CI. THE PROPORTION OF SKELETAL TISSUES IN INSECTS.

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THE proportion of the skeleton to the weight of the whole insect might be needed for many purposes. My own reason for enquiring is that I have subjected insects to controlled atmospheric conditions, determining the loss of weight and the proportion of water in the insect before and after exposure. But when it has been demonstrated that under certain conditions the insect loses a particular amount of water and of dry material, it is not known how much of the remaining dry material is living matter on which the insect could perhaps call for energy, and how much of it is "dead" skeleton. It is the purpose of this note to discuss methods of estimating the weight of skeletal tissues in insects.

It is known that a considerable portion of the hard exo-skeleton of insects consists of substances other than chitin. Campbell [1929], for instance, has shown that in *Periplaneta* and several other insects the chitin makes up 30 to 40 % of the skeleton¹. It is not known what the other materials are, though it is generally held that they consist (in part at least) of scleroproteins. For the moment it would suffice if an empirical figure for "skeleton" could be obtained. The proportion of chitin to skeleton differs in different parts of the same insect, and doubtless also in different species of insects. It is therefore not possible to estimate the chitin and deduce the total weight of skeleton from it.

EXPERIMENTS WITH MEAL-WORMS.

It is clear that the proportion of skeleton can best be obtained by causing the soft parts of the insect to go into solution and estimating the residue. I have used digestive enzymes for dissolving the soft parts, and have also experimented with dilute alkalis and acid. The greater part of the work has been done with meal-worms (larvae of *Tenebrio molitor*, Coleoptera): nearly full grown larvae, weighing 60–190 mg., were used.

Enzymes. It seems clear that if a dried and powdered insect is treated successively with pepsin and pancreatin, what is left was not a part of the

¹ The proportion of skeleton in a certain batch of meal-worms was found to be about 8·3 % of the dry weight (below): the proportion of chitosan left after prolonged boiling in potassium hydroxide (60 g. KOH to 100 cc. water) was 5·05 %.

original living organs or tissues: in other words, the undigested portion is skeleton. But it is not certain that the whole of the skeleton is left, for some part of it may have been attacked by one or other of the enzymes.

I have carried out the work in the following manner. The insects are dried at 100° and reduced to a fine powder: it is necessary to break up the separate parts of the legs into fragments. If the proportion of fat is high, as it is in the meal-worm, it should be removed with ether. The material is then again dried at 100°, and a weighed quantity—say 200–500 mg.¹—is transferred to a flask. In order to make the small fragments of chitin, *etc.* sink in the liquid containing the enzyme, they should first be moistened with acetone and then boiled in a few cc. of the buffer solution. A large volume—say 300 cc.—of pepsin buffered to p_H 2.5 is then added, and the material incubated at 37°, the reaction being adjusted daily if necessary. After 3 days the clear liquid is pipetted off and passed through a Gooch filter. The remainder is washed several times with distilled water, which is removed and passed through the same filter. The material is then digested with pancreatin at p_H 7.5 in the presence of toluene. After 3 days it is diluted with distilled water and passed through the same Gooch filter, which is then dried at 100° and reweighed.

From the results given in Table I, it seems that the method is accurate, for the meal-worm, to about 0.5 % of the dry weight (say 1.5 % of the original wet weight of the insect): for other insects the discrepancy is sometimes greater. It appears to be immaterial which enzyme is used first. It seems also that an exposure of 3–4 days to each enzyme at 37° is sufficient. A still longer exposure does not materially alter the result, from which I argue that the enzymes do not attack the non-chitinous material in the skeleton. This may readily be shown by taking the Gooch filter containing the solid remains and standing it in a beaker of pepsin or pancreatin for several additional days. At the end of that time there is generally a very slight reduction in weight. The *Tenebrio* material, which was treated with pepsin for 4 days and pancreatin for 2, contained 8.10 % of skeleton. Further treatment for 2 days with pepsin and 4 with pancreatin only reduced the percentage to 7.98.

Acid and alkali. It would be a much simpler procedure if the dried material could be treated with a dilute acid or alkali, and a result obtained consistent with that given above. The results of a series of experiments with 1 % hydrochloric acid, 1 % potassium hydroxide in water, and 5 % potassium hydroxide in absolute alcohol are shown in Table I. It is clear that treatment with 1 % KOH, at 100°, gives a result consistent with that obtained by the use of enzymes. The results obtained by the other methods are inconsistent. The 1 % KOH at 100° gives a result in 4 hours, and the proportion of “skeleton” is not reduced if treatment be continued for 24 hours. This method, therefore,

¹ It would be easy to work with greater quantities of meal-worm and obtain rather more accurate results. But it is essential with many insects to work with great economy. It must be remembered that many insects weigh less than 10 mg. (of which about two-thirds is water), and that it is often impracticable to obtain large numbers.

Table I. *Skeleton left after treating dried powdered meal-worm with enzymes, and dilute acids and alkalis.*

The material had been previously freed from fat with ether, but the results are expressed as percentages of the total dry weight.

ENZYMES.					
Pepsin (days)	Pancreatin (days)	Skeleton %	Pancreatin (days)	Pepsin (days)	Skeleton %
1	0	19.8	3	0	10.1
4	0	14.3	5	0	9.5
4	1	10.1	1	2	12.2
4	2	8.1	1	4	8.35
			3	4	8.7
ACID AND ALKALI.					
Reagent		Temp. ° C.	Time (hrs.)	Skeleton %	
1 % HCl, aqueous		100	4	40	
"		"	24	22	
"		"	72	13	
5 % KOH, abs. alc.		100	1	7.4	
"		"	3	6.4	
"		"	24	6.4	
"		"	48	5.1	
1 % KOH, aqueous		100	4	8.2	
"		"	24	8.3	

is the best to use, if it can be shown with other insects that the enzyme and potash methods give consistent results, and that an end-point is reached in a definite period.

RESULTS OBTAINED WITH OTHER INSECTS.

The dried powdered remains of other insects, freed from fat when necessary, have been subjected to digestive enzymes, and to 1 % KOH at 100°, by the methods already described. The results are presented in Table II. The *Peri-*

Table II. *The proportion of "skeleton" in certain insects as determined by the enzyme method and by digesting in 1 % aqueous KOH at 100° for 24 hours.*

Name	Mean live weight mg.	Weight % (dry = 100)			
		Live	Fat	Skeleton by enzymes	Skeleton by KOH
<i>Tenebrio</i> (larv.)	120.0	242	36.4	8.1- 8.7	8.2-8.3
<i>Periplaneta</i>	960.0	298	6.2	15.1-16.7	11.6
<i>Culex</i>	2.63	224	47.0	11.6-12.7	13.0
<i>Lucilia</i>	26.4	329	Trace	19.4-20.0	—
<i>Rhodnius</i>	110.0	340	Trace	11.8-12.2	10.8-11.2
<i>Cimex</i>	4.0	330	Trace	36.0	17.5

planeta americana (Blattidae, Orthopt.) were adults of both sexes. The *Culex pipiens* (Culicidae, Dipt.) were hibernating females, examined in November. The *Lucilia sericata* (Muscidae, Dipt.) were adults under 48 hours old. The *Rhodnius prolixus* (Reduviidae, Hemipt.) were adult males under 24 hours old. The *Cimex lectularius* (Cimicidae, Hemipt.) were adults of unknown age fed 10 days before.

With several of these insects I was able to show that either method reduces the amount of insoluble material to a definite point. For instance *Rhodnius*, after 4 days in pepsin and 1 in pancreatin, left a residue of 12.5 %; 2 more days in pancreatin reduced the figure to 11.8 %; after 3 further days in pepsin it remained unaltered. Samples of the same material treated with potassium hydroxide gave 11.05 % after 3 hours, and 11.2 % after 24 hours.

With each species of insect there is a general agreement between the results obtained with enzymes and with potassium hydroxide, having regard to the small quantities used. The figures for *Rhodnius* and *Cimex* show less residue by the potassium hydroxide method. This is probably due to the fact that the insects contained haematin, the remains of a previous meal on blood. This is not attacked by the enzymes, and is therefore reckoned as skeleton when they are used; but in the 1 % KOH it passes quickly into solution.

SUMMARY.

It is found that if dried powdered meal-worm is digested with pepsin and pancreatin, the reaction stops or becomes very slow when 8.1–8.7 % of the original dry material is left. The same proportion (8.3 %) of residue is left if the material is exposed to 1 % aqueous KOH at 100° for 24 hours, and here again the reaction reaches an end-point. When the same methods are applied to other insects, they also give consistent results.

The potassium hydroxide method is simpler and quicker, and has the additional advantage (in dealing with blood-sucking insects) of dissolving haematin.

REFERENCE.

Campbell (1929). *Ann. Entom. Soc. Amer.* **22**, 401.