	
122	52	66	57	63	−5
125	47	67	44	70	4
126	40	80	41	79	−1
127	30	68	35	65	−4
129	39	74	35	73	−1
131	78	59	75	60	2
132	104	73	93	76	4
134	52	76	46	76	0
					Mean −1

* As in the tables of Fox and Smith (1933), this gives the number of larvae alive at the end of the experiment. The same remark applies to Tables II, III and IV.

Apparently, then, heat destroys the growth stimulating substance in the fatigued muscles, but the result might mean that heat causes the production of a growth stimulant in the resting muscle. To test this possibility, larvae were fed on heated and on unheated resting muscle. The results are given in Table II. Growth was apparently better on the unheated muscle, but the difference is not significant. The conclusion, therefore, is that the growth stimulant is thermolabile.

Table II. *Average weights, at the peak of the growth curve, of larvae fed on unheated and on heated resting muscle*

Exp. no.	Unheated muscle		Heated muscle		Increase in weight %
	No. of larvae	Mean weight of 1 larva mg.	No. of larvae	Mean weight of 1 larva mg.	
136	45	82	35	75	−9
137	39	73	40	69	−5
138	56	75	46	72	−4
140	33	71	34	73	3
141	46	77	56	73	−5
142	65	79	65	77	−3
144	39	75	33	74	−1
145	39	99	45	93	−6
146	54	82	60	84	2
					Mean $-3{\cdot}1 \pm 1{\cdot}3$

This is not parallel to the result of Bêlehrádek (1924), who found that heat did not destroy the stimulative effect of fatigued frog muscle on the growth of tadpoles.

III. GROWTH ON BLOOD

The next point investigated was whether the growth stimulant passes from the contracting muscle into the blood stream.

Frogs were decerebrated and the muscles of half the number of frogs were fatigued by stimulation through electrodes inserted into the spinal cord. Stimulation lasted for two periods of 10 min. with an interval of 5 min. between them. The blood, which had of course continued to circulate through the muscles, was then drained from the aorta into a tube containing cotton-wool. This was the food of the larvae. Experiments were made both with *Rana temporaria* and *R. esculenta*. Fresh blood was used each day, and for a complete experiment using fifty larvae in each of the two batches, a total of 23–40 *R. temporaria* or 15–20 *R. esculenta* were required.

Tables III and IV show the results with each of the frog species. It is clear that the blood of the fatigued frogs acts as a growth stimulant. The mean percentage excess weight of the larvae fed on fatigued frogs' blood was $10{\cdot}1 \pm 2{\cdot}5$ for *R. temporaria* and $5{\cdot}8 \pm 2{\cdot}0$ for *R. esculenta*.

Table III. *Average weights, at the peak of the growth curve, of larvae fed on blood of resting and fatigued* Rana temporaria

Exp. no.	Resting frogs' blood		Fatigued frogs' blood		Increase in weight %
	No. of larvae	Mean weight of 1 larva mg.	No. of larvae	Mean weight of 1 larva mg.	
92	35	53	36	53	0
95	39	50	43	61	22
101	52	37	47	44	19
104	39	56	35	66	18
105	37	49	35	58	18
106	30	60	30	60	0
107	39	50	38	56	12
108	90	41	82	48	17
110	101	40	97	44	10
113	55	42	54	48	14
114	44	52	44	48	−8
117	76	49	75	51	4
119	43	58	47	61	5
					Mean $10{\cdot}1 \pm 2{\cdot}5$

IV. LIVER

Since the growth stimulant passes into the blood, ten experiments were made to see if it accumulates in the liver. Frogs were treated as in the blood experiments described in the last section, but the livers were finally removed instead of the blood. They were used as food for larvae in the usual way. Larvae fed on livers from resting frogs showed a percentage excess weight, at the peak of their growth curve, over those fed on the livers of fatigued frogs of $3{\cdot}2 \pm 1{\cdot}5$. This is not significant.

Table IV. *Average weights, at the peak of the growth curve, of larvae fed on blood of resting and fatigued* Rana esculenta

Exp. no.	Resting frogs' blood		Fatigued frogs' blood		Increase in weight %
	No. of larvae	Mean weight of 1 larva mg.	No. of larvae	Mean weight of 1 larva mg.	
170	69	46	65	50	9
171	37	66	51	69	4
172	37	68	49	73	7
173	46	60	40	57	−5
174	43	57	48	59	3
175	50	59	70	64	8
176	59	61	62	61	0
177	32	67	45	72	7
178	38	54	30	61	13
180	64	65	55	64	−1
181	89	43	90	51	19
					Mean $5\cdot8 \pm 2\cdot0$

V. BACTERIA

Numerical estimates were made of the bacterial populations on the two kinds of muscle after larvae had fed on them for 24 hours. A weighed quantity of muscle was shaken with a known volume of sterile water which was then successively diluted, and finally an aliquot part was mixed with peptone agar in a Petri dish. After incubation at 23° C. the colonies were counted. In the seventeen experiments performed there was a mean percentage excess density of bacteria on the resting muscle of $1\cdot6 \pm 6\cdot5$. It is clear that this is not significant: there are no more bacteria on the resting than on the fatigued muscle.

VI. SUMMARY

It had previously been shown that blow-fly larvae fed on fatigued frog muscle grow to a larger size than controls fed on resting muscle.

I have now demonstrated that the growth-stimulating substance is thermolabile and that it passes from the contracting muscle into the blood stream. It does not accumulate in the liver.

The bacterial flora is equally dense on resting and on fatigued muscle on which fly larvae have fed.

In conclusion I wish to thank Prof. H. Munro Fox, Mr H. G. Newth and Dr D. L. Gunn for advice and help. My gratitude is also due to Dr W. T. Hillier for his valuable assistance in the bacteriological work.

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