DOCTOR OF PHILOSOPHY

Studies on the nutrition and feeding of the larvae of the large cabbage white butterfly, Pieris brassicae L.

Akhtar, Muhammad

Award date:
1966

STUDIES ON THE NUTRITION AND FEEDING OF THE LARVAE OF THE LARGE CABBAGE WHITE BUTTERFLY PIERIS BRASSICAE L.

A THESIS

SUBMITTED TO THE UNIVERSITY OF WALES

BY

MUHAMMAD AKHTAR, M.Sc. (PANJAB)

IN CANDIDATURE FOR THE DEGREE OF

PHILOSOPHIAE DOCTOR

Department of Agricultural and Forest Zoology,

University College of North Wales,

BANGOR.

January, 1966

ABSTRACT

The review of literature showed that nutritional studies have been made on a number of insects but that the phytophagous insects have largely remained neglected, there being very few examples of these insects having been successfully reared on synthetic diets. Stress has been laid upon the qualitative and quantitative formulation of synthetic diets containing the essential nutrients, the various substances necessary as feeding stimuli, and possessing an acceptable physical texture.

The larva of a phytophagous insect, Pieris brassicae L., was therefore selected as the subject of the present investigations. It is a pest of economic importance almost all over the world, and has the advantage of being readily reared in the laboratory at all times of the year.

A semi-synthetic diet was devised, the 'unknown' factor of which was cabbage leaf powder, included in the diet after attempts to produce a purely synthetic diet had failed. A leaf powder concentration of 2 grams per 25 grams of medium was the optimum required by the caterpillars. The nutritional value of various substances was assessed by inclusion in, or omission from, this basic diet.

In the case of sugars, the effects of various pentoses, hexoses, di-, tri-, and polysaccharides and also one of the sugar

alcohols, mannitol, were examined. It was found that sucrose was a strong phagostimulant as well as apparently being nutritionally essential. The performance of larvae on diets containing glucose or fructose was poor, and this was contrary to most of the findings of various authors. On the other hand, pentoses, usually considered to be poorly utilised by insects, proved almost as good as sucrose in the diet, and an equimolecular mixture of glucose + fructose was also found to be equally effective. A sucrose concentration of 500 mgm. per 25 grams of medium was found to be adequate, and this was in reasonable agreement with the sugar requirements of those other phytophagous insects for which published results are available.

The work of a number of authors showed that mustard oil glucosides are responsible for evoking the feeding response in Pieris brassicae L. larvae, whereas, in the present investigation the inclusion of sinigrin - a mustard oil glucoside - in the diet did not produce any significant improvement in the performance of the larvae. Variations in the sinigrin concentration in the diet also failed to give a positive response.

The quantitative nitrogen requirements of the insect were studied using casein as the protein source. The omission of casein from the diet resulted in significantly lower larval weights and proportionately fewer larvae moulting than in the case of larvae reared on diets containing casein. The fifth instar larvae ingested significantly lower amounts of food when casein was omitted from the

diet. The concentration of casein in the diet finally adopted lay within the range of concentrations used in the diets of other phytophagous insects studied by other workers.

Cystine, one of the amino acids not normally included in the basic list of ten essential amino acids, has been shown by various authors to be connected with successful moulting in some non-phytophagous insects. In the present studies, the addition of cystine in the diet produced a proportionately higher percentage of larvae moulting in the earlier instars, and its omission from the diets of the fifth instar larvae caused emergence failures in the adults subsequently produced. This appears to be the first observation of such an effect in a phytophagous insect.

The effects of β-carotene, B vitamins and ascorbic acid were studied. The only detectable effects being retarded larval growth with the addition of β-carotene, and a higher mortality resulting from the inclusion of ascorbic acid. These results are very curious as all these materials have been added with advantage in the diets of various insects, particularly phytophagous ones, but the possibility of their effects being completely obscured in these short term experiments was discussed.

Most of the insects studied have been shown to require cholesterol in their diets. Hence, cholesterol and β-sitosterol were incorporated in the diets for Pieris brassicae L. in two

different concentrations. The higher concentration of 500 mgm. per 25 grams of medium proved deleterious, while the lower one (50 mgm. per 25 grams of medium) produced no detectable improvement, although this concentration was more or less in agreement with the requirements of those of other phytophagous insects studied previously. Similarly, the inclusion of Osbourne and Mendel salt mixture in the diet did not produce any observed effects.

Agar is commonly used in formulating artificial diets and in some cases it is said to affect food consumption. Nutrient agar was tried at different concentrations and a level of 2 per cent was found to make the diet cohesive and manageable. In this case no effect on food consumption was observed.

Cellophas was incorporated in the diet to give the medium a leaf like structure but it absorbed too much water, and the medium dried up very quickly. Cellulose powder, however, proved useful in increasing the amount of food consumed on the artificial diet to the level of that on cabbage. The addition of cellulose lowered the coefficient of utilisation considerably, and it was not found possible to correct this without affecting the consumption values. On the other hand, the coefficient of growth was much higher on cellulose diet than on cabbage, the coefficient of metabolism being correspondingly lower. Although the cellulose diet maintained adequate growth of the larvae in these short term experiments, yet the dietary characteristics of cabbage could not be completely reproduced.

STUDIES ON THE NUTRITION AND FEEDING OF THE LARVAE OF THE LARGE CABBAGE WHITE BUTTERFLY PIERIS BRASSICAE L.

CONTENTS

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

A. GENERAL

It is a well known fact that insects are able to obtain the chemicals necessary for the maintenance of life from a vast range of materials. The feeding habits and dietary requirements of insects have long been of interest to the academic entomologist and some of the findings made in this field are proving of practical value in other studies. Standardised diets for experimental insects are an important consideration in many genetic, physiological and toxicological investigations and in the sphere of economic entomology the connection between insect feeding, nutrition and such important factors as plant resistance, to quote but one example, have attracted a good deal of attention.

Many insects are of direct economic importance by virtue of the food materials to which they are adapted. As a result, some of these pests (those attacking various stored products being a good example) have attracted a good deal of attention from nutritional physiologists. However, in the case of one major group of pests, the insects attacking growing plants, the amount of fundamental information on nutritional requirements is comparatively small. Much of the information available is found in very recent publications and the reasons for the comparatively new development of this field are discussed later. There is no doubt that much valuable data on the feeding and nutrition of this large group of insect pests remains to be collected.

Many of these pests are larval forms, a large proportion of which belong to the Lepidoptera. One example was chosen as an experimental animal for this present contribution to the study of phytophagous insect nutrition, this was the almost ubiquitous pest of crucifers, the larva of the large cabbage white butterfly, Pieris brassicae L.

The number of publications dealing with various aspects of insect nutrition as a whole has also grown very rapidly over the past 10 to 15 years and there are already several reviews dealing with various aspects of this large amount of literature. Prior to the recent interest in this field, the first major review was that of Uvarov (1928) who included in his work a comprehensive account of the chemical composition of insects and of their products, together with the major information available at that time on the physiology and biochemistry of feeding and metabolism. The insects which had then been most thoroughly studied were Drosophila and the cockroach for which rather inadequate synthetic diets had been devised.

In recent years outstanding reviews of the modern findings have included publications by Trager (1953), Lipke and Fraenkel (1956), Gilmour (1961) and House (1961, 1965a) who have dealt with insects in general, and by Friend (1958) who has concentrated on phytophagous insects. It is the purpose of this present review to bring these publications up to date where necessary and to summarise the major findings in this field.

The main obstacles in nutritional studies have always been the lack of proper rearing techniques and the lack of chemically defined diets, Friend (1958) and House (1961) emphasised the need for a knowledge of the exact chemical composition of the diet and for the ability to vary the composition precisely in order to examine the consequent effects upon the insect. They also stressed the need for making the diet chemically and physically attractive for the insect in order to evoke satisfactory feeding responses. These needs are felt most strongly in the case of the investigations on phytophagous insects because of the great difficulty of obtaining nutritional data by any means other than the use of artificial food. Thus, it is not possible to control to any great extent the composition of the plant itself in order to study the effects of these variations on the insect. Furthermore, the straightforward analysis of the plant material most suitable for an insect's development is of restricted value, partly because of the limitations of analytical techniques and partly because of the largely unknown fluctuations in composition imposed on the plant by such factors as soil, weather and even time of day. To quote one example, Uvarov (1928) has reviewed the early work on silkworm nutrition which showed how mulberry leaves vary greatly in their phosphate, sugar and protein content according to whether they are picked during the evening or at day-break. Similarly, soil fertilisation has been shown to influence the development of phytophagous insects, this work being well reviewed by Lipke and Fraenkel (1956). Studies on insect

resistance in plants suggest that in some cases particular varieties may have foliage which is nutritionally inadequate for some insect species. This probably extends to different leaves on the same plant since the chemical composition of leaves changes as they age and many insects show a definite preference for leaves at a particular stage.

Therefore, the study of the nutritional requirements of phytophagous insects on the basis of leaf analysis presents the investigator with a host of variables over which he has no more than partial control. The development of artificial diets would seem to be an essential stage in the progress of this field of entomology. Even this approach, however, is greatly complicated by the physiological variation of the insect itself. For example, Beck (1956b) has shown variations in the requirements for sugar in different larval stages of the European corn borer, Ostrinia (Pyrausta) nubilalis Hbn. The difficulties of formulating a diet which is suitable and acceptable to the insect from both the chemical and physical point of view are therefore considerable, and these difficulties have been reviewed in some detail by Friend (1955).

As a result, there are comparatively very few really successful studies made on phytophagous insects. The notable examples being:

European corn borer	Ostrinia nubilalis Hbn.	(Bottger 1942, Beck et al. 1949, Chippendale and Beck 1964)
Pink bollworm	Pectinophora gossypiella (Saunders)	(Vanderzant 1957)

Boll weevil	Anthonomus grandis (Boh.)(Vanderzant 1965)
Asiatic rice borer	Chilo suppressalis (Walker)(Ishii et al 1959, Kamano 1961)
Desert locust	Schistocerca gregaria (Forsk.)(Dadd 1960a)
Migratory locust	Locusta migratoria L. (Dadd 1960a)
Onion maggot	Hylemya antiqua (Meig.)(Friend 1956)
Peach and potato aphid	Myzus persicae (Sulzer)(Dadd and Mittler 1965)

The nutritional studies on insects have shown that their qualitative requirements are more or less uniform in all species examined and that these are not very different from the requirements of other types of animals (Trager 1953). The substances required include, generally, water and certain minerals, carbohydrates, fats or proteins, sterols and B vitamins. However, Dadd (1957) was first to show a dietary requirement for A and C vitamins in locusts in addition to the usual B vitamins.

More or less chemically defined diets have been developed for quite a few insects and the results obtained up to 1954 have been tabulated by Albritton (1955). Although during the past decade a fair knowledge has been obtained concerning the nutritional requirements of several species, in some cases additional chemicals may be required in diets in order to elicit the correct feeding response in the insect. Uvarov (1928) mentioned that insects could be guided to their natural food by the tastes and odours of various chemical

substances which in themselves have no nutritional value. Similarly, Fraenkel (1953) stated that "the plants differ in the presence or absence of odd chemical substances like glucosides, essential oils, alkaloids, saponins or tannins which determine the host specificity of a food plant by acting as chemical stimuli". Thorsteinson (1955) reviewing the chemotactic basis of host specificity quotes Grevillius (1905) who induced the larvae of the brown-tail moth, Euproctis chrysorrhoea L. (Nygmia phaeorrhoea)(Donov.), to feed on otherwise unacceptable plants by smearing their leaves with a paste containing tannin from chickweed (Stellaria sp.), the usual host plant of the insect. Verschaffelt (1911) and Thorsteinson (1953) showed that larvae of Pieris brassicae L., Pieris rapae L. and Plutella maculipennis Curt. will feed on plants containing mustard oil glucosides. Thorsteinson (1956), however, states that "various nutrients have an independent stimulus for insect feeding and that all the chemical constituents of leaves, whether nutrients or not, may contribute to selection, acceptance or rejection of food", and he reported sucrose acting as a phagostimulant in seven polyphagous species of insects. Ito (1960) showed similar results with sucrose in Bombyx mori L.

In fact, the relative importance in insect feeding of non-nutritional chemicals or 'token stimuli' when compared with the general nutrient composition of the leaf is a subject upon which there has been some considerable controversy. This controversy has been well reviewed by

Thorsteinson (1960) and since it lies mainly beyond the scope of the present investigation a detailed account is not included here.

B. QUALITATIVE REQUIREMENTS

1. Carbohydrates.

The qualitative requirements for different sugars which can support the growth and development of an insect are given in Table 1.

There are a few insects in which it has been shown that carbohydrates in some form are an essential part of the diet, examples being, Tenebrio molitor L., Anagasta (Ephestia) kuehniella Zell., Oryzaephilus surinamensis (L.) (Fraenkel and Blewett 1943a,b; Fraenkel et al.1950, Leclerq 1948), adults of Calliphora vicina (=erythrocephala)(Meig.) (Fraenkel 1936) and Agria (Pseudosarcophaga) affinis (Fall.) (House 1954, 1956). On the other hand Tribolium confusum (Duv.), Lasioderma sericorne (Fab.), Ptinus tectus Boield, Attagenus sp., and the larvae of Phormia regina Meig., Calliphora vicina (Meig.), Aedes aegypti (L.) and Musca domestica L. do not require any carbohydrate in their diets, although they can utilise some of the sugars if present (see Table 1) (Fraenkel and Blewett 1943a,b; Moore 1946, McGinnis et al.1956, Sedee 1956, Akov 1962, Brookes and Fraenkel 1958).

From the information available it can be seen that generally pentoses were not required or were very poorly utilised; whereas, hexoses, trisaccharides, disaccharides and polysaccharides were, with

TABLE 1

Qualitative carbohydrate requirements of insects

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
PENTOSES																																
Xylose	−	−	+																	+	−	+		+				−	+			
Arabinose	−	−																		?		−						−				
Ribose	−	−							+																	−						
Rhamnose	−	−																		+		−						−				
HEXOSES																																
Glucose	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−			+		+	
Fructose	+	+					+		+	+							+			+	+	+		+		−		+	+		+	
Mannose	?	?							+											+		+		+				+				
Sorbose	−	−																		+		−										
Galactose	+	−	+	+					+											?		+		+				+	+			
TRISACCHARIDES																																
Melezitose	+	+																		+	+	+						+			+	
Raffinose	+	+			+			+													+	+				−		+				
DISACCHARIDES																																
Maltose	+	+			+		+	+	+								+				+	+		+				+	−		+	
Cellobiose	+	+							+													−										
Trehalose	+	+																				+						+			+	
Sucrose	+	+	+	+		+		+	+	+		+	+	+						+	+	+		+	+	−	+	+	+	+	+	+
Lactose	+	+		+			+		+												−	+		+				−	+			
Melibiose	+	+							+													+						?				
Saccharose																						+										
POLYSACCHARIDES																																
Dextrin	+	+	−	+			+	+									+			+		+						+				
Starch	+	+	−	+	+	+	+	+	+											+		+			+	−		−		+		
Glycogen								+												+		+	+			−						
SUGAR ALCOHOLS																																
Mannitol					+		+	+												+	+	+						−			+	
Glycerol																															+	
Sorbitol							+													+		+									+	
∝-GLUCOSIDES																						+									+	

Key to Table 1

+ utilised
- not utilised
? doubtful; addition may prove beneficial

1. Schistocerca gregaria (Forsk.) (Dadd 1960b)
2. Locusta migratoria L. (Dadd 1960b)
3. Melanoplus bivittatus (Say) (Brown 1937)
4. Blatella germanica (L.) (Noland and Baumann 1949)
5. Stegobium paniceum (L.) (Fraenkel and Blewett 1943a,b)
6. Lyctus sp. (Parkin 1936)
7. Oryzaephilus surinamensis (L.) (Fraenkel and Blewett 1943a,b)
8. Tenebrio molitor L. (Evans and Goodliffe 1939, Leclercq 1948 and Fraenkel et al. 1950)
9. Anthonomus grandis (Boh.) (Vanderzant 1965)
10. Ostrinia nubilalis Hbn. (Bottger 1942, Beck 1950 and Beck et al. 1949)
11. Prodenia eridania Cram. (Crowell 1941)
12. Pieris brassicae L. (Evans 1939)
13. Aglais urticae L. (Evans 1939)
14. Phalera bucephala L. (Evans 1939)
15. Galleria mellonella L. (Dadd 1964)
16. Plodia interpunctella (Hbn.) (Fraenkel and Blewett 1946b)
17. Anagasta kuehniella Zell. (Fraenkel and Blewett 1946b)
18. Ephestia elutella (Hbn.) (Fraenkel and Blewett 1946b)
19. Cadra (Ephestia) cautella Wlk. (Fraenkel and Blewett 1946b)
20. Agria affinis (Fall.) larva (House 1956, House and Barlow 1956, 1960)
21. Agria affinis (Fall.) adult (House 1956)
22. Calliphora vicina (Meig.) adult (Fraenkel 1936)
23. Calliphora vicina (Meig.) larva (Sedee 1956)
24. Phormia regina Meig. adult (Rasso and Fraenkel 1954, Evans and Dethier 1957)
25. Phormia regina Meig. larva (McGinnis et al. 1956)
26. Musca domestica L. larva (Brookes and Fraenkel 1958)
27. Musca domestica L. adult (Monroe 1960)
28. Anastrepha ludens (Loew.) (Baker et al. 1944)
29. Drosophila melanogaster Meig. (Sang 1956)
30. Aedes aegypti (L.) (Akov 1962)
31. Apis mellifera L. (Bertholf 1927)
32. Myzus persicae (Sulzer) (Dadd and Mittler 1965)

a few notable exceptions, utilised in almost all cases.

A more detailed discussion of some of the findings recorded in Table 1 will be left until a later chapter when comparisons can be made with the results of the present work on Pieris brassicae L.

2. Nitrogen compounds.

All insects require nitrogen for their development and growth and it is usually supplied in artificial diets in the form of the proteins casein and egg albumen, or as individual amino acids. Casein, because of its ready availability in standardised form, is the protein most frequently used in compounding insect diets. Thus casein has been used in studies on Blatella germanica (L.) (Noland and Baumann 1949), Attagenus sp. (Moore 1946), Lasioderma serricorne (Fab.), Stegobium paniceum (L.), Dermestes maculatus Deg., Oryzaephilus surinamensis (L.), Ptinus tectus Boield (Fraenkel and Blewett 1943a,b), Tenebrio molitor L. (Fraenkel et al.1950), Anthonomus grandis (Boh.) (Vanderzant et al.1962), Ostrinia nubilalis Hbn. (Bottger 1942, Beck et al.1949, Beck and Stauffer 1950, Chippendale and Beck 1964), Pectinophora gossypiella (Saund.) (Vanderzant and Reiser 1956b), Anagasta kuehniella Zell., Ephestia elutella (Hbn.), Cadra cautella Wlk., Plodia interpunctella (Hbn.), Tineola bisselliella (Hum.) (Fraenkel and Blewett 1946a,b), Bombyx mori L. (Ito 1960), Galleria mellonella L. (Dadd 1964), Drosophila melanogaster Meig. (Sang 1956), Aedes aegypti (L.) (Akov 1962), Phormia regina Meig. (McGinnis et al.1956).

In some instances casein alone is an inadequate source of organic nitrogen and in the locusts, Schistocerca gregaria (Forsk.) and Locusta migratoria L., for example, satisfactory development required a diet having a mixture of casein, egg albumen and peptone (Dadd 1960a).

The nitrogen requirements can also be satisfied with amino acids and basically, the insects require the same ten amino acids classified as essential for the rat, namely arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine and threonine. The work on which this general conclusion is based has been tabulated in the recent review of House (1965a) who has also noted the few known instances of departures from this basic requirement. In Tribolium confusum (Duv.) it was shown that a mixture of 19 crystalline amino acids known to occur in casein satisfied the nitrogen requirements of the larvae equally as well as purified casein (Lemonde and Bernard 1951a). However, this is not always readily shown and Dadd (1961b) for example, failed to adequately replace the protein content of his migratory locust diet with a similar mixture of nineteen amino acids.

3. Vitamins.

For a long time the view, that insects require vitamins only of the B-group, was generally accepted and most of the work was confined to the classification of the B vitamins into essential or non-essential for a particular insect. This work is summarised in Table 2 for ease of reference.

TABLE 2

Qualitative requirements for B vitamins

	+Thiamine	+Riboflavin	+Nicotinic acid	+Pantothenic acid	Biotin	+Pyridoxine	Folic acid	+Choline	Vitamin B12	Carnitine	Inositol	BT	p-amino benzoic acid	
Blatella germanica (L.)	+	+	+	+	+	+	+	+			+			18, 26
Schistocerca gregaria (Forsk.)	+	+	+	+	+	+	+	+			+			8
Locusta migratoria L.	+	+	+	+	+	+	+	+			+			8
Attagenus sp.	+	+	+	+	+	+	+	+						24, 25
Carpophilus hemipterus L.	+	+	+	+		+		+						30
Lasioderma serricorne (Fab.)	+	+	+	+	+	+		+						2
Tribolium confusum (Duv.)	+	+	+	+	+	+	+	+			+?		+?	10, 13, 14
Dermestes maculatus Deg.	+	+	+	+	+	+	+	+						10
Tribolium castaneum (Herbst)	+	+	+	+	+	+	+							14
Oryzaephilus surinamensis (L.)	+	+	+	+										10
Palorus ratzeburgi Wissman	+	+	+	+	-	+	-	+			-			7
Ptinus tectus Boield.	+	+	+	+	+	+		+			+?	+	+?	10
Tenebrio molitor L.	+	+	+	+	+	+	+	+		-			-	14, 15
Stegobium paniceum (L.)	+	+	+	+		+		+			-		-	2
Anthonomus grandis (Boh.)	+	+	+	+	+	+	+	+	+		+			31
Ostrinia nubilalis Hbn.	+	+	+	+	+	+	+	+	+		+			5
Chilo simplex Butler	+		+	+	+	+	+							21
Trichoplusia ni (Hbn.)	+	+	+	+	+	+	+		+		+			6
Plodia interpunctella (Hbn.)	+	+	+	+	+	+	+				-		-	12, 14
Anagasta kuehniella Zell.	+	+?	+	+	+	+	+	+			+		-	12, 13, 14
Ephestia elutella (Hbn.)	+	+	+	+	+	+	+	+			-		-	12, 14
Cadra cautella Wlk.	+	+	+	+	+	+					-		-	12
Galleria mellonella L.	+	+	+	+	+	+	+	+	+		+			9
Tineola bisselliella (Hum.)	+	+	+	+	+?	+		+			-		-	11
Pectinophora gossypiella (Saund.)	+	+	+	+		+	+							27
Drosophila melanogaster Meig.	+	+	+	+	+	+	+	+	+		-		-	29
Aedes aegypti (L.)	+	+	+	+	+	+	+	+	-		-		+?	1, 4
Culex pipiens L.	+	+												4
Phormia regina Meig.	+	+	+	+		+		+						23
Musca domestica L. (larva)	+	+	+	+	+	+	+	+	-		-		-	3, 20, 28
Phaenicia sericata (Meig.)	+	+	+	+	-	+	-	+			-		-	22
Agria affinis (Fall.) (larva)	+	+	+	+	+	+	-	+	+?		-		-	19
Apis mellifera L.		+	+											17
Hylemya antiqua (Meig.)	+	+	+	+	+	+	+	+	+					16

Key to Table 2

\+ essential
\- not essential
+? not essential but addition may prove beneficial

1. Akov (1962)
2. Blewett and Fraenkel (1944)
3. Brookes and Fraenkel (1958)
4. Buddington (1941)
5. Chippendale and Beck (1964)
6. Chippendale et al.(1965)
7. Cooper and Fraenkel (1952)
8. Dadd (1961a)
9. Dadd (1964)
10. Fraenkel and Blewett (1943c)
11. Fraenkel and Blewett (1946a)
12. Fraenkel and Blewett (1946b)
13. Fraenkel and Blewett (1946c)
14. Fraenkel and Blewett (1947a)
15. Fraenkel et al.(1950)
16. Friend and Patton (1956)
17. Haydak (1949)
18. House (1949a)
19. House (1965a)
20. House and Barlow (1958)
21. Ishii and Urushibara (1954)
22. Kadner and La Fleur (1951) reported by House (1965a)
23. McGinnis et al.(1956)
24. Moore (1943)
25. Moore (1946)
26. Noland et al.(1949)
27. Ouye and Vanderzant (1964)
28. Perry and Miller (1965)
29. Sang (1956)
30. Stride (1953)
31. Vanderzant (1959)

However, Dadd (1957) was the first to show that β-carotene (a precursor of vitamin A) was essential for adequate growth and pigmentation in an insect, in this case the desert locust, Schistocerca gregaria (Forsk.). Vitamin A acetate was an adequate substitute for β-carotene as far as the growth effects were concerned but not for pigmentation. Dadd (loc. cit.) postulated an unknown essential factor which could be derived from β-carotene or vitamin A.

On the other hand vitamins A and D were tried in the diet of Tribolium confusum (Duv.) and Acanthoscelides obtectus (Say) but did not prove of value (Chiu and McCay 1939). However, House (1965c) supported Dadd's findings when he showed that dietary vitamin A acetate apparently accelerated the growth rate in the larvae of Agria affinis (Fall.) and enabled the adults subsequently obtained to produce young.

Dadd (1957, 1960d) has also shown that ascorbic acid (vitamin C) is essential for normal growth and development in Schistocerca gregaria (Forsk.) and this finding has recently been confirmed in several other species, notably in the boll weevil, Anthonomus grandis (Boh.), the bollworm, Heliothis zea (Boddie), and the salt marsh caterpillar, Estigmene acrea (Drury) (Vanderzant et al. 1962), the European corn borer, Ostrinia nubilalis Hbn. (Chippendale and Beck 1964), the rice stem borer, Chilo suppressalis (Wlk.) (Kamano 1964) and the cabbage looper, Trichoplusia ni (Hbn.) (Chippendale et al. 1965).

A number of insects, however, have been reared satisfactorily on diets in which ascorbic acid was excluded and in other cases it has been suggested that this vitamin merely performs a phagostimulant function (Thorsteinson 1956, House 1965a).

4. Fats.

Studies on the fat requirements show that only a few insects have a special need for this material in their diets (Friend 1958). Most of these insects are lepidopteran and their requirements can be met by the addition of pure linoleic acid or one of the natural oils containing linoleic acid (e.g. corn oil or wheat germ oil). This requirement has been demonstrated in the European corn borer (Beck et al.1949), the bollworm (Vanderzant and Reiser 1956b) and Anagasta kuehniella Zell., Ephestia elutella (Hbn.) and Cadra cautella Wlk. (Fraenkel and Blewett 1946d, 1947b). In addition an identical fat requirement has been demonstrated in two isolated examples of the cyclorrhaphous flies, Agria affinis (Fall.) (House and Barlow 1960) and Calliphora vicina (Meig.) (Sedee 1956).

5. Sterols.

House (1965a) showed that all but one of the insects studied up to the time of his review require a sterol in their diets, the only exception being Ctenolepisma sp. (Clayton et al.1962). The sterol which best fulfills the immediate needs of insects is apparently

frequently cholesterol and Levinson (1962) demonstrated that a variety of phytophagous species distributed among the Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera and Orthoptera can convert the sterols of their food into cholesterol, whereas, non-phytophagous insects like Dermestes or Attagenus are incapable of doing this. He added that cholesterol was required for larval growth and reproduction.

However, in some insects cholesterol has been shown to be less suitable than various other sterols of plant origin. Thus, the silk-worm, Bombyx mori L. cannot be reared on a sterol-free diet and although cholesterol when added to this diet, results in improved survival and growth, the effect of a number of plant sterols is very much more marked (Ito et al.1964). They suggested that the dietary sterols were primarily nutritional requirements but also played a minor part as phagostimulants. On the other hand, Dadd and Mittler (1965) failed to improve growth of the aphid , Myzus persicae (Sulzer) with the inclusion of cholesterol in the diet, rather, the incorporation of cholesterol suspension retarded growth.

6. Minerals.

The minerals in insectan synthetic diets are usually provided by the inclusion of one of the well-known mammalian salt mixtures and comparatively few attempts have been made to study the relevant effects of different minerals. However, Dadd and Mittler (1965) showed that in the absence of magnesium chloride and potassium phosphate the larval

growth of the aphid, Myzus persicae (Sulzer) was stopped. Potassium, phosphorus, magnesium and sodium have been termed as essential for the development of Drosophila melanogaster Meig. (Sang 1956) and zinc was found to be an essential trace element for Tenebrio molitor L. (Fraenkel 1958). Satisfactory growth of locust nymphs was obtained with four salts comprising the eight radicals Na, Ca, K, Mg, CO_3, Cl, PO_4 and SO_4 (Dadd 1961b). Magnesium was shown to be the only indispensable mineral for proper growth of the boll weevil (Vanderzant 1965).

Reduction in larval weight, reduction of relative growth rate, increased larval mortality and delayed pupation were noticed in the larvae of Pieris brassicae L. fed on the leaves of plants showing symptoms of deficiency of nitrogen, phosphorus, potassium or iron (Allen and Selman 1957). House and Barlow (1965) have shown the importance of the inorganic composition of the food of insects by introducing cobalt, copper, manganese and zinc into a new salt mixture added to the diet of Agria affinis (Fall.). The body weight and protein content of the larvae reared on the diet with the new mixture were superior to that of larvae reared on the same diet with the addition of a commercial salt mixture, and in fact equalled that of larvae reared on pork liver.

7. Other substances.

a) Agar

Agar has been in common use for formulating the diets for insects. Bottger (1942) and Beck et al.(1949) used it as an inert carrier in the

diet of the European corn borer. Tanton (1965) maintained that a suitable physical texture of the medium increased the intake of synthetic food and he tried to achieve this with agar in the diet of the mustard beetle, Phaedon cochleariae (Fab.). Agar was also used in the diet of silkworm by Ito (1960).

b) Cellulose

The use of cellulose for making up the 'roughage' or 'bulk' in a synthetic diet is frequently reported in the literature, two of the most recent examples being diets for the cabbage looper (Chippendale et al.1965) and the wax moth (Dadd 1964).

Cellulose may be obtained in various forms and one of these, called Cellophas, can be readily formed into sheets. It was used by Stride (1953) to give a proper structure to the diet of Carpophilus hemipterus L. and because of its physical properties he suggested its use in synthetic diets for leaf eating insects.

An alternative method of obtaining cellulose in a 'leaf-like' form has been used by Niimura and Ito (1964) in their study of phagostimulants for silkworm. The larvae were fed on sucrose impregnated on filter paper previously heated to 200°C for fifteen hours.

C. QUANTITATIVE REQUIREMENTS

The quantitative inter relationships of the constituents of a diet may have a marked effect on the insect. Even the essential nutrients in inappropriate proportions can adversely affect the growth and

development of an insect as has been well shown in the case of the European corn borer (Beck 1956b), the pink bollworm (Vanderzant and Reiser 1956a) and in that of Celerio euphorbiae (L.) larvae (House 1965b).

1. Carbohydrates.

There is little detailed information in the literature on the optimum quantities of carbohydrates required in the diets of various insects. Table 3 is a summary of data from some of the published work in which carbohydrate quantities have been varied in artificial diets.

The table deals solely with the total percentage amount of carbohydrate used by various authors, since more detailed information on the quantitative inter relationships of individual carbohydrates in insect diets is extremely scarce. Furthermore, the percentage values quoted are not necessarily the optimum levels of carbohydrate for the growth and development of a particular insect. An author may have used only two concentrations, the figure quoted in the table being the one which gave the best results. The value of these figures is also affected by the fact that in most cases authors have not attempted to separate the optimum concentration of sugar required from a purely nutritional point of view, from that required for a diet to exert its maximum phagostimulant effect.

TABLE 3

Total quantity of utilisable carbohydrate in diets for various insects

Species	Total %age of carbohydrate in the diet	Nature of carbohydrate	Author
Attagenus sp. (larva)	Not required	-	Moore (1946)
Dermestes vulpinus Fab.	" "	-	Gay (1938)
Tineola bisselliella (Hum.)	" "	-	Fraenkel and Blewett (1946a)
Calliphora vicina (Meig.) (larva)	" "	-	Sedee (1956)
Phormia regina Meig. (larva)	" "	-	McGinnis *et al*.(1956)
Aedes aegypti (L.)	" "	-	Akov (1962)
Lasioderma sericorne (Fab.)	" ")	Although development slowed in its absence	Fraenkel and Blewett (1943a,b)
Ptinus tectus Boield.	" ")		Fraenkel and Blewett (1943a,b)
Tribolium confusum (Duv.)	" ")		Chiu and McCay (1939)
	45% starch) used in the) diet)		
Agria affinis (Fall.)	Not essential Optimum growth on 0.5	glucose	House and Barlow (1956)
Drosophila melanogaster Meig.(larva)	0.75	fructose	Sang (1956)
Pectinophora gossypiella (Saund.)	2.5	glucose (amino acid medium)	Vanderzant (1957)
	4-6	sucrose (casein medium)	Vanderzant and Reiser (1956b)
Ostrinia nubilalis Hbn.	0.7	early instars) fructose,	Beck (1956b)
	5.3	late instars) glucose or sucrose	
Anthonomus grandis (Boh.) (larva)	3.5	fructose, maltose or sucrose	Vanderzant (1965)

TABLE 3 (Continued)

Total quantity of utilisable carbohydrate in diets for various insects

Species	Total %age of carbohydrate in the diet	Nature of carbohydrate	Author
Myzus persicae (Sulzer)	10-20	sucrose	Dadd and Mittler (1965)
Hylemya antiqua (Meig.)	24 (dry)	dextrose	Friend (1956)
Schistocerca gregaria (Forsk.)	26 (dry)	glucose, sucrose	Dadd (1960c)
Locusta migratoria L.	26 (dry)	glucose, sucrose	Dadd (1960c)
Blatella germanica (L.)	32	glucose, dextrin	Noland et al. (1949)
	65 (dry)	sucrose, lactose, dextrin	House (1949a)
Galleria mellonella L.	Approx. 25 (wet)	glucose	Dadd (1964)
	35-45 (dry)	glucose	Dadd (1964)
Stegobium paniceum (L.)	45-50	starch or glucose	Fraenkel and Blewett (1943b)
Oryzaephilus surinamensis (L.)	45-50	starch or glucose	Fraenkel and Blewett (1943b)
Plodia interpunctella (Hbn.)	33-66 (wet)) 50-80 (dry))	glucose	Fraenkel and Blewett (1946b)
Ephestia elutella (Hbn.)	50-80	glucose	Fraenkel and Blewett (1946b)
Cadra cautella Wlk.	50-80	glucose	Fraenkel and Blewett (1946b)
Anagasta kuehniella Zell.	66 (wet)) 80 (dry))	glucose	Fraenkel and Blewett (1946b)
Palorus ratzeburgi Wissman	80	glucose, starch	Cooper and Fraenkel (1952)
Tenebrio molitor L.	80-85	glucose, starch	Fraenkel et al. (1950)

In spite of all these qualifications certain tentative conclusions are probably valid. At one end of the scale are insects feeding on organic debris of various sorts, such as carrion, fur, wool and blood, which can be reared in the absence of carbohydrate or on very low levels of this material. At the other end of the scale are the highly adapted stored products pests which seem to have become dependent upon a very high level of carbohydrate, values in excess of 50 per cent being optimal in synthetic diets.

Phytophagous insects seem to require a moderately low percentage of carbohydrate in their diet and this may be due to the fact that the major constituent of their natural diet is water. Insects like the cockroach and the wax moth seem to lie mid way between the phytophagous group and those living on high carbohydrate food.

2. Nitrogen.

The total percentage nitrogen supplied in synthetic diets for various insects is given in Table 4. The same qualifications apply to the figures quoted in this table as to those of Table 3. However, again tentative conclusions can be drawn in this case.

Insects feeding normally on protein rich food, such as the larder beetle, fly larvae and clothes moth larvae are obviously best reared on protein rich synthetic diets. At the other end of the scale are the phytophagous insects which appear to require a very low percentage of nitrogen because of the high proportion of water which must be supplied

TABLE 4

Total quantity of utilisable nitrogen in diets of various insects

Species	Total %age 'nitrogen' in the diet*	Nature of nitrogen compounds	Author
Aedes aegypti (L.)	1	casein	Akov (1962)
Anthonomus grandis (Boh.)	1.9	amino acid mixture	Vanderzant (1965)
Pectinophora gossypiella (Saund.)	2.1	amino acid mixture	Vanderzant (1957)
Hylemya antiqua (Meig.)	2.42	amino acid mixture	Friend (1956)
Myzus persicae (Sulzer)	3	amino acid mixture	Dadd and Mittler (1965)
Ostrinia nubilalis Hbn.	2.9-3.7	casein	Chippendale and Beck (1964)
Drosophila melanogaster Meig.	5	casein	Sang (1956)
Galleria mellonella L.	15	casein	Dadd (1964)
Tribolium confusum (Duv.)	15-45	brain protein, casein, cotton seed protein, fibrin, gluten wheat, glycine, lactalbumen, liver protein, peanut protein, soybean protein	Chiu and McCay (1939), Fraenkel and Blewett (1943a,b)
	20	amino acid mixture	Lemonde and Bernard (1951b)
Palorus ratzeburgi Wissman	20	casein	Cooper and Fraenkel (1952)
Tenebrio molitor L.	10	casein, lactalbumen,	Fraenkel et al.(1950)
	20	peanut protein	
Anagasta kuehniella Zell.	Approx. 20	casein	Fraenkel and Blewett (1946b)
	45	casein	Fraenkel and Blewett (1943b)

TABLE 4 (Continued)

Total quantity of utilisable nitrogen in diets of various insects

Species	Total %age 'nitrogen' in the diet*	Nature of nitrogen compounds	Author
Blatella germanica (L.)	22-24	casein, yeast	Haydak (1953)
	30	casein or amino acid mixture	House (1949a)
Bombyx mori L. (larva)	27-30 dry	casein	Ito and Horie (1962)
Phormia regina Meig. (larva)	10	amino acid mixture)	McGinnis et al. (1956)
	29	casein)	
Calliphora vicina (Meig.) (larva)	10 wet 80 dry	casein	Sedee (1956)
Dermestes vulpinus Fab.	44.4	casein, cystine, yeast	Gay (1938)
Stegobium paniceum (L.)	45	casein	Fraenkel and Blewett (1943b)
Oryzaephilus surinamensis (L.)	45	casein	Fraenkel and Blewett (1943b)
Ptinus tectus Boield.	45	casein	Fraenkel and Blewett (1943b)
Tineola bisselliella (Hum.)	Approx. 45	casein, yeast	Fraenkel and Blewett (1946a)
Musca domestica L. (larva)	Approx. 70	casein	Monroe (1962); Perry and Miller (1965)

*Figures represent the percentage of the compounds listed in column 3

in the diet.

3. Vitamins.

The quantitative amounts of B vitamins in various synthetic diets are given in Table 5. However, in addition to B vitamins the insects may require vitamins A or C for their development. The larvae of the boll weevil did not develop to adults when the eggs were obtained from the parents fed on a diet containing less than 0.05 per cent ascorbic acid (Vanderzant et al.1962); the European corn borer required 0.5 per cent ascorbic acid in its diet (Chippendale and Beck 1964); ascorbic acid at the rate of one per cent of the dry diet increased survival especially in the late stages of the larvae of the silkworm and proved to be a relatively strong stimulant for feeding of the larvae (Ito and Horie 1962); 0.2 to 0.4 per cent was found to be essential in the diet of Chilo suppressalis (Wlk.) (Kamano 1964), and 0.5 per cent in that of the cabbage looper, Trichoplusia ni (Hbn.) (Chippendale et al.1965). In the diet of the desert locust, Schistocerca gregaria (Forsk.), omission of ascorbic acid caused complete mortality of hoppers by the fourth instar. Omission of β-carotene also caused high mortality in the early instars and the surviving nymphs which reached the fifth instar lacked yellow colouration (Dadd 1957). Dadd used ascorbic acid and β-carotene at concentrations of 0.44 per cent and 0.11 per cent respectively.

TABLE 5

Total quantity of B vitamins in diets of various insects

Species	Total %age of B vitamins in the diet	Author
Tribolium confusum (Duv.)	0.00163	Fraenkel and Blewett (1947a)
Musca domestica L.	0.00392	House and Barlow (1958)
Drosophila melanogaster Meig.	0.00437	Sang (1956)
Hylemya antiqua (Meig.)	0.007756	Friend and Patton (1956)
Pectinophora gossypiella(Saund.)	0.0075	Ouye and Vanderzant (1964)
Aedes aegypti (L.)	0.01286	Akov (1962)
Tenebrio molitor L.	0.0335	Fraenkel *et al.*(1950)
Trichoplusia ni (Hbn.)	0.0327	Chippendale *et al*. (1965)
Schistocerca gregaria (Forsk.)	0.06-0.18	Dadd (1961a)
Anthonomus grandis (Boh.)	0.073	Vanderzant (1965)
Tineola bisselliella (Hum.)	0.075	Fraenkel and Blewett (1946a)
Lasioderma serricorne (Fab.)	0.1	Blewett and Fraenkel (1944)
Stegobium paniceum (L.)	0.1	Blewett and Fraenkel (1944)
Myzus persicae (Sulzer)	0.1231	Mittler and Dadd (1962)
Palorus ratzeburgi Wissman	0.13	Cooper and Fraenkel (1952)
Galleria mellonella L.	0.1732	Dadd (1964)
Ostrinia nubilalis Hbn.	0.2327	Chippendale and Beck (1964)
Blatella germanica (L.) app.	0.26	House (1949a)

4. Fats and fatty acids.

Not much work has been done on the requirements for fats and fatty acids in insects. However, these have been included in the diets of a few insects. For instance linoleic acid or a mixture of linoleic, oleic and linoleinic together with a small amount of various fully saturated acids, was found to be a necessary component of the diet of the locusts (Dadd 1961b), linoleic and linoleinic acids being used in the concentration of five milligrams per gram of the diet. Similarly, 0.25 gram corn oil was used per 100 grams of medium for Pectinophora gossypiella (Saund.) larvae since linoleic acid makes up about 58 per cent of the commercial corn or 'Mazola' oil (Vanderzant and Reiser 1956a,b), while Beck (1950) used one per cent linoleic acid in the diet of the European corn borer Ostrinia nubilalis Hbn. In the cabbage looper, Trichoplusia ni (Hbn.) certain lipids were shown to contain a wing development factor and a lipid extract of cabbage tissue used at the rate of 0.064 gram in 100 grams of the medium gave satisfactory results. However, Ishii and Urushibara (1954) could not find any fat requirements in the larvae of the rice stem borer, Chilo simplex Butler.

5. Sterols.

The work on requirements for sterols in different insects has been reviewed very recently by House (1965a). It appears that insects are generally supplied with cholesterol in artificial diets, but the quantities vary in different species. A few examples may be quoted

to show the optimum percentage of cholesterol in diets for various species (Table 6).

6. Minerals.

The quantitative requirements for minerals in the diets of insects have not been given much attention and the published work does not lead to any general conclusions. However, Fraenkel (1958) found that the zinc concentration may be a limiting factor for the growth of Tenebrio molitor L. with 6 p.p.m. required for optimal growth, but as little as 0.37 p.p.m. giving a growth response. The potassium requirements fell between 1.7 and 2.9 per cent of the diet.

The diet of Hylemya antiqua (Meig.) contained a 0.2 per cent mixture of various mineral salts (Friend 1956). The aphid , Myzus persicae (Sulzer) required 43 to 85 milligrams of phosphate, 35 to 70 milligrams of potassium and 25 to 30 milligrams of magnesium chloride per 100 millilitres of the diet (Dadd and Mittler 1965).

Dadd (1961b) obtained satisfactory growth of locust hoppers on a diet with a simplified salt mixture. This mixture included sodium carbonate, calcium chloride, potassium phosphate and magnesium sulphate mixed at 20, 15, 50 and 15 parts by weight respectively. A medium containing 1.2 per cent of salts seemed to have an optimum effect on the development of the pink bollworm, Pectinophora gossypiella (Saund.) (Vanderzant and Reiser 1956b). The boll weevil's (Anthonomus grandis (Boh.) needs were satisfied by 0.1 per cent magnesium sulphate in a synthetic diet. Doubling this amount caused higher mortality and ommission

TABLE 6

Total quantity of cholesterol in diets of various insects

Species	Total %age cholesterol in the diet	Author
Drosophila melanogaster Meig.	0.01 to 0.05	(Sang 1956)
Aedes aegypti (L.)	0.02 to 0.032	(Akov 1962)
Hylemya antiqua (Meig.) (larva)	0.1	(Friend 1956)
Musca domestica L. (larva)	0.2	(Monroe 1962)
Pectinophora gossypiella (Saund.) (larva)	0.01 to 0.3	(Vanderzant and Reiser 1956b)
Bombyx mori L. and *Trichoplusia ni* (Hbn.) (larvae)	0.3	(Ito *et al.* 1964, Chippendale *et al.* 1965)
Dermestes maculatus Deg.	0.32	(Levinson 1962)
Schistocerca gregaria (Forsk.)	0.84	(Dadd 1960b)
Anagasta kuehniella Zell., *Ephestia elutella* (Hbn.), *Cadra cautella* Wlk., *Plodia interpunctella* (Hbn.) and *Ostrinia nubilalis* Hbn. (larva)	1	(Fraenkel and Blewett 1943d, 1946d, Beck 1950)

resulted in smaller adults, slower development and again a lower survival (Vanderzant 1965).

7. Other substances.

a) Agar

Tanton (1965) showed that an increase in the agar concentration of the diet of the mustard beetle, Phaedon cochleariae Fab., proved beneficial. Quantities of up to 6 per cent agar were tried and the highest amount of food was consumed at that level.

D. COMPARISON OF DIETS

As artificial diets are developed for various insects it becomes essential to have precise methods of evaluating these diets in comparison with one another and with the natural food. One method of achieving this is to obtain some quantitative estimate of the proportion of food actually utilised by the insect for energy production and the building of body tissues.

The first attempt made in this direction was that of Evans (1939) who calculated three parameters which he termed the coefficients of utilisation, growth and metabolism. These were derived as follows:-

$$\text{Coefficient of utilisation} = \frac{A - B}{A}$$

$$\text{Coefficient of growth} = \frac{C}{A - B}$$

$$\text{Coefficient of metabolism} = \frac{(A - B) - C}{A - B}$$

where:-

A = Dry weight of food consumed

B = Dry weight of faeces produced

C = Increase in dry weight of larvae

Four species of insects were used, these being Phalera bucephala L. which was fed on hornbeam foliage, Malacosoma neustria (L.) fed on willow foliage, Aglais urticae L. fed on nettle and Pieris brassicae L. fed on cabbage leaves. It was noticed that the larvae of the species studied utilised and consumed food at very different rates. The amounts of carbohydrate, fat and ash utilised per gram of larva per day varied very much in the four species.

Davey (1955) investigated the amounts of food eaten by the hoppers and adults of Schistocerca gregaria (Forsk.) and showed that the coefficient of utilisation falls from 78 per cent in the first instar to 35 per cent in the fifth instar. The food consumption per hopper increased with the number of hoppers per cage.

Dadd (1960e) found that in the locust, Schistocerca gregaria (Forsk.), growth could occur on diets lacking yeast, sugar and wheat

germ oil, (the only dietary components found to have phagostimulatory properties), and it appeared that special gustatory stimuli were necessary for initiating feeding activity in hungry hoppers. So, he compared the amounts of various complete diets eaten and the faeces produced from them. No major differences in feeding attributable to palatability were apparent, but the amounts of food taken were found to be related to differences in utilisability. With both the species, i.e. Schistocerca and Locusta, the lower the utilisability of the food the greater the amount eaten and for a range of diets of various utilisabilities, including fresh grass, the values obtained by multiplying the amount eaten (dry weight) by the coefficient of utilisation were similar for each species. He suggested that the amount of food eaten appears to be largely regulated by its overall utilisability.

Smith (1959) fed the migratory grasshopper, Melanoplus bilituratus (Walker), on either wheat, western wheat grass or oats for 40 days after hatching. The amounts of wheat and western wheat grass consumed were approximately equal and were twice that of oats. The final weights of hoppers were less on oats by about one-third. Overall utilisation was 32 per cent on each of the three foods. The coefficient of growth was 38 per cent for oats, 32 per cent for wheat and 27 per cent for western wheat grass and was negatively correlated with the amount of food utilised. So, the various criteria indicated that the wheat was a good food.

Waldbaur (1964) calculated the consumption, digestion and utilisation of solanaceous and non solanaceous plants by larvae of the tobacco hornworm, Protoparce sexta (Johan.). The coefficient of growth was higher on solanaceous plants i.e. potato and Solanum dulcamara (where it was 64 and 64.4 per cent respectively), but it was only 53.4, 47.9 and 38.5 per cent respectively on the non solanaceous plants, Taraxacum officinale, Arctium minus and Verbascum thapsus.

E. CONCLUSION

Obviously much information has been obtained on the requirements of insects by carefully controlled feeding tests, and considerable skill has been developed in rearing, often aseptically, insects on synthetic diets. Few insects, apparently, prefer such diets in place of their natural diets. However, the information obtained with these chemical diets is applied by the worker or the dietician in selecting combinations that supply the optimum daily quantities of protein, vitamins and other dietary essentials.

One conclusion to be drawn from this review is that comparatively little has been done as far as nutritional studies on phytophagous insects are concerned. This was the major governing factor in the choice of the cabbage white butterfly larva as the subject of the present investigation. Moreover, this particular phytophagous insect is a pest of economic importance all over the world and also has the

advantage for this type of work of being readily reared in the laboratory at all times of the year.

There is exceedingly little published information on the nutrition of this insect which could provide a starting point for this work. It was not possible to take up all the aspects simultaneously, although an attempt has been made to investigate, as far as possible, the basic food requirements of the larvae and to evolve a chemically and physically suitable diet. This has, to some extent, involved investigations into the phagostimulant effects of certain nutrients. An attempt has been made to evaluate quantitatively the requirements for several components of the insect's diets and also to provide a quantitative comparison between some of the diets formulated and the insect's natural food.

CHAPTER II

MATERIALS AND METHODS

A. MASS CULTURE TECHNIQUES

The insects used in this work were all descendants of an original culture of butterflies reared from eggs supplied by Dr. W.A.L. David of the Agricultural Research Council Unit of Insect Physiology, Cambridge.

1. The Adults.

The culture of adults was maintained in a cage measuring 40 x 30 x 36 inches high. The top of the cage and one of the 30 inches wide walls were made of terylene netting stretched on light wooden frames. The other sides were made of glass and the floor made of hard-board. Access to the cage was through a sleeve fixed in the lower half of the terylene covered side (Plate 1).

The cage was placed in a greenhouse to allow maximum daylight but supplementary sources of light, as described by David and Gardiner (1952, 1961a), were provided. These consisted of two Mazda fluorescent 80 watts daylight strips set to a sixteen-hour day length and a Philips 400 watts mercury fluorescent reflector lamp which was used to give a sunlight effect on cloudy days.

The greenhouse was heated during the winter by means of a Humex Autoheat fan heater. No attempt at strict temperature control was made, the variation during the course of a year being from 13°C to 28°C.

PLATE 1

Showing cage containing the culture of adult butterflies.

The butterflies were fed on a 10 per cent solution of honey in water. This was placed in 2 x 3/8 inch specimen tubes which were fixed to perspex artificial 'flowers' of the type described by David and Gardiner (1961b). These 'flowers' were arranged in groups of four, and each group (Plate 2) was made from a perspex plate 1/8 inch thick and 4 inches square. Four holes were drilled in this plate for the four honey tubes, the centre of each hole being 1¼ inches from the corresponding corner of the plate. This hole was approximately 3/8 inch in diameter and was surrounded by a blue or yellow painted circle 1½ inches in diameter. This coloured circle was in its turn surrounded by a white painted band, half an inch in width. The flowers were painted blue or yellow as the butterflies are attracted to these two colours (David and Gardiner 1952, 1961b). Usually four groups of flowers were placed in the cage at two different levels each supported by a rod fixed below in a pot. The tubes and the flowers were cleaned regularly and fresh honey solution was added every day.

Eggs were obtained by placing either young potted cabbage plants or large cabbage leaves in the cage, David and Gardiner (1962) having shown the necessity for bringing the fore-legs into contact with the cabbage tissue in order to evoke oviposition.

2. The Caterpillars.

The leaves on which the eggs were laid were taken out of the cage and were kept in the greenhouse in small round tins, three inches high

PLATE 2

Showing groups of artificial flowers.

and with a diameter of eight inches. On hatching, the caterpillars were fed on cabbage leaves and when they entered the fourth instar the tins were covered with terylene netting to prevent escapes. The fifth instar caterpillars were transferred to large biscuit tins measuring $10\frac{1}{2}$ x $10\frac{1}{2}$ x 12 inches high in each of which was placed a piece of wire gauze resting about three inches from the bottom. The caterpillars and the cabbage leaves remained above the gauze, while the faeces dropped through and were regularly removed. These tins were also covered with terylene netting. The caterpillars were allowed to pupate inside the large tins and the adults on emergence were transferred to their cage. The tins were thoroughly washed and cleaned before use with each new batch of caterpillars.

B. EXPERIMENTAL CULTURE TECHNIQUES

1. Laboratory Rearings.

For experimental purposes caterpillars were removed from the culture and placed on the media under investigation which were contained in glass tubes. The size of the tubes used depended upon the stage in the larval life under investigation, small caterpillars being maintained in 3 x 1 inch tubes and the last instar in 8 x $1\frac{3}{4}$ inch tubes. If the caterpillars were to be kept for a long time on the media, the tubes along with the media were autoclaved for twenty minutes at a pressure of ten pounds per square inch, the temperature being 115°C. The tubes were always plugged with non-absorbent cotton wool and sterilized absorbent gauze.

vitamins destroyed cf p 29

For the experiments designed to investigate the amount of food consumed and utilised by the caterpillars, only the fifth instar larvae were used and they were allowed to feed on the media for twenty four hours. In these experiments the media were poured into glass petri dishes 1¾ inches deep and with a diameter of 1 7/8 inches. Each dish was covered with terylene netting, after adding the larvae, and was placed in the insectary at a temperature of 28 ± 2°C. under fluorescent lights which gave a sixteen-hour day length.

2. Preparation of Experimental Media.

a) Leaf powder

Cabbage leaves were subjected to steam over a water bath for five minutes in order to kill the enzymes, and were then dried in an oven overnight at a temperature of 40°C. The dried leaves were ground into powder which was then used to make the media. Freeze dried cabbage was also used to make the leaf powder and in this case, it was oven dried without any previous steam treatment. The response of the larvae was not affected by the method of preparation of the leaf powder.

b) Vitamins

B vitamins were weighed and dissolved in distilled water, appropriate quantities of the solution being used in making media.

c) Mixing

All the ingredients of each medium, with the exception of agar and vitamins, were weighed, stirred together while still dry and

ground with a pestle and mortar for thorough mixing. Nutrient agar was always dissolved in the required amount of distilled water with the aid of a boiling water bath and then added to the rest of the ingredients while hot. After cooling, the specified quantity of vitamin solution was added and the complete medium thoroughly mixed. Small quantities of the medium were mixed by hand and larger quantities were mixed in an M.S.E. 'Atomix' homogeniser.

C. METHODS OF RECORDING RESULTS

1. Measurement of Larval Weight.

a) Live weight of caterpillars

An estimate of the growth of the caterpillars on a given medium was obtained by weighing them on a torsion balance (100 x 0.2 mgm.) at the beginning and end of the experimental period.

b) Dry weight of caterpillars

The utilisation of food was measured on the basis of dry weight readings. The caterpillars were dried at 110°C for sisteen hours, then removed from the oven, placed in a desiccator, and finally weighed on the torsion balance.

In order to measure the dry weight gain during the course of an experiment, initial dry weights are required in addition to final dry weights. These initial dry weights, obviously, have to be estimated and for this purpose a larval drying factor was calculated at the beginning of each experiment. In order to obtain this factor a batch

of approximately twenty larvae, which were as similar as possible to the experimental larvae, was removed from the same stock culture. After recording the individual live weights of these larvae, they were dried to constant weight at 110°C. The drying factor was simply the ratio of dry weight to live weight and could be used to estimate the initial dry weights of experimental larvae with known live weights. In order to do this the live weight of each caterpillar was multiplied by the average drying factor for the caterpillars of that particular batch.

2. Measurements of Weights of Food Consumed and Faeces Produced.

The consumption of food by the larvae and their consequent production of faecal material were measured in one of the two ways, either gravimetrically or colorimetrically.

a) Gravimetric method

Food consumption was measured directly by subtracting the final dry weight of the medium at the end of a particular experiment from the estimated dry weight of the same medium at the beginning of the experiment. The estimated initial dry weight was obtained by the use of a drying factor in an identical manner to that described for estimating the initial dry weight of larvae. Faecal production was obtained by carefully collecting all faecal material and drying it to constant weight.

b) Colorimetric method

An alternative method of measuring food consumption was also used, the colorimetric method of McGinnis and Kasting (1964a,b). This method avoids the necessity for estimating initial dry weights for the media by means of a drying factor, and also avoids the direct measurement of the final dry weights of the media. A known amount of chromic oxide (Cr_2O_3) was mixed with the medium and at the end of each experiment the faeces were collected, dried and the faecal concentration of Cr_2O_3 estimated. The weight of Cr_2O_3 remaining in the insect was also measured and the dry matter consumed was calculated according to the following formula:-

$$\frac{(CF \times WF) + WCI}{CM} = \text{Dry weight of food consumed}$$

where:-

CF = Concentration of Cr_2O_3 in faeces

CM = Concentration of Cr_2O_3 in medium

WF = Weight of faeces

WCI = Weight of Cr_2O_3 in insect

All concentrations were expressed in micrograms per milligram and all weights in milligrams.

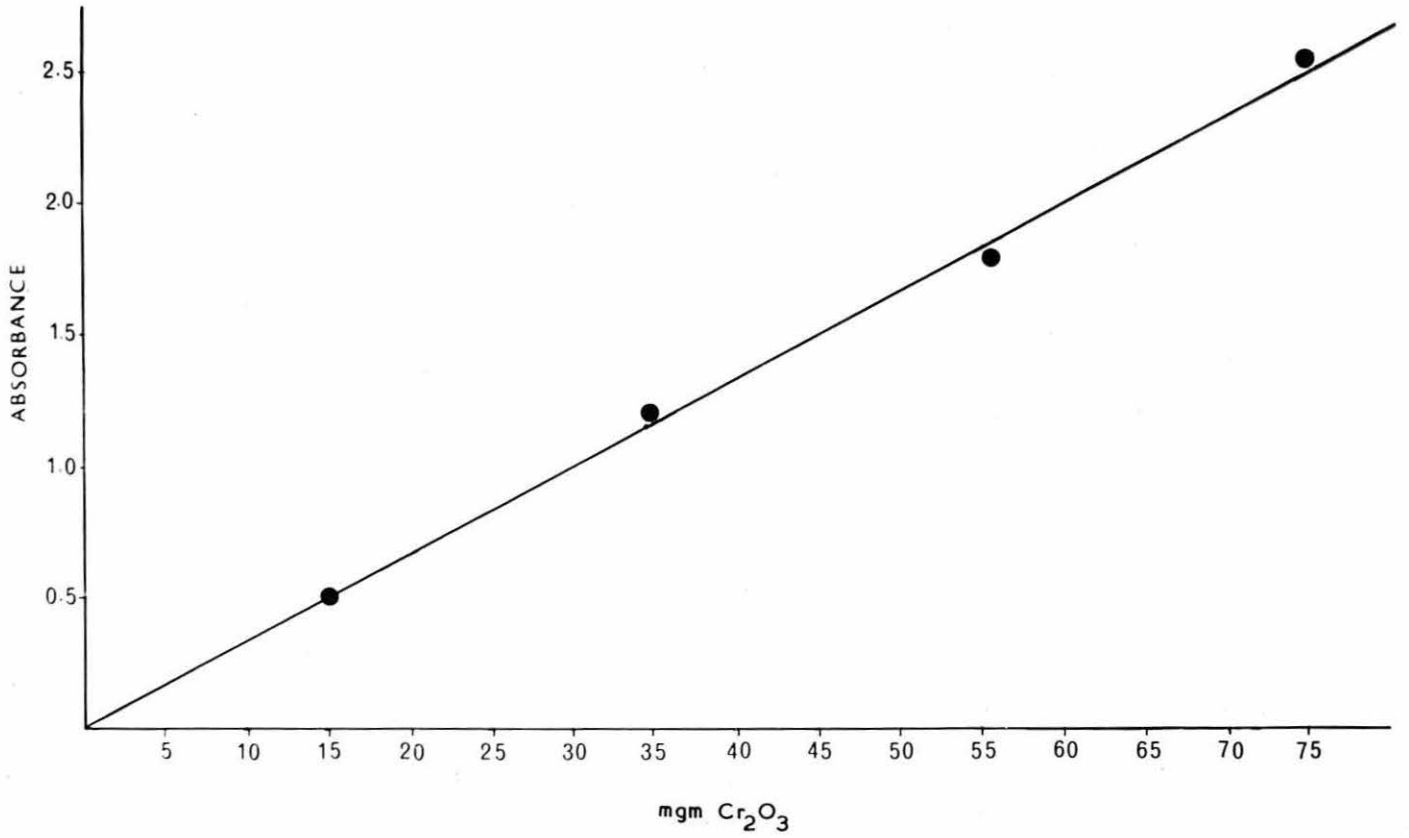
The concentrations of chromic oxide were measured with an 'Eel' portable colorimeter using the Ilford 625 (maximum 540 mμ transmission) filter. A calibration curve was prepared by measuring the absorbance

of a known quantity of chromic oxide in the medium. For this purpose chromic oxide was included in several batches at concentrations of 15, 35, 55 and 75 milligrams per gram of the medium. After drying, samples of twenty milligrams were taken from each concentration and digested according to the method prescribed by McGinnis and Kasting (1964a,b). Using the calibration curve derived in this way (Figure 1) the concentration of chromic oxide in dried samples of faeces and media could readily be estimated. A full account of the digestion procedure has been given by McGinnis and Kasting and is repeated here in Appendix I. Kasting and McGinnis (1965) have also evolved a radioisotopic method for measuring the consumption of food in insects. But in their experiments with Agrotis orthogonia Morr. larvae they found that results obtained by all the three methods namely, gravimetric, colorimetric and radioisotopic, agreed well with one another.

3. Comparative Estimates of Feeding.

Certain of the media were compared with one another on the basis of the amount of material ingested by larvae in a given time. The method adopted was to incorporate a dye into the medium and then to dissect out the guts for examination after a known feeding time. In the initial experiments neutral red was mixed with the medium, at a concentration of 0.1 per cent by weight, to impart a red colour easily detectable in the gut. However, the colour changed to orange in the mid gut due to a high pH in that region and subsequently, therefore,

Fig.1 Showing absorbance of chromic oxide at different concentrations.



neutral red was replaced with carmine which maintained its original colour throughout.

The relative amounts of coloured food ingested were assessed by recording the extent of the coloration of the alimentary canal of the caterpillars. The system of scoring the relative extent of the coloration that was adopted was similar to that described for aphids by Mittler and Dadd (1963). Arbitrary values of 0, ½, 1, 1½, 2, 2½ or 3 were allocated to the individual guts examined under a dissecting microscope. Although the seven colour grades were part of a continuous series, they could be defined as follows (see also Figure 2):-

0 - guts in which the red stain was not observable.

½ - some red colour in the fore gut or a just discernible pink coloration in the anterior part of the mid gut.

1 - fore gut and/or one quarter of the mid gut coloured.

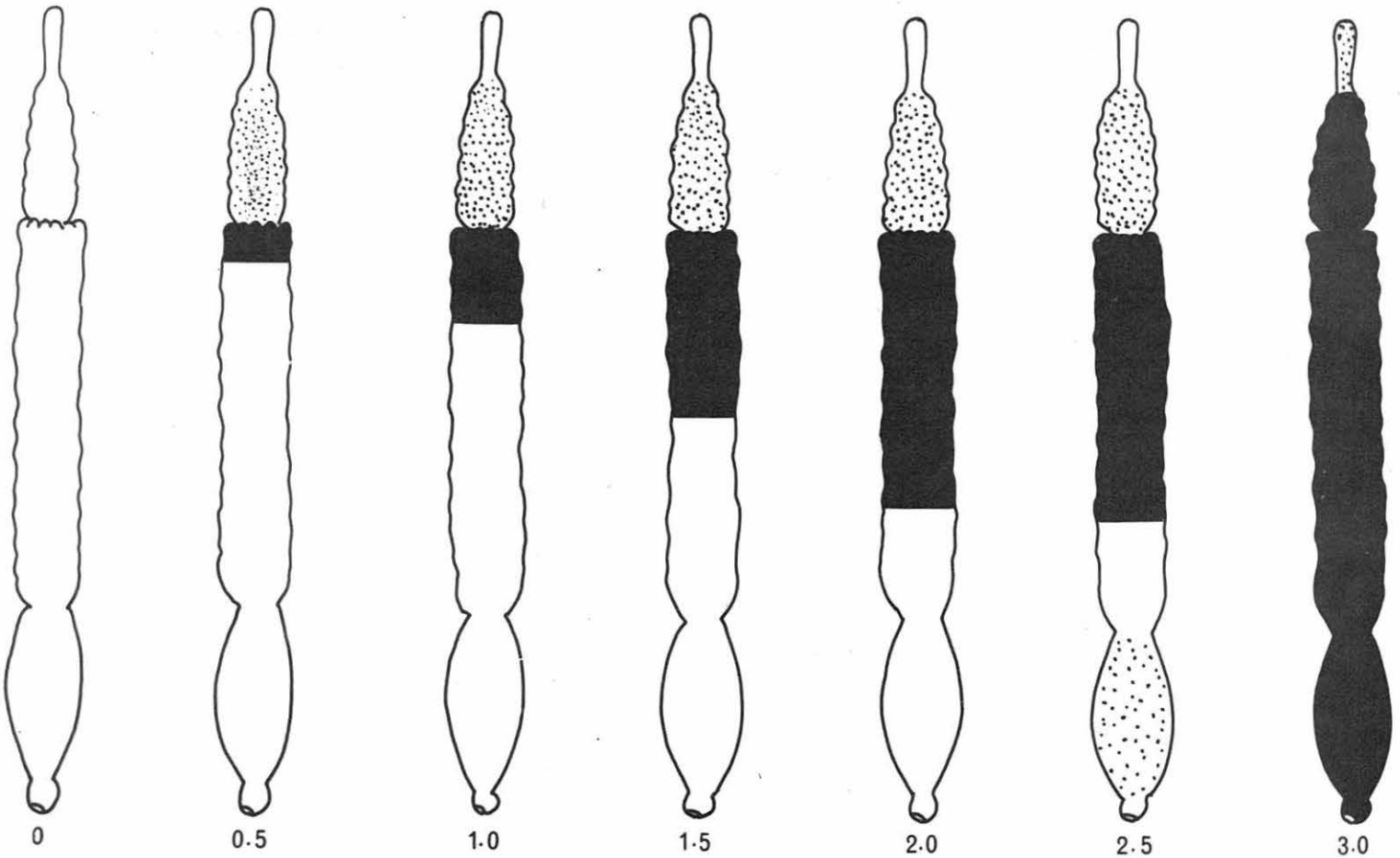
1½ - half of the mid gut coloured.

2 - about three quarters of the mid gut coloured.

2½ - coloration observed in about three quarters of mid gut in addition to hind gut and/or fore gut.

3 - the entire alimentary canal is stained red.

Fig. 2 Showing arbitrary values for different colour grades in the gut.



CHAPTER III

THE DEVELOPMENT OF A BASIC DIET

The need for devising a suitable synthetic diet has been emphasised in Chapter I, this chapter describes attempts directed towards this goal i.e. evolving a suitable diet for the caterpillars of the large cabbage white butterfly, Pieris brassicae L.

A. ORIGINAL FORMULA

The first synthetic diets tried were modified from one supplied by Miss Arlene McMorran of the Insect Pathology Research Institute in Sault Ste. Marie, Ontario, Canada. This diet had originally been used in Canada for rearing pine shoot moth larvae and had the following composition.

1. Distilled water	110.0 ml.
2. Ten Pinus resinosa buds or equivalent in current shoots	30.0 gms.
3. Casein (vitamin free)	17.5 gms.
4. 4 M. potassium hydroxide (Mol. wt. 56.1)	2.5 ml.
5. Alphacel (non-nutritive bulk)	2.5 gms.
6. Wesson's salt mixture	5.0 gms.
7. Sucrose	17.5 gms.
8. Wheat embryo	15.0 gms.
9. Choline chloride	0.5 gm.

* 10. Vitamin solution	5.0 ml.
11. Ascorbic acid	2.0 gms.
12. Methyl para hydroxy benzoate	0.75 gm.
13. Aureomycin	0.15 gm.

14. 12.5 grams nutrient agar dissolved (heated over steam or in beaker immersed in boiling water) in 320 ml. water. This is added to mixture while hot.

* Vitamin solution

Distilled water	100 ml.
Niacin	100 mgm.
Calcium pantothenate	100 mgm.
Riboflavin	50 mgm.
Thiamine hydroxide	25 mgm.
Pyridoxal hydrochloride	25 mgm.
Folic acid	25 mgm.
Biotin	2 mgm.
Vitamin B-12	0.2 mgm.

An attempt was made to find out the qualitative and quantitative requirements for different materials by eliminating and including various ingredients and by changing their concentrations in the diet. Tests with different modifications were conducted by allowing the cabbage white caterpillars to feed on them and observing the effect

on the growth, development and mortality of the test insects.

B. MODIFICATIONS

Obviously pine buds were not required for Pieris brassicae L. larvae and initial attempts were made to rear second and third instar larvae on Miss McMorran's diet with this plant material excluded. Survival was extremely poor and the addition of the mustard oil glucoside, sinigrin, did not significantly improve the situation (diets nos. 1, 3, 5, 6, 7, 8, 23, 24, 25, 26, 27, 28, Table 7), although this substance is said to induce the chemotactic response to food by the larvae (Reviewed in Chapter I).

Following the negative results with sinigrin attempts were made to modify the physical structure of the medium by making it into a form which imitated in structure the plant leaf (Table 7, nos. 1 and 3). This was accomplished by adding cellophas B (a sodium carboxymethyl cellulose - a product of I.C.I.) which was sufficiently viscous to enable the material to be spread as thin membranous sheets. The result was very disappointing, since the cellophas absorbed such enormous quantities of water that it was not possible to prevent the diet drying up overnight.

Several attempts were then made to bring about an improvement by altering the relative amounts of the original constituents of the diet, e.g. casein (Table 7, nos. 13 and 14), sucrose (nos. 23, 26, 29 and 32), nutrient agar (nos. 21 and 22); by introducing new ingredients,

TABLE 7

Composition of different synthetic diets

Diet No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Distilled water 110 ml.	+	+	+	+	+	+	+	+	-	70	-	-	-	-	-	-	-	-	-	-
Sinigrin 25 mgm.	+	+	-	5	10	25	50	-	-	-	-	-	-	-	-	-	-	-	-	-
Casein 17.5 gm.	+	+	+	+	+	+	+	+	+	+	+	+	35	50	+	+	+	+	+	+
KOH 2.5 ml.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	1	-	-	+	+
Cellophas 5 gm.	+	+	-	2.5	+	+	+	+	-	-	3.5	-	-	-	-	-	-	-	-	-
Osbourne and Mendel salt mixture 5 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose 17.5 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Wheat embryo (Bemax) 15 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Choline chloride 0.5 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B vitamins solution 5 ml.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascorbic acid 2 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ethyl p-hydroxy benzoate 0.75 gm.	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Nutrient agar 12.5 gm. in 320 ml. water	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HCl 10% 1 ml.	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cystine 100 mgm.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.75 gm.	4.375 gm.	8.75 gm.

TABLE 7 (Continued)

Composition of different synthetic diets

Diet No.	21	22	23	24	25	26	27	28	29	30	31	32	33
Distilled water 110 ml.	-	-	-	-	-	-	-	-	-	-	-	-	-
Sinigrin 25 mgm.	-	-	6	6	6	6	6	6	-	-	-	-	-
Casein 17.5 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+
KOH 2.5 ml.	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellophas 5 gm.	-	-	-	-	-	-	-	-	-	-	-	-	-
Osbourne and Mendel salt mixture 5 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose 17.5 gm.	+	+	8.5	+	+	8.5	+	+	8.5	+	+	8.5	+
Wheat embryo (Bemax) 15 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+
Choline chloride 0.5 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+
B vitamins solution 5 ml.	+	+	+	+	+	+	+	+	+	+	+	+	+
Ascorbic acid 2 gm.	+	+	+	+	+	+	+	+	+	+	+	+	+
Ethyl p-hydroxy benzoate 0.75 gm.	+	+	-	+	+	-	+	+	-	+	+	-	-
Nutrient agar 12.5 gm. in 320 ml. water	+	+	+	+	+	+	+	+	+	+	+	20	5
HCl 10% 1 ml.	-	-	-	-	-	-	-	-	-	-	-	-	-
Cystine 100 mgm.	-	-	+	+	+	-	-	-	+	+	+	-	-

+ denotes included in the quantity given above. Variations in quantities of materials from those given at the top are indicated in figures in appropriate columns.

- not included

e.g. cystine (nos. 18, 19, 20, 23, 24, 25, 29, 30 and 31); and by changing the pH through altering the concentrations of potassium hydroxide (nos. 15, 16 and 17) or adding hydrochloric acid (no. 17).

All the constituents of the various diets are given in Table 7 and the results obtained from these diets are summarised in Table 8. Thirty three variations of the basic diet were tried but the results remained disappointing.

C. INCLUSION OF PLANT MATERIAL

Since no information on nutritional requirements can be obtained if the insects fail to survive, a decision to incorporate plant material had to be made at this stage.

A powder was prepared from cabbage leaves as detailed in Chapter II and a fresh start was made on the selection of nutrients to incorporate with the minimum quantity of cabbage leaf powder in order to make a suitable diet for the larvae.

The experiments were started with first instar caterpillars because their initial weights were so uniform, but it was very difficult to handle these minute creatures and weighing errors may be significantly large in proportion to the small total weights. Therefore, third instar caterpillars were subsequently used because they were comparatively convenient to handle and weigh, and the weights were still more or less uniform.

TABLE 8

Effect of synthetic diets on the mortality and weights of the caterpillars

Diet No.	No. of larvae used	Deaths after days 2	4	6	8	10	12	Change in the weight of the surviving larvae
1	-	Medium spread over glass sheets dried completely in 48 hours						
2	8	0	0	7	7	7	7	gained app. 3 times (one larva)
*3	8	0	0	6	6	6	6	gained app. 3 times (2 larvae)
4	6	0	3	5	6	6	6	-
5	6	0	2	2	2	5	6	only one larva gained a little weight but died ultimately
6	9	0	2	5	5	8	9	very little gain in two larvae only
7	9	0	6	7	7	9	9	no gain
8	9	0	8	9	9	9	9	lost weight
9	10	4	5	7	10	10	10	lost weight
10	10	6	9	10	10	10	10	-
11	10	0	10	10	10	10	10	-
12	10	10	10	10	10	10	10	-
13	10	10	10	10	10	10	10	-
14	10	10	10	10	10	10	10	-
15	10	10	10	10	10	10	10	-
16	10	10	10	10	10	10	10	-
17	10	10	10	10	10	10	10	-
18	10	10	10	10	10	10	10	-
19	10	10	10	10	10	10	10	-
20	10	10	10	10	10	10	10	-
21	10	10	10	10	10	10	10	-
22	10	10	10	10	10	10	10	-
23	6	5	5	5	5	5	6	one larva lived for 10 days and gained 30.1 mgm.
24	6	4	4	4	4	6	6	-
25	6	3	3	3	3	5	5	one continued but gained only 4.7 mgm. in 10 days
26	6	4	4	4	4	6	6	-
27	6	3	3	3	3	5	5	one continued and gained 11.9 mgm. in 10 days
28	6	3	3	3	3	5	5	one continued and gained 33.9 mgm. in 10 days
29	6	5	5	5	5	6	6	-
30	6	6	6	6	6	6	6	-
31	6	6	6	6	6	6	6	-
32	6	5	5	5	5	6	6	lost weight
33	6	6	6	6	6	6	6	-

*When diet no. 3 was spread over 2% cellophas sheets, it dried completely in 48 hours and all the larvae were dead

In all, 17 diets (Table 9) were tried on first instar larvae and 59 (Table 10) on third instar larvae. The results with these, however, are reported in the following sections. The outcome of this work was a diet which gave the optimum growth and survival and was used as a standard for comparison with the results of further experiments. It is no. 16 in Table 10 and may be called the 'normal' diet or the 'basal' diet.

TABLE 9

Composition of diets tested with first instar caterpillars

Diet No.	Distilled water 10 ml.	Agar compound 20 gm.	Leaf powder 1.2 gm.	Sucrose 0.6 gm.	Casein 1 gm.	Ethyl p-hydroxy benzoate 40 mgm.	B vitamins solution 0.25 ml.
1	-	30	+	-	-	-	-
2	5	25	+	-	-	-	-
3	+	20	+	-	-	-	-
4	15	15	+	-	-	-	-
5	20	10	+	-	-	-	-
6	+	+	+	0.3	-	-	-
7	+	+	+	+	-	-	-
8	+	+	+	0.9	-	-	-
9	+	+	+	1.2	-	-	-
10	+	+	+	1.5	-	-	-
11	+	+	0.3	+	-	+	-
12	+	+	0.6	+	-	+	-
13	+	+	+	+	-	+	-
14	+	+	2.4	+	-	+	-
15	+	+	4.8	+	-	+	-
16	+	+	2.4	+	+	+	-
17	+	+	2.4	+	+	+	+

+ denotes the amount as given at the top of the column. Variations from this amount are denoted by figures in appropriate lines.

- Compound omitted

TABLE 10

Composition of diets tested with third instar caterpillars

Diet No.	Distilled water 25 ml.	Nutrient agar 0.5 gm.	Cabbage leaf powder 2 gm.	Sucrose 0.5 gm.	Casein 0.875 gm.	Ethyl p-hydroxy benzoate 37.5 mgm.	L-cystine 50 mgm.	
1	+	+	+	+	-	+	-	
2	+	+	+	+	+	+	-	+ sinigrin 1 ml. (2mgm. dissolved in 10 ml. water)
3	+	+	+	+	1.75	+	-	
4	+	+	+	+	+	+	-	
5	+	+	+	+	+	+	-	medium spread over filte paper
6	+	+	+	+	+	+	200	
7	+	+	4	+	+	+	+	
8	+	+	+	+	+	+	100	
9	+	+	+	+	+	+	400	
10	+	+	+	+	+	+	200	+ 100 mgm. ascorbic acid
11	+	+	+	+	+	+	200	+ 25 mgm. β-carotene
12	+	+	+	+	+	+	200	+ 100 mgm. ascorbic acid and 25 mgm. β-carotene
13	+	+	+	+	+	+	+	+ 25 mgm. β-carotene
14	+	+	+	+	+	+	+	+ 10 mgm. β-carotene
15	+	+	+	+	+	+	+	+ 500 mgm. cholesterol
× 16	+	+	+	+	+	+	+	
17	+	+	+	+	+	+	25	
18	+	+	0.25	+	+	+	+	
19	+	+	0.5	+	+	+	+	
20	+	+	1	+	+	+	+	
21	+	+	+	+	+	+	+	+ 250 mgm. Osbourne and Mendel salt mixture
22	+	+	+	-	+	+	+	+ 0.5 gm. glucose
23	+	+	+	-	+	+	+	+ 0.5 gm. fructose
24	+	+	+	+	-	+	+	
25	+	+	+	1	+	+	+	
26	+	+	+	0.25	+	+	+	
27	+	+	+	+	+	+	+	+ 0.25 ml. B vitamin solution
28	+	+	+	0.02	+	+	+	
29	+	+	+	0.05	+	+	+	

TABLE 10 (Continued)

Composition of diets tested with third instar caterpillars

Diet No.	Distilled water 25 ml.	Nutrient agar 0.5 gm.	Cabbage leaf powder 2 gm.	Sucrose 0.5 gm.	Casein 0.875 gm.	Ethyl p-hydroxy benzoate 37.5 mgm.	L-cystine 50 mgm.	
30	+	+	+	0.1	+	+	+	
31	+	+	+	0.15	+	+	+	
32	+	+	+	0.2	+	+	+	
33	+	+	+	-	+	+	+	
34	+	+	+	0.025	+	+	+	
35	+	+	+	0.075	+	+	+	
36	+	+	+	0.3	+	+	+	
37	+	+	+	0.4	+	+	+	
38	+	+	+	0.75	+	+	+	
39	+	+	+	-	+	+	+	+ 0.5 gm. maltose
40	+	+	+	-	+	+	+	+ 0.5 gm. trehalose
41	+	+	+	-	+	+	+	+ 0.5 gm. melibiose
42	+	+	+	-	+	+	+	+ 0.5 gm. cellobiose
43	+	+	+	-	+	+	+	+ 0.25 gm. glucose + 0.25 gm. fructose
44	+	+	+	+	+	+	+	+ 500 mgm. sitosterol
45	+	+	+	-	+	+	+	+ 0.5 gm. lactose
46	+	+	+	-	+	+	+	+ 0.5 gm. galactose
47	+	+	+	-	+	+	+	+ 0.5 gm. raffinose
48	+	+	+	-	+	+	+	+ 0.5 gm. melezitose
49	+	+	+	-	+	+	+	+ 0.5 gm. glycogen
50	+	+	+	-	+	+	+	+ 0.5 gm. starch
51	+	+	+	+	+	+	+	+ 25 mgm. sinigrin
52	+	+	+	+	+	+	+	+ 2.5 mgm. sinigrin
53	+	+	+	+	+	+	+	+ 50 mgm. cholesterol
54	+	+	+	+	+	+	+	+ 50 mgm. sitosterol
55	+	+	+	-	+	+	+	+ 0.5 gm. mannose
56	+	+	+	-	+	+	+	+ 0.5 gm. mannitol
57	+	+	+	-	+	+	+	+ 0.5 gm. ribose
58	+	+	+	-	+	+	+	+ 0.5 gm. arabinose
59	+	+	+	-	+	+	+	+ 0.5 gm. xylose

Key to Table 10

+ denotes the amount as given at the top of the column. Variations from this amount are denoted by figures in appropriate lines.

- denotes compound omitted from the diet.

The constituents of the normal diet (no. 16) make up a final weight of about 25* grams of the medium. Hence, all the additions or alterations to this diet are referred to as per '25 grams of medium'.

* actually 28.962 g

CHAPTER IV

QUALITATIVE AND QUANTITATIVE NUTRIENT REQUIREMENTS

A. CARBOHYDRATES

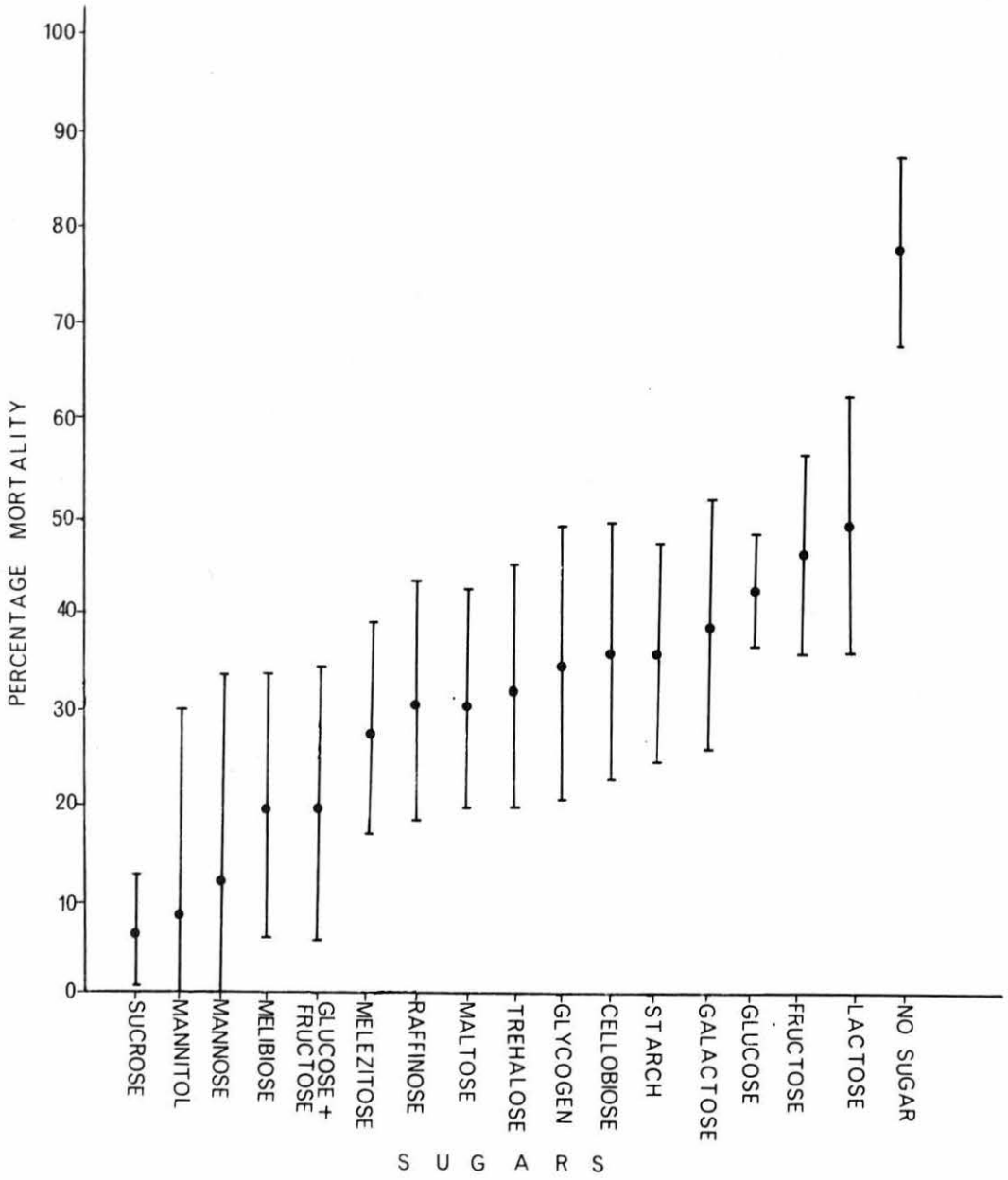
1. Sugars.

a) Qualitative requirements

The nutritional importance of the different sugars for Pieris brassicae L. larvae was examined by including each one in turn in the basal diet in place of sucrose. All these experiments were conducted on third instar caterpillars fed on different diets for four days, after which their percentage mortality was recorded. The composition of the diets is given at nos. 16, 22, 23, 33, 39, 40, 41, 42, 43, 45, 46, 47, 48, 49, 50, 55, 56, 57, 58 and 59 in Table 10 in Chapter III. The results are presented graphically in Fig. 3 with confidence limits plotted at the 5 per cent level.

It is clear that the omission of all sugars from the diet resulted in a significantly greater mortality indicating that sugars play an essential role in the diet of these insects. The inclusion of sucrose in the diet showed a mortality of only 6.6 ± 5.6 per cent which was significantly lower than on diets containing any other sugar excepting melibiose, a mixture of glucose + fructose, and probably mannose and mannitol. In these cases there was a higher mean mortality than in the case of sucrose but the differences are not significant.

Fig. 3 Showing larval mortality on diets containing different sugars.



The inclusion of glucose, fructose or lactose in the diets resulted in a mean mortality above 35 per cent, which was significantly greater than that obtained with sucrose, melibiose, the glucose + fructose mixture, mannose or mannitol. All other sugars studied produced mean percentage mortalities which were not significantly different from one another, the probable values lying between the limits of about 17 and 51.5 per cent. These arbitrary groupings may be summarised as follows:

Group I	sucrose, melibiose, glucose + fructose and probably mannose and mannitol	Mortality minimal
Group II	melezitose, raffinose, maltose, trehalose, cellobiose, glycogen, starch and galactose	Mortality significantly greater than sucrose; overlaps remainder of Group I
Group III	glucose, fructose and lactose	Mortality overlaps Group II but significantly greater than all of Group I

It is thought necessary to qualify the results for mannose and mannitol since the mean values in these two cases are based on only 25 larvae (5 batches of 5 larvae) and the confidence limits are therefore large.

Preliminary experiments have also been carried out using pentose sugars in the diet and these results are also subject to the same qualification. No mortality occurred in larvae reared on diets containing arabinose or xylose and only 12 per cent mortality in

larvae reared on diets containing ribose. It, therefore, appears at this stage that these three pentoses might also be included in Group I, above. The significance of these results will be discussed later.

b) Quantitative requirements for sucrose

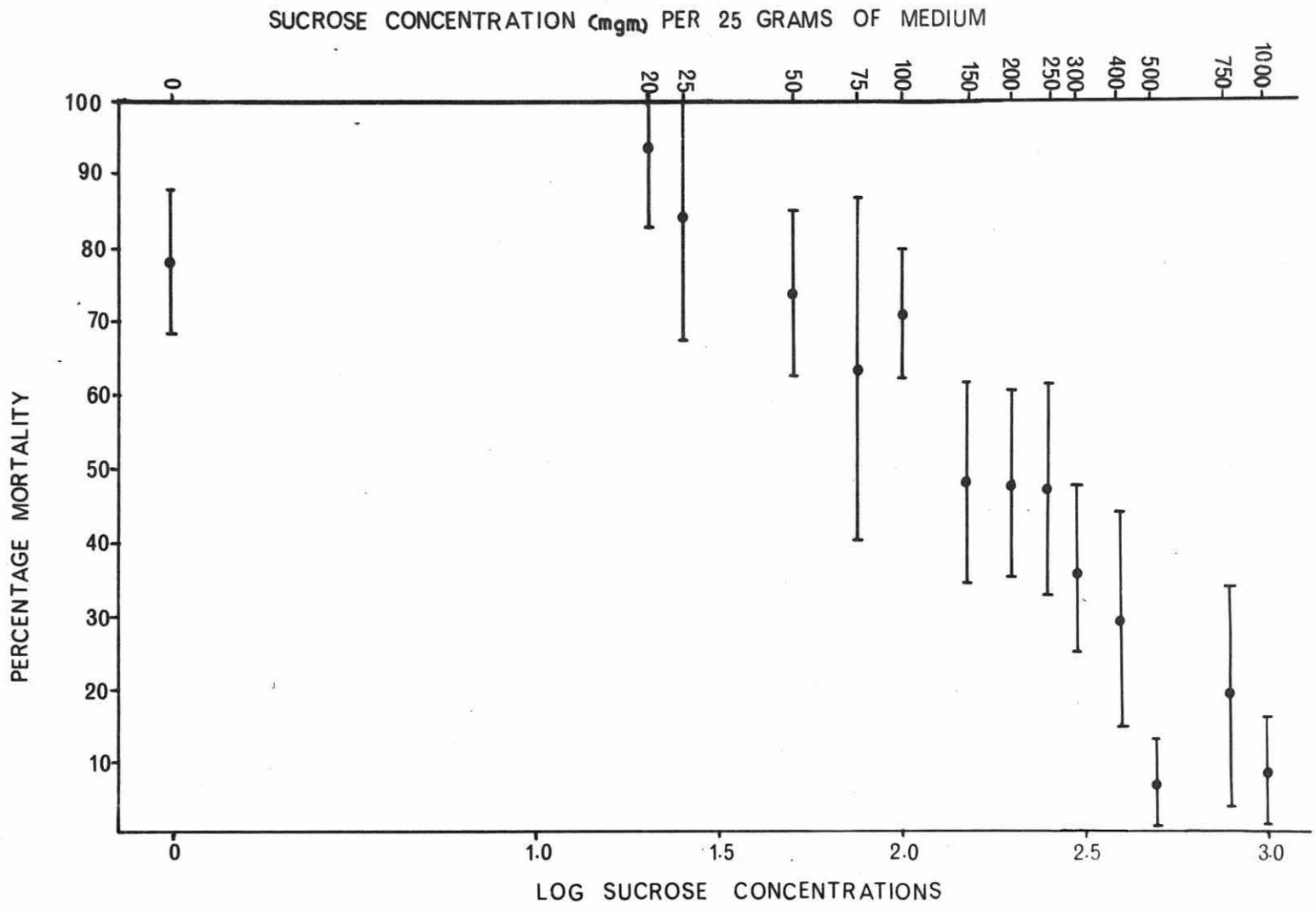
An investigation was made of the optimum quantity of sucrose required in the basal diet. A range of 14 concentrations was tried between 0 to 1 gram per 25 grams of medium (nos. 16, 25, 26, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37 and 38 in Table 10 in Chapter III). Third instar caterpillars were fed on these diets for four days and then the percentage mortality on each diet was recorded. The results are represented in Fig. 4 with confidence limits plotted at the 5 per cent level.

The results showed that 500 milligrams sucrose per 25 grams of medium was the optimum quantity. Concentrations higher than 500 milligrams did not decrease the mortality while the lower ones showed a significantly higher mortality, the values increasing steadily with a decrease in sucrose concentration. At concentrations of 25 milligrams per 25 grams, or less, the mortality was very high.

c) Phagostimulant action of sugars

To ascertain the phagostimulant action of sugars third instar caterpillars were allowed to feed on very similar diets to those used to investigate the relationship between dietary sugars and

Fig. 4 Showing larval mortality on diets containing sucrose at different concentrations.



mortality. The only difference was the addition of 3 grams of cellulose powder per 25 grams of the medium in each case as this made the medium more suitable physically (see later). Ten caterpillars were used in each case and after two hours feeding they were dissected to record the colour score in the gut according to the method described in Chapter II. The maximum possible score was 30 and in Table 11 are given the scores on each diet.

It is clear from Table 11 that diets without any sugar and those with fructose, glucose + fructose, lactose, galactose or starch failed to provoke any feeding reaction in the caterpillars during a period of two hours. A little response was shown to diets containing melibiose, trehalose, maltose, glucose, glycogen, raffinose, melezitose and cellobiose, whereas, the highest amount was eaten from the diet with sucrose in it. Thus, the role of sucrose as a phagostimulant is quite obvious. Furthermore, although melibiose and the glucose + fructose mixture were almost as important as sucrose for survival of the caterpillars, they failed to give any indication of their action as phagostimulants.

Further experiments were conducted to investigate whether, or not, sucrose was capable of maintaining sustained feeding. Only two diets were tried in this case, one with sucrose and the other without. Ten caterpillars were used for each reading and the colour scores were recorded in the same way after 2, 4, 6 and 12

TABLE 11

Colour score on diets with different sugars

Dietary sugar	Colour score
No sugar	0
Fructose	0
Glucose + fructose	0
Lactose	0
Galactose	0
Starch	0
Melibiose	0.5
Trehalose	1
Maltose	2
Glucose	2
Glycogen	3
Raffinose	3.5
Melezitose	4
Cellobiose	4.5
Sucrose	13.5

hours of feeding on a particular diet. It was observed that feeding started sooner on the sucrose containing diet and at the end of 12 hours the overall colour score was 98 per cent of the maximum possible. This suggests that the caterpillars have been eating the food almost continuously. On the other hand those feeding on the diet lacking sucrose showed only about 58 per cent of the maximum possible score. These results confirmed the phagostimulant action of sucrose in the diet of the caterpillars of Pieris brassicae L. The significance of these findings will be discussed further in Chapter VI.

2. Sinigrin.

a) Qualitative requirements

Sinigrin is a mustard oil glucoside which is said to be the chemical responsible for stimulating the feeding of the caterpillars of Pieris brassicae L. and the published work on this topic has been reviewed in Chapter I. The present experiments were designed to investigate its effect on the caterpillars feeding on artificial diets. Two diets were prepared, one with sinigrin and the other without it (nos. 2 and 4, Table 10, Chapter III). Thirty, third instar caterpillars were fed on each diet for four days and observations on mortality, moults and weights were made. On both diets the mortality was nil and in each case 14 of the larvae moulted to the fourth instar. The final weights were:-

On the diet containing sinigrin 15.27 ± 2.09 mgm.

On the diet without sinigrin 15.04 ± 1.67 mgm.

These results showed no significant differences and the initial weights were the same in each case, therefore, the addition of sinigrin had no effect whatsoever on the growth and development of the caterpillars. This lack of response to sinigrin on the part of the caterpillars could be due to the presence of leaf powder in the diet which might contain sufficient material to make the additional sinigrin ineffective. However, further experiments were continued with different quantities of sinigrin and these are reported below.

b) Quantitative requirements

Media were made up containing sinigrin at concentrations of 0, 2.5 and 25 milligrams per 25 grams (nos. 16, 51 and 52, Table 10, Chapter III). Fifteen third instar caterpillars were fed on each diet for four days and then their weights, moults and deaths were recorded. The results are summarised in Table 12.

The results recorded in Table 12 did not show any significant difference in weight, deaths or moults of the caterpillars. However, to confirm these results another experiment was designed in which only the lower concentration of sinigrin was compared with the 'basal' diet. All of the 25 caterpillars fed on each diet lived

TABLE 12

Effect of sinigrin on the growth and development of third instar caterpillars

	Diet with no sinigrin	2.5 mgm. sinigrin	25 mgm. sinigrin
No. of caterpillars used	15	15	15
No. of caterpillars dead	1	0	0
No. of caterpillars moulted	5	10	8
Percentage moults	33.3	66.6	53.3
Average weight per caterpillar (mgm.) *	14.9 ± 1.9	18.6 ± 5.6	18.3 ± 4.8

*The initial weights were uniform and these figures therefore indicate the relative weight gains of the larvae.

but none moulted. The mean weights at the end of four days were 20.1 ± 1.6 milligrams on sinigrin and 20.2 ± 1.8 on that without sinigrin.

It suggested therefore, that the caterpillars neither responded to concentration changes of sinigrin in the diet, nor showed any adverse effects arising from the omission of sinigrin since there was no significant difference in weights, percentage moults or mortality of the caterpillars.

B. PROTEINS AND AMINO ACIDS

1. Casein.

a) Qualitative requirements

Casein is often used as the protein source in the diets of insects and in the present studies 'fat and vitamin free' casein has been used throughout.

(i) First instar caterpillars: The caterpillars were put on media with and without casein (nos. 14 and 16, Table 9, Chapter III). Newly hatched caterpillars were allowed to feed on the media for four days and then weighed individually. Observations on mortality and moulting were also recorded. The results are summarised in Table 13.

These results on the mortality and moulting on the two diets were tested in each case by means of a twofold frequency test. This showed no significant difference in the mortality on the diets

TABLE 13

Development of first instar caterpillars on casein diet

	Diet with casein	Diet without casein
No. of caterpillars used	240	180
No. of caterpillars dead after 4 days	71	57
Mortality percentage	29.6	31.7
Total weight of the living caterpillars (mgm.)	142.5	86.3
Average weight of one caterpillar (mgm.)	0.84 ± 0.07	0.70 ± 0.06
No. of caterpillars moulted	110	53
Percentage moults	65.1	43.1

but the value of X^2 was very highly significant in the case of the moults ($P < 0.001$). Furthermore the omission of casein from the diet resulted in larvae with a significantly lower mean weight. The addition of casein therefore, had a positive effect on growth and development of the first instar caterpillars.

(ii) Third instar caterpillars: Newly moulted caterpillars of the third instar were weighed and put on the media (nos. 1 and 4, Table 10, Chapter III). They were allowed to feed for four days and then weighed individually. Moulting and mortality was also recorded and the results are summarised in Table 14. The initial weights were uniform and therefore, once again, the final weights recorded in the table provide a quantitative indication of the weight gain on these media.

The results recorded in Table 14 did not reveal any significant difference in the final weights of the caterpillars, yet moulting was significantly higher ($P < 0.01$) in the case of the caterpillars reared on the casein diet.

(iii) Fifth instar caterpillars: Ten caterpillars of the fifth instar were fed on similar media to those used for the first and third instar caterpillars but with the addition of cellulose powder. The caterpillars were starved before being placed on the media and then they were allowed to feed for 24 hours. The results are reported in Table 15.

TABLE 14

Development of third instar caterpillars on casein diet

	Diet with casein	Diet without casein
No. of caterpillars used	30	30
No. of caterpillars dead after 4 days	0	0
Total weight of the living caterpillars (mgm.)	563.9	464.4
Average weight per caterpillar (mgm.)	18.8 ± 2.0	15.5 ± 1.9
No. of caterpillars moulted	22	10
Percentage moults	73.3	33.3

TABLE 15

Development of fifth instar caterpillars on casein diet

Caterpillar No.	Larval weights on diet with casein: Initial calculated dry wt. (mgm.)	Final dry wt. (mgm.)	Increase in dry wt. (mgm.)	Larval weights on diet without casein: Initial calculated dry wt. (mgm.)	Final dry wt. (mgm.)	Increase in dry wt. (mgm.)
1	12.30	15.25	2.95	8.92	11.30	2.38
2	16.12	20.00	3.88	13.95	12.80	-
3	11.46	16.25	4.79	10.66	9.30	-
4	12.84	17.95	5.11	13.57	13.00	-
5	9.98	12.35	2.37	12.44	11.60	-
6	11.33	9.80	-	15.46	18.45	2.99
7	11.58	14.65	3.07	17.86	15.00	-
8	13.63	10.80	-	11.78	10.75	-
9	12.20	14.50	2.30	13.18	10.90	-
10	11.99	13.50	1.51	8.57	8.55	-

It is clear from Table 15 that the caterpillars on the casein diet have put on weight while those on that without casein have lost weight although all the caterpillars have been eating food excepting the caterpillar no. 6 on the casein diet and no. 8 on the other which did not produce any faecal pellets.

From all these results it is clear that the caterpillars of the cabbage white butterfly required a dietary source of protein for their growth and development and that the cabbage leaf powder was not sufficient to fulfill the protein requirements.

b) Quantitative requirements

A group of thirty larvae were put on to each of two different concentrations of casein in the diet (nos. 3 and 4, Table 10, Chapter III). Newly moulted third instar larvae were put into five tubes on each diet with six larvae per tube. They were allowed to feed for four days and then their weights, moults and deaths were recorded and the results are summarised in Table 16. The initial weights of the larvae were also recorded and these were almost constant, therefore the final weight is proportional to the weight gain.

The observations recorded in the table do not show any significant difference in the weights of the caterpillars or the percentage moults. Thus, doubling the amount of casein, apparently, had no effect, and therefore, the 0.875 gram of casein per 25 grams of medium was selected as being quite satisfactory for the development and growth of the larvae.

TABLE 16

Effect of different concentrations of casein on third instar larvae

	Diet with 1.75 gm. casein	Diet with 0.87 gm. casein
No. of caterpillars used	30	30
Average weight per caterpillar (mgm.)	14.4 ± 1.3	15.0 ± 1.7
Moults	12	14
%age moults	40	46.6

2. Cystine.

a) Qualitative requirements

Cystine is not included in the list of the ten essential amino acids, although it has been used as a supplement in the diets of a few insects. Thus, in the case of Aedes aegypti (L.) its omission from the diet inhibited pupation (Golberg and DeMeillon 1948, Singh and Brown 1957). Similarly, Kasting and McGinnis (1962) determined the amino acid requirements of Agrotis orthogonia Morr. by injecting glucose-U-C^{14} into fifth instar caterpillars, and concluded that cystine was nutritionally essential in that insect. Lack of cystine in the diets had a deleterious effect on moulting in Blatella germanica (L.) (House 1949b) and Phaenicia (Lucilia) sericata (Meig.) (Michelbacher et al.1932), also on the growth of larval Calliphora vicina (Meig.) (Sedee 1956).

With these findings in view, observations were made on the effect of cystine on Pieris brassicae L. caterpillars. For this purpose cystine was included in the diets of the third instar caterpillars in the first instance (no. 4 and 6, Table 10, Chapter III). Thirty newly moulted third instar caterpillars, each of similar weight, were released on each medium. The media were enclosed in twelve tubes, each containing five caterpillars. The observations on development, mortality and moulting were recorded after four days and these are summarised in Table 17.

TABLE 17

Effect of cystine on third instar caterpillars

	Diet with cystine	Diet without cystine
No. of caterpillars used	30	30
Deaths	0	0
Average final weight per caterpillar (mgm.)	20.4 ± 2.6	11.6 ± 1.1
Moults	19	1
Percentage moults	63.3	3.3

The results in Table 17 clearly indicate the superiority of the cystine diet, the weights of the caterpillars and the percentage moults being significantly higher on this diet.

In order to examine the possible effect of cystine on pupation and emergence of adults, similar diets were fed to fifth instar caterpillars. Twenty newly moulted fifth instar caterpillars were put on each diet i.e. that with cystine and that without cystine. They were allowed to grow on the diets until pupation. The diet was changed when necessary i.e. either when it had become too dry or at the first appearance of fungal contamination. The results are recorded in Table 18.

The X^2 value calculated for the comparison between successful pupations on the two diets is slightly less than that required to indicate significance at the 5 per cent level. However, once the pupal period was completed the number of emergence failures on the diet lacking cystine was significantly higher than on the diet containing this amino acid.

The appearance of these emergence failures is shown in Plate 3 and a discussion of this phenomenon is reserved for a later chapter.

b) Quantitative requirements

It has been shown above that the addition of cystine improved the diet, and this was investigated quantitatively. For this purpose five concentrations of cystine were tried i.e. 25, 50, 100, 200 and 400 milligrams per 25 grams of medium (nos. 6, 8, 9, 16 and

TABLE 18

Effect of cystine on pupation and emergence of adults

		Diet with cystine	Diet without cystine
Larval mortality	No.	8	8
	%age	40	40
Successful pupation	No.	11	6
	%age	91.6	50
Partial pupation	No.	1	6
	%age	8.3	50
Perfect emergence	No.	10	1
	%age	90.9	16.6

PLATE 3

Showing:

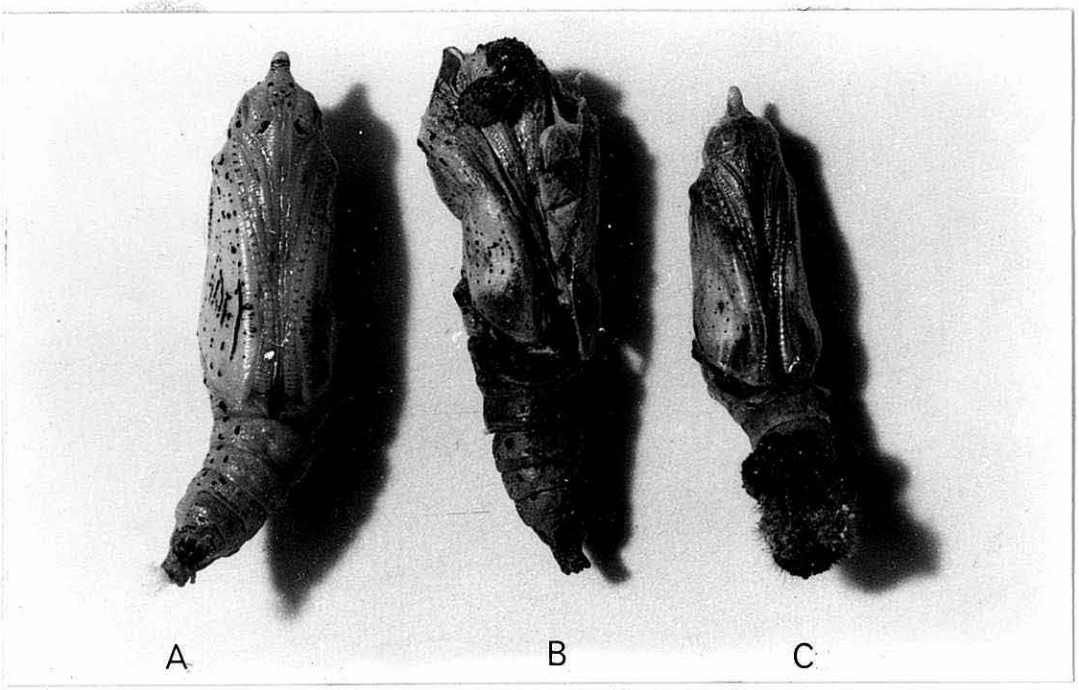
A Normal pupa

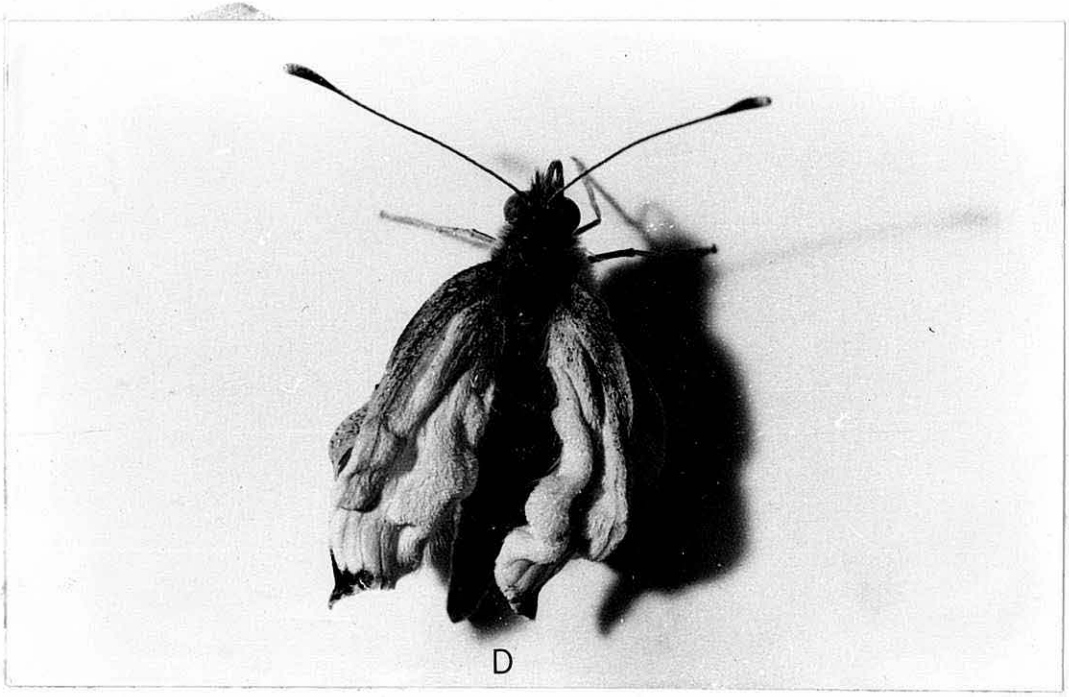
B Emergence failure of adult from an apparently normal pupa

C Partial pupation

D Adult emerged with crippled wings

PLATE 3





17, Table 10, Chapter III). The experiment was conducted in the usual way with thirty larvae of the third instar, placed on each diet for four days. The observations recorded on the final weights of the larvae, their mortality and moults are summarised in Table 19.

The results recorded in Table 19 show that there is no significant difference between the weights of the caterpillars on all these media and all the caterpillars grew equally satisfactorily on all the diets, except for the moults. The numbers of larvae moulting on the 50, 100, 200 and 400 milligrams per 25 grams concentrations of cystine were not significantly different from one another. However, the value of X^2 for the numbers of larvae moulting was significantly higher on these media as compared to that with 25 milligrams of cystine.

This, once again confirmed the connection of cystine with the process of moulting and 50 milligrams of cystine in 25 grams of medium was found to be a perfectly adequate concentration as the higher concentrations did not bring any further improvement in weights or moults.

C. VITAMINS

1. Carotene.

a) Qualitative requirements

It was as recently as 1957 that Dadd first showed the dietary importance of β-carotene (a precursor of vitamin A) for adequate growth and pigmentation in the desert locust, a phytophagous insect.

TABLE 19

Development of third instar larvae on different concentrations of cystine

	Amount of cystine per 25 gm. of medium (mgm.)				
	25	50	100	200	400
No. of larvae used	30	30	30	30	30
Deaths	0	0	0	0	0
Average weight per larva (mgm.)	17.7 ± 2.1	20.4 ± 1.5	20.9 ± 2.4	19.2 ± 2.9	20.9 ± 2.2
Moults	11	21	19	16	21
%age moults	36.7	70.0	63.3	53.3	70.0

In the present studies, β-carotene was included to examine its effect on the caterpillars and diets with and without β-carotene were prepared (no. 6 and 11, Table 10, Chapter III). Thirty caterpillars of the third instar were allowed to feed on each of these diets for four days and then their weights, and the numbers of deaths and moults recorded. The data are summarised in Table 20.

The results recorded in Table 20 show that neither the final weights nor the number of moulted of the caterpillars were significantly affected by the inclusion of β-carotene in the diet. However, there was a mortality of 13.3 per cent on the diet containing β-carotene and no deaths when the carotene was omitted. Thus addition of β-carotene to the diet did not bring about any significant improvement.

b) Quantitative requirements

Two quantities of β-carotene viz. 10 milligrams and 25 milligrams per 25 grams of medium were tried and the effect of these concentrations was compared with that of the 'normal' diet without β-carotene (nos. 13, 14, and 16, Table 10, Chapter III). Thirty, third instar caterpillars were allowed to feed on each of these diets for four days and then the observations were recorded on the different aspects which are summarised in Table 21.

The results recorded in Table 21 reveal that the survival was equally good on all the diets. There was no significant difference in the larval weights on the two β-carotene diets but these were

TABLE 20

Effect of β-carotene on third instar caterpillars

	Diet with β-carotene	Diet without β-carotene
No. of caterpillars dead	4	0
%age mortality	13.3	0
Average weight per caterpillar (mgm.)	16.5 ± 9.3	19.2 ± 2.9
No. of caterpillars moulted	7	16
%age moults	26.9	53.3

TABLE 21

Effect of different quantities of β-carotene on the development of third instar caterpillars

	Normal diet	Normal + β-carotene 10 mgm.	Normal + β-carotene 25 mgm.
No. of caterpillars dead	0	1	1
%age mortality	0	3.3	3.3
Average weight per larva (mgm.)	20.4 ± 1.5	14.2 ± 2.0	16.6 ± 2.0
No. of larvae moulted	21	5	6
%age moults	70	17.2	20.7

significantly lower than those on the normal diet. Similarly the number of larvae which had moulted was significantly higher on the normal diet than on the two β-carotene diets but there was no difference in the proportion of moulted larvae between the two β-carotene diets. It indicates that the two quantities of β-carotene tried did not have any effect on the development of caterpillars; rather the growth was retarded with the addition of -carotene at the two concentrations tried.

2. B vitamins.

The mixture of B vitamins was prepared according to McMorran (1964) and used in various diets during the course of the investigations. The constituents of this mixture are given in Chapter III.

a) First instar caterpillars

Vitamin B mixture was added to the diet of the first instar caterpillars (no. 16 and 17, Table 9, Chapter III). Sixty newly hatched caterpillars were put on each diet for four days and then they were weighed individually and observations on mortality and the number of larvae which had moulted were recorded. Thedata are summarised in Table 22.

The observations recorded in Table 22 did not show any significant difference in the weights of the caterpillars, the number of larvae which had moulted or the mortality on the diets. Hence, in the presence of leaf powder, at least, the addition of B vitamins appears to be unnecessary.

TABLE 22

Effect of B vitamins on the growth and development of first instar caterpillars

	Diet with B vitamins	Diet without B vitamins
No. of caterpillars dead	2	0
%age mortality	3.33	0
Average weight per caterpillar (mgm.)	0.80 ± 0.09	0.99 ± 0.13
No. of caterpillars moulted	47	52
%age moults	81.0	86.7

b) Third instar caterpillars

The mixture of B vitamins was also tried in the diet of the third instar caterpillars. The newly moulted caterpillars were allowed to feed on two diets of similar composition to those of the previous experiment (no. 16 and 27, Table 10, Chapter III) for four days and then their weights were recorded individually and the number of larvae which had moulted and the deaths noted. The data collected is summarised in Table 23.

The results recorded in Table 23 did not indicate any difference in the final weights of the larvae, the percentage moults or deaths on the diets in any of the experiments excepting Experiment no. III, where the mean weight per larva was significantly higher in the case of those feeding on the diet containing a mixture of B vitamins. However, the pooled results of all three experiments did not show any significant difference in the recorded figures. Thus, it can be said that the addition of B vitamins to the diet did not improve the medium to give a significant difference in development of the third instar larvae.

3. Ascorbic acid (Vitamin C).

Ascorbic acid was incorporated in the diet for third instar caterpillars to examine the effect on the development of the larvae. Thirty newly moulted third instar caterpillars were put on each diet i.e. one containing the ascorbic acid and the other without it (no. 6

TABLE 23

Development of third instar caterpillars on diets with B vitamins

Experiment	Diet	No. of larvae used	No. of larvae dead	%age mortality	No. of larvae moulted	%age moults	Average weight of each larva (mgm.)
I	Vit.	30	1	3.3	22	75.9	20.6 ± 2.2
	Nor.	30	0	0	22	73.3	18.8 ± 2.0
II	Vit.	15	2	13.3	13	100	19.5 ± 3.6
	Nor.	15	0	0	14	93.3	24.7 ± 6.5
III	Vit.	25	0	0	6	24	24.0 ± 1.6
	Nor.	25	0	0	0	0	20.2 ± 1.7
Pooled results	Vit.	70	3	4.3	41	61.2	21.7 ± 1.3
	Nor.	70	0	0	36	51.4	20.6 ± 1.7

Vit. - diet containing B vitamins

Nor. - 'normal' diet

and 10, Table 10, Chapter III). The caterpillars were taken off the diets after four days and weighed individually. Observations on the number of caterpillars moulted and those dead were also recorded. The results are summarised in Table 24.

The results recorded in Table 24 did not reveal any significant difference in the larval weights and percentage of moults on either diet. However, the mortality was 26.7 per cent on the ascorbic acid diet, while it was nil on the other. Thus, the addition of ascorbic acid had a slightly adverse effect in terms of mortality.

4. Ascorbic acid plus β-carotene.

An experiment was conducted to examine the combined effect of vitamins A and C on the development of third instar caterpillars and the results were compared with those from a diet without these vitamins (no. 6 and 12, Table 10, Chapter III). Observations recorded after four days of feeding are summarised in Table 25.

The results in Table 25 show that the combination of ascorbic acid and β-carotene had a deleterious effect as it gave 26.7 per cent mortality and only 16.7 per cent moults, whereas, these values were nil and 58.3 per cent respectively on the other diet. The larval weights on the vitamin containing diet were also significantly lower than those on the diet without ascorbic acid and β-carotene.

TABLE 24

Effect of ascorbic acid on the development of third instar caterpillars

	Diet with ascorbic acid	Diet without ascorbic acid
No. of caterpillars dead	8	0
%age mortality	26.7	0
Average weight per larva (mgm.)	20.4 ± 3.4	19.2 ± 2.9
No. of larvae moulted	15	16
%age moults	68.2	53.3

TABLE 25

Effect of β-carotene plus ascorbic acid on the development of third instar caterpillars

	Diet without β-carotene and vit. C	Diet with β-carotene and vit. C
No. of caterpillars used	60	30
No. of caterpillars dead	0	8
%age mortality	0	26.7
Average weight per larva (mgm.)	19.8 ± 2.0	15.0 ± 1.9
No. of larvae moulted	35	5
%age moults	58.3	16.7

D. STEROLS

It has been shown in Chapter I that the inclusion of cholesterol in synthetic diets for insects is a common practice, although in some cases other sterols have proved better than cholesterol or at least can replace dietary cholesterol. In the present investigations the effect produced by two dietary sterols, namely cholesterol and the naturally occurring plant sterol β-sitosterol, was studied.

Two concentrations of each sterol i.e. 500 and 50 milligrams per 25 grams of medium (nos. 15, 44, 53 and 54, Table 10, Chapter III) were tried with third instar caterpillars and these were compared with the 'normal' diet. Cholesterol was also used at a concentration of 125 milligrams per 25 grams of medium in the diet of fourth instar caterpillars. The observations recorded on larval weights, number moulted and mortality are given in Table 26.

It is clear from the results that larval weights and moulting were significantly lower on the diets containing 500 milligrams of cholesterol or sitosterol than those on the normal diet. However, there was no significant difference in weights, percentage moults or mortality between larvae reared on normal diet and those reared on the diet containing 50 milligrams of either sterol.

In the case of the 125 milligrams concentration of cholesterol there was no significant difference in the mean larval weights of the fourth instar caterpillars on either diet (i.e. with or without

TABLE 26

Effect of dietary sterols on growth and development of caterpillars

Experiment No.	Diet with sterol	Quantity of sterol used per 25 gm. of medium (mgm.)	Mean initial larval weights (mgm.)	Mean final larval weights (mgm.)	No. of caterpillars used	No. of caterpillars dead	Percentage mortality	No. of larvae moulted	Percentage moults
Third instar									
I	Cholesterol	500	5.8 ± 0.2	9.8 ± 1.4	30	2	6.6	0	0
	Normal	-	5.9 ± 0.2	20.4 ± 1.5	30	0	0	21	70
II	Sitosterol	500	5.5 ± 0.4	9.3 ± 1.4	25	2	8	0	0
	Normal	-	6.0 ± 0.3	28.3 ± 5.7	25	2	8	20	87
III	Cholesterol	50	10.0 ± 1.1	20.0 ± 1.4	25	3	12	12	54.5
	Sitosterol	50	9.8 ± 1.3	22.9 ± 3.3	25	0	0	13	52.0
	Normal	-	8.9 ± 1.1	21.2 ± 2.0	25	0	0	9	36.0
Fourth instar									
IV	Cholesterol	125	25.4 ± 1.0	38.6 ± 6.3	25	13	52	0	0
	Normal	-	25.4 ± 1.2	38.9 ± 4.3	25	3	12	0	0

cholesterol) but the mortality was significantly higher on the cholesterol containing diet.

Thus it can be concluded that 500 milligrams of cholesterol or β-sitosterol in the diet adversely affected the growth and development of the caterpillars, whereas, concentrations of 50 milligrams per 25 grams of medium had no observed effect at all. The concentration of 125 milligrams did not affect the growth but had an adverse effect shown in terms of a high mortality.

E. MINERALS

Only one salt mixture namely 'Osbourne and Mendel salt mixture' was included in the diet in the concentration of 250 milligrams per 25 grams of medium and its effect was compared with that of 'normal' diet (no. 16 and 21, Table 10, Chapter III). Twenty five third instar caterpillars were fed on each diet for four days and then their weights, percentage moults and deaths were recorded. The results are summarised in Table 27.

The results recorded in Table 27 do not show any significant difference in mean larval weights, percentage moults or mortality on either diet. Hence, the addition of salt does not seem to have any effect on the growth and development of third instar larvae.

TABLE 27

Effect of salt mixture on growth and development of third instar caterpillars

	Diet	
	Normal	Normal + salt mixture
No. of larvae dead	0	1
No. of larvae moulted	9	10
Percentage moults	36	41.6
Mean initial larval weights (mgm.)	8.9 ± 1.1	8.8 ± 1.1
Mean final larval weights (mgm.)	21.2 ± 2.0	18.9 ± 2.4

F. OTHER SUBSTANCES

1. Agar.

Agar has been used in the diets of many insects and very recently it has been shown to contribute towards the physical structure of the diet of Phaedon cochleariae Fab. where the amount of food eaten increased with the concentration of agar in the diet (Tanton 1965). In the present studies nutrient agar was added to produce a diet of a consistency acceptable to the caterpillars. It was used, in the first instance, in the diet of first instar caterpillars, where the concentration of agar was varied while that of the cabbage leaf powder remained constant, this diet consisting only of these two substances and water. Five concentrations of agar were tried and the composition of all the five diets is given at nos. 1 to 5 in Table 9 in Chapter III. The caterpillars were kept individually in separate tubes and there were five tubes containing each diet. The larvae were weighed individually before and after feeding on the experimental diets for three days. The results are presented below

Agar concentration (%age)	1	1.5	2.0	2.5	3.0
Mean weight gain (mgm.)	2.20	2.20	3.17	2.22	2.00

Overall mean starting weight 2.53 mgm.

These observations did not reveal any significant difference in larval weight gains or mortality. Therefore, any of these

concentrations of agar could be adopted for inclusion in further experiments but it was noticed that higher concentrations tend to make the medium sticky and somewhat unmanageable. For practical convenience, therefore, a concentration of 2 per cent was adopted for all subsequent experiments.

2. Cellulose.

Cellulose is added to make the 'bulk' and it is not an essential nutrient. In some of the early experiments filter paper was used to provide a feeding surface, or in other words the medium was spread over the filter paper to give it, very approximately, the structure of a leaf (nos. 4 and 5, Table 10, Chapter III). The composition of the diet was the same in both the cases, but whereas in no. 5 the medium was spread over a filter paper, in no. 4 it was simply poured into tubes. Thirty, third instar, caterpillars were allowed to feed for four days in each case and then their weights, percentage moults and deaths were recorded. The results are summarised in Table 28.

The results recorded in table reveal that the larval weights were significantly lower on the medium spread on filter paper and there was no significant difference in the value of X^2 calculated for the mortalities or the percentage moults on either medium. It is evident, therefore, that filter paper did not help to produce any improvement, but probably made it difficult for the caterpillars to ingest the food.

TABLE 28

Development of third instar caterpillars on medium spread over filter paper

	Medium spread over filter paper	Medium alone
No. of caterpillars used	30	30
No. of caterpillars dead	0	0
No. of caterpillars moulted	20	14
%age moults	66.7	46.7
Average weight per larva (mgm.)	11.4 ± 1.2	15.0 ± 1.7

Further experiments on the effect of inclusion of cellulose powder in the diet on feeding and food consumption of the caterpillars were conducted and the results are reported later in Chapter V.

3. Cabbage leaf powder.

a) First instar caterpillars

After the failures with the purely synthetic diet (Chapter III) the cabbage leaf powder was included and initial observations were made with first instar caterpillars. A pilot experiment was carried out using only leaf powder, sucrose and agar (nos. 11 to 15, Table 9, Chapter III) in the media and the results suggested that the caterpillars would survive reasonably well on the diet when leaf powder concentration was as little as 8 per cent.

Having established that the caterpillars would not thrive on the synthetic diet lacking leaf powder it became equally important to be certain that development was sub-optimal on a diet of leaf powder lacking supplementary synthetic nutrients. Accordingly, the development of caterpillars on the 8 per cent leaf powder-agar-sucrose diet was compared with that on cabbage. The results are shown in Table 29.

These results suggest that although the majority of larvae on the agar diet did in fact survive for a week or more, there was very little feeding taking place. After the first two days or so

TABLE 29

Development of caterpillars on 8 per cent leaf powder-agar-sucrose diet

Day of feeding	No. of surviving caterpillars on		Average weight per larva (mgm.)		Percentage mortality	
	Med.	Cabb.	Medium	Cabbage	Med.	Cabb.
1st	40	40	0.3	0.2	0	0
2nd	39	37	0.4	0.5	2.5	7.5
3rd	39	37	0.6	1.0	2.5	7.5
4th	39	37	0.7	1.4	2.5	7.5
5th	36	35	0.9	3.8 ± 0.3	10.0	12.3
6th	33	35	0.9	4.8 ± 0.4	17.5	12.3
7th	31	35	1.1 ± 0.3	10.6 ± 1.4	22.5	12.3
8th	29	35	1.1 ± 0.3	20.5 ± 2.3	27.5	12.3
9th	29	35	1.0 ± 0.2	30.0 ± 3.7	27.5	12.3
10th	29	35	0.9 ± 0.2	68.8 ± 10.2	27.5	12.3
11th	26	35	0.9 ± 0.2	98.1 ± 10.2	35.0	12.3

the mean weight remained constant.

b) Third instar caterpillars

An experiment was then conducted to determine the optimum amount of leaf powder required to supplement the normal synthetic diet (nos. 7, 16, 18, 19 and 20, Table 10, Chapter III). Third instar larvae were used in batches of 30 individuals and observations recorded after four days' feeding are given in Table 30.

The results in Table 30 indicate that the diet containing only one per cent leaf powder was totally inadequate, giving 100 per cent mortality; 2 per cent was similar with 90 per cent mortality. On the 4 per cent leaf powder diet, again the mortality was very high (66.6 per cent) and the larval weights significantly lower than those on higher concentrations. There was no significant difference between the larval weights or the values of X^2 for moulting or mortality at either of the higher concentrations (8 and 16 per cent) nor were these significantly different from the corresponding values on cabbage leaves, with the exception of the moults which were significantly higher on cabbage. The concentration of 8 per cent was taken as the most suitable minimum for inclusion in the diets for the caterpillars.

The moulting in the case of larvae feeding on cabbage occurred one day earlier than in the case of those on artificial diets. The weights were recorded immediately after moulting and therefore the results in Table 30 show that there was no difference in the weights

TABLE 30

Development of third instar caterpillars on different leaf powder concentrations

Leaf powder concentration in the medium	No. of larvae dead	%age mortality	No. of larvae moulted	%age moults	Average larval weights (mgm.)
1%	30	100	0	0	0
2%	27	90	2	66.7	12.9
4%	20	66.7	10	100	15.1 ± 2.1
8%	0	0	19	63.3	20.4 ± 2.6
16%	0	0	26	86.7	25.4 ± 5.1
Cabbage*	0	0	30	100	23.6 ± 2.0

*Weight results recorded at time of moult (see text)

of the newly moulted fourth instar larvae whether reared on the artificial diet or on cabbage. However, the weights of the cabbage fed larvae in Table 30 were recorded one day earlier than the other weights.

4. Ethyl para-hydroxy benzoate.

This chemical was added to the diets merely as a fungicide and is of no nutritional value. The recommendations of McMorran (1964) were followed and 37.5 milligrams added per 25 grams of medium. No experiments were carried out on the effects, if any, of varying this quantity.

CHAPTER V

CONSUMPTION AND UTILISATION OF FOOD

Most of the techniques for quantitatively evaluating insect diets in terms of consumption and utilisation are of recent origin and much of the published work has been reviewed in Chapter I. Usually, this type of work has been carried out solely on natural foods, but the present investigation was undertaken to evaluate the semi-synthetic diet developed for the larvae of Pieris brassicae L., in comparison to the natural food of the insect and to try, if possible, to improve the diet.

A. CONSUMPTION AND UTILISATION ON THE SEMI-SYNTHETIC DIET

1. Normal medium compared with cabbage.

First of all the 'normal' medium was compared with the natural food, the experimental techniques being those described in Chapter II. Twenty-five fifth instar caterpillars were placed in batches of five on each diet for 24 hours and the amounts of food consumed and faeces produced were measured.

The results are recorded in Table 31 and they show that the food consumption, faecal production and larval weight gains were significantly higher on cabbage, but that the coefficient of utilisation on cabbage was significantly lower. The result obtained in this experiment for the coefficient of utilisation of cabbage is an

TABLE 31

Consumption of food on 'normal' medium and cabbage

Mean dry weight (mgm.)	Medium	Cabbage
Food consumed by batch of 5 larvae	75.9 ± 58.0	300.9 ± 60.0
Faeces produced by batch of 5 larvae	36.8 ± 27.3	222.3 ± 33.2
Food utilised by batch of 5 larvae	39.1 ± 30.8	78.6 ± 40.3
Calculated initial dry weight per larva	10.5 ± 0.9	10.5 ± 0.9
Final dry weight per larva	13.3 ± 2.5	26.0 ± 3.3
Coefficient of utilisation (%age)	50.9 ± 2.4	25.4 ± 9.0

anomalous one, since subsequent experimentation failed to confirm this low value of 25.4 ± 9.0 (see below) and no explanation can be offered.

It was clear from these results that the medium could only become strictly comparable to cabbage if the larvae could be made to ingest the medium in far larger amounts. The work of Dadd (1960e) suggested that the consumption of food may be controlled directly in some circumstances, by the amount of food that an insect was able to pass through the gut.

In other words the hypothesis was put forward that the low consumption figures for Pieris brassicae L. larvae fed on the artificial medium were, in part at least, the result of a purely physical failing in the constitution of the diet, perhaps a lack of 'bulk' or 'roughage'.

2. Effect of cellulose on consumption and utilisation of food.

An experiment was designed to test this hypothesis by adding 'roughage' in the form of nutritionally inert cellulose powder. Five grams of cellulose powder were added to the 'normal' medium and varying amounts of water were used to examine the effect of diets of different consistencies in comparison with cabbage. The following diets were prepared and 25 fifth instar caterpillars were allowed to feed on each diet, in batches of five, for 24 hours.

Diet A - Normal medium

Diet B - " " + 5 gm. cellulose

Diet C - " " + " " + 5 ml. water

Diet D - " " + " " + 15 ml. water

Diet E - " " + " " + 25 ml. water

Diet F - Cabbage leaves

The results, recorded in Table 32, show that the amounts of food consumed and faeces produced on diets A and D were not significantly different from one another and were significantly lower than those on diets B and C. These latter were not significantly different from one another in terms of these factors. In the case of diet E these values were extremely low, presumably because the diet was too moist. The small weight of faeces produced by each larva on diet E, in most cases scarcely differed from the equally small weight of food consumed. This diet was, therefore, quite unsuitable for the larvae and it is impossible to calculate a meaningful coefficient of utilisation. The coefficient of utilisation on diet A was significantly higher than those of diets B and C but was not significantly different from that of D.

Because of the discrepancy between the coefficient of utilisation recorded for this series of experiments and the low result recorded previously for cabbage, two confirmatory experiments were carried out with the sole purpose of comparing the cabbage

TABLE 32

Effect of cellulose on consumption and utilisation of food

	Diets					
	A	B	C	D	E	F
Food consumed*	128.4 ± 87.0	664.2 ± 214.1	485.2 ± 122.5	122.4 ± 85.5	15.2 ± 15.3	580.5 ± 47.8
Faeces produced*	72.5 ± 48.0	547.6 ± 177.7	400.6 ± 21.5	93.6 ± 70.9	16.6 ± 16.7	308.7 ± 16.8
Food utilised*	55.9 ± 41.4	116.6 ± 49.5	84.7 ± 15.9	28.8 ± 17.0	-	271.7 ± 33.1
Coefficient of utilisation (%age)	42.0 ± 7.9	17.6 ± 3.9	17.7 ± 2.8	24.4 ± 10.8	-	46.7 ± 2.1

*Dry weight in mgm. per 5 larvae

coefficient with that of the medium containing 5 grams of cellulose powder (Diet B above). The coefficients of utilisation were as follows and these were calculated from readings on 10 individual larvae in each case.

Experiment I	Medium	22.8 ± 3.4
	Cabbage	37.7 ± 11.5
Experiment II	Medium	17.3 ± 2.3
	Cabbage	43.7 ± 16.2

The diet with additional cellulose was quite comparable to cabbage in terms of the amount consumed by the larvae and the problem was, therefore, to regain the higher value for the medium coefficient of utilisation in order to make the two diets completely comparable. In an attempt to raise the medium coefficient without lowering the food consumption intermediate quantities of cellulose, between 0 and 5 grams were added to the diet.

The results of these experiments are given in Table 33. These show that any lowering of the cellulose content below 5 grams per 25 grams of medium resulted in a significant decrease in the amount of food consumed. On the other hand, the cellulose content had to be reduced to only 1 gram per 25 grams of medium before any significant rise in the coefficient of utilisation was obtained and even then, the value was significantly lower than that for cabbage. Thus, the attempt to reproduce the cabbage characteristics in the

TABLE 33

Effect of different concentrations of cellulose on consumption and utilisation of food

Cellulose added (gm.)	No. of individual larvae	Mean initial weight of larvae (mgm.)	Mean weight gain in larvae		Food consumed (mgm.)	Faeces produced (mgm.)	Coefficient of utilisation (%age)
			Total (mgm.)	%age			
0	9	15.0 ± 1.6	4.5 ± 2.3	28.6 ± 13.6	29.1 ± 7.1	15.6 ± 3.9	46.3 ± 1.7
1	11	19.2 ± 2.4	6.8 ± 2.9	31.7 ± 11.9	38.1 ± 9.9	26.0 ± 6.7	31.2 ± 5.0
2	15	19.5 ± 1.9	4.4 ± 2.2	20.3 ± 5.4	29.1 ± 6.6	22.6 ± 5.4	21.0 ± 4.6
3	16	26.7 ± 3.5	5.1 ± 1.9	20.9 ± 8.5	43.6 ± 14.5	39.7 ± 9.8	14.6 ± 4.0
4	22	18.3 ± 2.5	4.2 ± 1.5	23.1 ± 7.0	48.2 ± 8.3	37.1 ± 5.8	20.4 ± 5.0
5	10	20.2 ± 3.0	10.6 ± 3.7	49.6 ± 13.4	82.0 ± 23.3	65.0 ± 20.1	17.5 ± 2.0
4 + 3 gm. Potato starch	15	17.1 ± 1.7	4.9 ± 2.4	26.9 ± 12.0	44.5 ± 14.0	36.8 ± 11.2	16.3 ± 4.0
2 + 2 gm. Potato starch	9	13.6 ± 0.9	1.2 ± 0.3	9.1 ± 1.0	30.8 ± 5.5	21.6 ± 4.9	30.6 ± 6.6

All weights are dry weights

artificial diet was not successful, if cellulose was omitted from the medium the consumption was too low, and if 5 grams of cellulose per 25 grams of medium were added, the consumption discrepancy was corrected but the coefficient of utilisation falls to too low a value.

The effect of other polysaccharide materials was considered and potato starch was also included together with cellulose at two different concentrations. As is clear from the results in the table, the addition of starch had no effect and all the values on cellulose plus starch diets were not significantly different from those on the corresponding concentrations of cellulose alone.

The increased food consumption obtained by adding 5 grams cellulose to the diet resulted in a significant increase in both the absolute weight gain and the percentage weight gain of the larvae as compared to those of larvae reared on other cellulose containing diets or on the 'normal' diet.

The corresponding figures for parallel experiments carried out on cabbage are given in Table 34. There was good agreement between the batches of larvae for the various values obtained except in the case of experiment D. In this case the faecal production had a significantly higher value than that for B and as a consequence the coefficient of utilisation was significantly lower. Nevertheless, with this reservation in mind, it was felt that in view of the small numbers in each batch, pooled results for these observations on

TABLE 34

Food consumption and utilisation on cabbage

Experiment	No. of individual larvae	Mean initial weight of larvae (mgm.)	Mean weight gain in larvae		Food consumed (mgm.)	Faeces produced (mgm.)	Coefficient of utilisation (%age)
			Total (mgm.)	%age			
A	9	-	-	-	80.2 ± 19.9	49.4 ± 8.8	37.7 ± 11.5
B	10	19.8 ± 3.3	15.5 ± 4.3	77.7 ± 14.3	85.1 ± 25.8	30.6 ± 6.5	43.7 ± 21.6
C	9	24.2 ± 8.5	10.0 ± 3.2	41.1 ± 13.5	80.7 ± 22.4	36.8 ± 11.5	52.9 ± 11.5
D	9	22.8 ± 2.3	19.9 ± 4.3	87.2 ± 19.0	81.3 ± 19.4	56.3 ± 10.4	28.5 ± 10.6
Pooled		22.2 ± 2.7	15.7 ± 2.7	71.7 ± 11.1	81.8 ± 9.3	51.7 ± 8.9	40.5 ± 6.5

1) All weight values are in terms of dry weights

2) Experiment A was originally designed to discover the food consumption and calculate the coefficient of utilisation, and therefore larvae were not dried and their weights are not given in the table.

cabbage would be of value. These are given in the table and it will be seen that the pooled coefficient is not significantly different from the values obtained in previous experiments (Table 32).

Neither the absolute weight gain, nor the percentage weight gain on cabbage are significantly different from those obtained with the diet containing 5 grams of cellulose per 25 grams of medium.

In view of the difference in the coefficients of utilisation, these results suggest that a greater proportion of the utilised cellulose diet is used by the larvae for weight gain. This is best illustrated by the coefficients of growth and metabolism calculated from the same data that was used in compiling Table 33 and 34.

	Medium	Cabbage
Coefficient of growth (%age)	73.0 ± 9.0	41.5 ± 8.9
Coefficient of metabolism (%age)	26.9 ± 9.0	58.5 ± 8.9

(For the method of calculation see Chapter II)

These results will be further discussed later.

3. Effect of sucrose on the consumption and utilisation of food.

A few experiments were undertaken to examine the short term effects of the omission of the major essential nutrients on these various methods of assessing the performance of the animals on the diets. The results of excluding sucrose from the diet are shown in Table 35. Four grams of cellulose were added per 25 grams of

TABLE 35

Effect of sucrose on consumption and utilisation of food

Dry weights (mgm.)		Diet with sucrose	Diet without sucrose
Mean initial weight per larva		15.4 ± 2.3	14.4 ± 2.5
Mean weight gains	(Total	5.3 ± 4.1	3.5 ± 3.2
	(%age	31.7 ± 21.2	22.2 ± 13.8
Mean food consumed		59.6 ± 26.5	53.9 ± 29.3
Mean faeces produced		15.1 ± 7.9	9.5 ± 4.5
Coefficient of utilisation (%age)		24.2 ± 4.4	18.4 ± 4.4

medium in each case and ten larvae were fed on each diet for 24 hours. The results showed that the omission of sucrose from the diet had no real effect on larvae fed for this short period.

4. Effect of nitrogen compounds on the consumption and utilisation of food.

Having examined the effects of the carbohydrate constituents of the diet, a brief examination of the other major nutrients, the proteins and amino acids, was made. Casein or cystine, or both, were omitted from the diet and the effect on larval weight gains, food consumption and food utilisation was reocrded. All the diets contained 4 grams of added cellulose per 25 grams of medium and ten larvae were fed on each diet for 24 hours. The results are shown in Table 36.

The omission of casein, or both casein and cystine together, resulted in loss of weight and reduced the consumption of food without affecting the coefficient of utilisation. Omission of cystine alone had no effect on these factors and the values obtained were comparable to those on the diet containing the corresponding concentration of cellulose powder.

These results will be further discussed later.

5. Effect of leaf powder.

Attempts to measure in a similar way the influence of leaf powder all failed since on omitting this material from the diet food consumption ceased.

TABLE 36

Effect of nitrogen compounds on consumption and utilisation of food

Mean dry weights (mgm.)		Diet without cystine	Diet without casein	Diet without casein and cystine
Initial weight per larva		12.3 ± 1.2	11.8 ± 1.0	12.6 ± 2.0
Weight gain	Total	3.2 ± 1.1	Lost weight	
	%age	26.4 ± 8.3	Lost weight	
Weight loss	Total	-	1.4 ± 0.6	1.8 ± 0.8
	%age	-	11.9 ± 4.6	10.7 ± 4.6
Food consumed		39.1 ± 2.3	19.9 ± 5.5	18.7 ± 5.8
Faeces produced		30.7 ± 5.9	14.4 ± 3.0	15.7 ± 5.1
Coefficient of utilisation (%age)		25.9 ± 12.2	29.5 ± 7.3	29.7 ± 18.2

CHAPTER VI

DISCUSSION

In Chapter I it has been shown that studies on the nutrition of insects benefit considerably from the use of artificial diets, although this approach does, nevertheless, raise numerous problems and difficulties. Stress has been laid upon three major considerations. These being, the introduction of the substances necessary as feeding stimuli, the qualitative and quantitative incorporation of the essential nutrients into the diet, and the production of a medium with a physical texture which is acceptable to the insect.

There are two types of materials which act as feeding stimuli for insects, those substances which have a nutritional value in addition to their phagostimulant effect, and those substances whose effect appears to be solely on the sensory receptors of the insect. These latter are often termed 'token stimuli'. In the larvae of Pieris brassicae L. sucrose seems to act as a phagostimulant, since the results of the 'colour score' work showed that the larvae started eating earlier on the diet containing sucrose than on those without sucrose. This finding conforms to that of Ito (1960) in the case of the silkworm larvae.

The results which have been reported earlier, clearly indicated that cabbage white larvae offered artificial diets containing various sugars showed a marked reluctance to begin feeding on diets containing

any sugar other than sucrose. The substances showing weak phagostimulant effects all contained glucose molecules linked in various ways, these were the monosaccharide glucose itself, the disaccharides trehalose, maltose and cellobiose (each of which consists solely of glucose molecules), the trisaccharides melezitose and raffinose, and the glucose polysaccharide glycogen. With the exception of glucose, none of the monosaccharides apparently induced feeding during the 2 hour period of the experiment.

The conclusion that was drawn was that diets containing sucrose were initially far more acceptable than other diets to larvae transferred from cabbage. However, the curious fact emerged that these sucrose containing diets did not maintain their superiority over a 24 hour feeding period. Measurements of the total quantity of food consumed during a 24 hour period failed to reveal any significant difference between sucrose containing and sucrose-free diets. This suggests perhaps that on sucrose deficient diets the larvae failed to feed initially, but subsequently responded to hunger stimulation and in fact, their food intake caught up with that of sucrose fed larvae during the first 24 hours. Results after 12 hours show that on a sucrose-free diet the colour score has reached 60 per cent of the maximum possible level. The interrelations of phagostimulation, hunger stimulation and food intake will obviously merit further detailed investigation.

The prolongation of the feeding period on the sucrose-free diet to four days resulted in a very much higher mortality among larvae as compared to those feeding on a sucrose containing diet. This strongly suggests that the phagostimulant action of sucrose and its nutritional value are both of considerable importance to the insect. The larvae may have eaten the non-sucrose diet in order to satisfy a hunger stimulus over a long period of four days but it failed to adequately support life and maintain growth of the caterpillars.

In general, the results with other sugars in the present studies support those observations recorded on different insects by various authors (reviewed in Chapter I) but there do exist some differences. The most outstanding difference was the response to the glucose molecule which appears to be the preferred sugar of almost all the various species of insects investigated by other workers. Although, as shown above, the glucose molecules occur in combination in all sugars showing a phagostimulant effect on the larvae of Pieris brassicae L., sucrose was several times more effective than uncombined glucose. In this respect these results seem to be in agreement with those on the silkworm (Ito 1960). Fructose, which evoked no response from the larvae of the cabbage white butterfly, was shown by other authors to be almost as equally attractive as glucose for other insects. In this case the results

differed completely from those recorded by Ito (loc. cit) on the silkworm larvae where fructose was almost as good a phagostimulant as sucrose.

Differences between insects of widely differing taxonomic groups and feeding habits are not perhaps altogether surprising. Rather more striking are the differences shown between the cabbage white larvae and the silkworm, insects belonging to the same order (Lepidoptera) both of which are leaf feeders. A survey of this topic in leaf feeding insects, made in conjunction with analytical studies on the host plants, might prove of some interest.

As far as the token stimuli are concerned, Verschaffelt (1911) and Thorsteinson (1953) showed that larvae of Pieris brassicae L., Pieris rapae L. and Plutella maculipennis Curt. will feed on plants containing mustard oil glucosides. In the present investigation, however, sinigrin - one of the main mustard oil glucosides of cabbages - failed to evoke any detectable response in the larvae of Pieris brassicae L. and their performance on the diets containing sinigrin was not significantly different from that on diets lacking this glucoside. Variations in the sinigrin concentration in the diets also failed to give a positive response.

This lack of effect exhibited by sinigrin was unexpected because the published work leaves little doubt that this chemical does stimulate the sensory apparatus of Pieris larvae. The unpublished work of Hussain (1963) is of interest in this connection.

He allowed larvae to feed on agar containing various concentrations of sinigrin and counted the number of faecal pellets produced. He showed an increase in the faecal count proportional to the sinigrin concentration up to a level of 31.2 p.p.m. From 31.2 p.p.m. to 250 p.p.m. the faecal count remained constant and above 250 p.p.m. the count declined in proportion to the increasing concentration.

There is no doubt that the leaf powder preparation contains sinigrin, and the effect of adding sinigrin to diets containing leaf powder may therefore well be expected to be adverse. One might assume that the selection of the optimum quantity of leaf powder was in effect a selection of the optimum quantity of sinigrin. However, if this were the case the addition of 100 p.p.m. sinigrin to diets lacking leaf powder should have brought about some improvement. No ingestion occurred on the diet lacking leaf powder whether sinigrin or sucrose were added individually or as a mixture. This is contrary to the finding of Hussain (1963), who showed an increased faecal count when larvae were fed on agar to which sinigrin or sucrose was added, and a very greatly increased count when both were added together.

There are at least three possible explanations for the function of leaf powder in this connection. Firstly, it may provide yet another phagostimulant in the absence of which sucrose and sinigrin are non-functional. This is contrary to all previous published work on the effect of sinigrin and also contrary to the findings of

Hussain (loc.cit.) described above. Secondly, it may provide some additional substance which modifies the effect of sinigrin and/or sucrose although not itself a phagostimulant. In this connection it is interesting to note the contention of Verschaffelt (loc.cit.) that it was the mustard oil derived from the glycolysis of the glucosides which was physiologically active and that the enzyme myrosinase, derived from damaged plant tissue, was necessary for the production of the phagostimulant mustard oil. However, Hussain (loc.cit.) disputes this finding. He observed an increased faecal count when pure sinigrin was included in the agar fed to the larvae, but found that pure mustard oils were repellent. The third, and possibly most reasonable explanation of the function of the leaf powder, is that the artificial mixture of nutrients prepared in these experiments differs from the pure agar used by Hussain in being unpalatable to the larvae. In other words we are dealing here not merely with the absence of phagostimulants but with the presence of some repellent property. Sucrose and sinigrin fail to adequately mask this repellency, whereas, leaf powder is successful in this respect.

Turning now to the nutritional importance of the constituents of the diet, the review of the literature has shown that many insects can live without carbohydrates in their diets, although they can utilise some of the sugars if they happen to be present in the diet (Chapter I). In the case of a few stored products pests some

carbohydrate in the diet is essential for adequate growth and survival. In view of the results given in this present work, it may well be that the list of insects for which some carbohydrate is essential may have to be extended to include Pieris brassicae L. It has been shown that mortality increases very considerably if sugar is excluded and the facts already discussed strongly discount the view that this is merely an effect brought about by the absence of phagostimulation. Furthermore, the value of various sugars for maintaining low mortalities (Fig. 3) in no way corresponds with their value as phagostimulants (Table 11). In fact, other phytophagous insects may well require adequate quantities of certain sugars as an essential part of their diet, glucose and sucrose being particularly important from this point of view. Thus Beck (1956a,c, 1957) showed that larval growth in Ostrinia nubilalis Hbn. was positively correlated with the sugar content of the host tissues and that feeding activity was prolonged in proportion to the sugar content of the diet.

From the review in Chapter I, it is clear that pentoses are usually said to be very poorly utilised by insects in general, although xylose is utilised by some Diptera and ribose has been shown to be utilised by the boll weevil, Anthonomus grandis (Boh.) (Vanderzant 1965). In preliminary experiments arabinose and xylose were shown to be utilised by Pieris brassicae L. larvae which continued feeding on the diets containing these sugars for more than

four days without showing any mortality or other adverse effect. Similarly, ribose supported life and the mortality was not significantly different from that on the sucrose diet. These results with pentoses are based on only five replicates of five insects in each case and are obviously in need of confirmation. If this confirmation is forthcoming, the results are surprising since the statement is frequently made that pentoses are poorly utilised. However, Table 1 illustrates how few insects have been examined from this point of view and proof of their value for Pieris brassicae L. would suggest that a comparative examination of other species might well be profitable.

Of the hexoses, glucose and fructose each have high nutritive values for most insects (Gilmour 1961) but the results with Pieris brassicae L. larvae are just the reverse. In fact, Figure 3 shows that with one exception, no hexose monosaccharides showed any individual promise as an adequate replacement for sucrose in the diet. The one exception was mannose which has previously been shown by other authors to adequately provide carbohydrate in the diets of the desert locust, the boll weevil and some Diptera (Table 1). The experiments with the cabbage white butterfly larvae suggest that mannose and its alcohol, mannitol, are suitable replacements for dietary sucrose, although these results are subject to the same reservations as applied to those obtained with pentoses. Galactose has been successfully used in the diets of various insects (Table 1)

but with cabbage white butterfly larvae its use produced a significantly higher mortality than that on a sucrose diet. A very interesting result is that shown for an equimolecular mixture of fructose and glucose which proved as good as sucrose in the diet.

The results obtained with disaccharides and trisaccharides (see Fig. 3) cannot be completely explained on the basis of the chemistry of the individual sugars. If one assumes that the sucrose molecule is the ideal sugar as far as this insect's nutrient requirements are concerned, it is not surprising perhaps that an equimolecular mixture of glucose and fructose is just as successfully utilised. Sucrase is a widely occurring digestive enzyme of insects and it may be postulated that ingested sucrose is readily broken down to an equimolecular glucose and fructose mixture in the gut. On this basis the results obtained with the trisaccharides are not too inexplicable. One need not postulate unusual enzymes in order to suppose that raffinose can be hydrolysed completely to galactose, glucose and fructose molecules and melezitose to fructose and two glucose molecules. Both trisaccharides were shown to be nutritionally inferior to either the sucrose molecule or the glucose-fructose mixture and it could be postulated that this was due to a contamination or imbalance of the necessary constituents, resulting from the presence of the galactose molecule in the case of digested raffinose, and the extra glucose molecule in the case of melezitose. The three disaccharides maltose, trehalose and cellobiose all yield

only glucose molecules on hydrolysis and it will be seen in Figure 3 that the percentage mortality obtained on diets containing one of these sugars did not in fact show any significant difference from that on diets containing glucose alone. The same is true of the polysaccharide glycogen which yields only glucose on hydrolysis.

The anomalous results are those obtained with the disaccharides melibiose and lactose. Melibiose in the diet resulted in a low percentage mortality, which did not differ significantly from that obtained using sucrose, or the glucose-fructose mixture. Melibiose on hydrolysis yields one molecule of glucose and one molecule of galactose, and since survival is significantly higher than on the diets containing glucose alone, this may suggest that the galactose molecule 'improves' the glucose molecule from a nutrient point of view. However, lactose also yields one molecule of glucose and one molecule of galactose on hydrolysis, and yet the mean mortality using this disaccharide in the diet was the highest recorded in all the experiments, except those excluding sugar altogether. One can speculate further on this anomaly. Melibiose is hydrolysed by an α-galactosidase, whereas lactose requires a β-galactosidase for its digestion. The former enzyme does seem to be of more common occurrence than the latter in the Insecta (Gilmour 1961). Further evidence on this point can be obtained by measuring the mortality on a diet containing an equimolecular galactose-glucose mixture, and by assaying the enzymes of the alimentary canal in this insect.

The quantitative sugar requirements of the larvae were examined using sucrose in the diet over a range of 0 to 1000 mgm. per 25 grams of medium. The results showed that 500 mgm. or 2 per cent was the optimum concentration. This is in reasonable agreement with the results obtained for other phytophagous insects, studied by different authors. Thus, Vanderzant and Reiser (1956b) used 4 to 6 per cent sucrose in the diet of the pink bollworm; Beck (1956b) used 0.7 to 5.3 per cent glucose, fructose or sucrose for the European corn borer; and Vanderzant (1965) included 3.5 per cent fructose, maltose or sucrose in the diet of the boll weevil. A study of Table 3 reveals that phytophagous insects, generally, require a low percentage of carbohydrates in their diet. Any variation in concentration outside the rather restricted range would probably result in the same adverse effects on the insects as have been shown in the case of Pieris brassicae L. larvae.

Similarly, the nitrogen requirements of Pieris brassicae L. larvae agree with those of other phytophagous insects studied so far. It has been shown in Chapter I that phytophagous insects generally, require a low dietary concentration of protein which apparently, normally, lies between 2 and 4 per cent, and in the present investigation about 3.5 per cent concentration proved adequate.

Ishii et al. (1959) showed that the larvae of the rice stem borer Chilo suppressalis (Wlk.) required a higher range of

concentration (the minimum level being 4 per cent) of casein in their diet throughout their life, whereas, Beck (1956b) demonstrated a requirement for a higher protein concentration (casein 5.3 per cent) in the early instars, and a lower concentration (0.7 per cent) in late instars in the larvae of the European corn borer. Beck (1956a) also showed that newly hatched larvae preferred to settle on a diet with a high protein content. In the present studies such effects were not fully investigated, yet the concentration of casein was increased in the case of the diet of third instar larvae without any detectable improvement in their performance. The omission of casein from the diets of the first instar larvae adversely affected their growth, resulting in significantly lower larval weights and a reduction in the number of insects moulting. The third instar larvae did not show any significant lowering of weight gain but the proportion of insects moulting was significantly lower on the diet lacking casein. In the fifth instar larvae the amount of food consumed was also affected, a significantly lower amount being ingested on diets lacking casein. This resulted in an actual loss of more than 10 per cent of the body weight of the larvae.

The advantages gained by including cystine in the diet have already been described. The nutritional singificance of this amino acid for insects is still a matter of some debate. Attention has been drawn to the work of various authors suggesting that the

presence of cystine is connected with successful moulting in some of the non-phytophagous insects, particularly within the Diptera (Chapter IV). It is not normally regarded as an essential amino acid in the accepted sense. Kasting and McGinnis (1962), however, determined the amino acid requirements of Agrotis orthogonia Morr. by injecting glucose-U-C-14 into fifth instar caterpillars and concluded that the amino acids which were subsequently shown to have failed to take up any radioactivity were nutritionally essential. Included among these amino acids was cystine. This was an indirect method and did not show whether or not dietary cystine actually had any physiological effect on the insect.

In the present investigation, the addition of cystine resulted in significantly higher mean final weights for the third instar larvae and also gave a significantly higher value for the percentage of insects moulting. In the fifth instar caterpillars, its effect was not clear up to the time of emergence of adults from the pupae. The fifth instar larvae did not show any significant reduction in food consumption, faecal production, coefficient of utilisation, or weight gain on the food from which cystine was excluded. Successful pupation occurred on the diets lacking cystine, but the number of emergence failures was significantly higher on those diets in comparison with pupae from larvae reared on diets containing cystine. This appears to be the first observation of such an effect in a phytophagous insect. McMorran and Grisdale (1964) reared the larvae of Pieris

rapae L. on a semi-synthetic diet and reported that a high percentage of male adults had crippled wings which the addition of corn oil or soybean oil could not prevent. This may well be due to lack of cystine in the diet since the deformities described by McMorran seem similar to those observed in the present studies. However, it must be pointed out that David and Gardiner (1965) have recently reported 80 per cent emergence of adults reared on McMorran's diet.

It was not possible to make a detailed qualitative and quantitative study of the effects of all the dietary components on the development of the insect in the limited period of time available. Nevertheless, a survey of other possibly important dietary factors was made and some consideration was given to the vitamins and sterol requirements of Pieris brassicae L. larvae. Thus, β-carotene (a precursor of vitamin A) was tried in two different concentrations but its addition resulted in retarded growth of the larvae. Earlier Chiu and McCay (1939) had included vitamin A in the diets of Tribolium confusum (Duv.) and Acanthoscelides obtectus (Say) but without any effect. On the other hand, Dadd (1957) reared the newly hatched nymphs of the desert locust to adults and showed that β-carotene or vitamin A acetate was essential for adequate growth and pigmentation. House (1965c) supported this view by showing that vitamin A acetate accelerated the growth rate in the larvae of Agria affinis (Fall.). No such requirement could be shown in the present

studies. Inclusion of a mixture of B vitamins in the diet had no effect at all, although B vitamins have been included with advantage in the diets of various insects studied by different authors (Table 2). Possibly the added B vitamins did not have any effect because the larvae were obtaining the necessary factors from the cabbage leaf powder. However, it must be emphasised that the object of the experiments which have been described was to detect short term effects of deficiencies in the diet and to this end all experiments were conducted over a maximum of four days. On this basis, one cannot discount the possibilities of more subtle responses to vitamin concentrations. Effects such as those described by Dadd on locust pigmentation would not reveal themselves in short term experiments of the type described above. More conclusive data will be obtained from long term rearing experiments on vitamin-containing and vitamin-free diets.

Ascorbic acid (vitamin C) was first incorporated into the diet of a phytophagous insect by Dadd (1957) using the desert locust and since then its importance has been stressed in the diets of several other species of mainly phytophagous insects. At the same time a number of insects have been reared satisfactorily without this vitamin and in other cases it has been suggested that it merely performs a phagostimulant function (Chapter I). In the present investigation, however, ascorbic acid did not improve the diet but its addition, in fact, resulted in a more than 25 per cent mortality of the larvae, as compared to zero mortality on the diet lacking this vitamin. This result is very curious since the weight

gains of larvae were not affected by the incorporation of ascorbic acid into the diet. This suggests, therefore, that one is not dealing with any repellent effect of this material in the diet, and experiments on food consumption would check this conclusion. In Chapter I it was shown that where ascorbic acid has been found to produce a beneficial effect, it has usually been incorporated into the diets at a concentration of between 0.2 and 0.5 per cent and in these present studies 0.4 per cent was used. Although no reference to the ill effects of overdosing with vitamin C has been found in the literature, it is possible that in this case a considerable proportion of the amount of this vitamin present in cabbage, survives the treatment by which leaf powder is made. It may well be that the added leaf powder contained the optimum quantity of the vitamin for the larvae, and in fact, the same argument may be applied here as was used in the case of sinigrin discussed above.

Only a few insects have been shown to require fats or fatty acids in their diets, and these requirements have not been investigated in the present study on the larvae of Pieris brassicae L. The only relevant data which have been obtained are that when wheat embryo, which contains fatty acids shown to be essential in some Lepidoptera, was incorporated into the diet when attempts were made to exclude leaf powder; these attempts were a failure (Chapter III).

The effects of incorporating cholesterol and β-sitosterol in the diet of Pieris brassicae L. larvae were examined, since the majority of insects have been shown to require a sterol in their diet. However, no improvement in the diet could be brought about in the present studies and in fact, a concentration of about 2 per cent of either sterol adversely affected the growth and development of the larvae. The lower concentration of 0.2 per cent did not produce any observed effect, although this concentration was more or less in agreement with the requirements of other phytophagous insects studied in the past (Table 6). The higher concentration of 2 per cent was perhaps an excessive quantity, and the adverse effects might have occurred as a result of overdosing. On the other hand, Dadd and Mittler (1965) failed to improve the growth of the aphid Myzus persicae (Sulzer), with the inclusion of cholesterol in the diet and, in fact the incorporation of cholesterol suspension retarded growth. One might expect that in the case of plant feeding insects β-sitosterol would be of more importance in the natural diet than cholesterol. However, the present studies have failed to demonstrate that any benefit is to be derived from either sterol in an artificial diet for this species. Here again one must emphasise that it is impossible to conclusively prove a negative result using a diet containing a constituent of unknown composition such as leaf powder. Conclusions can only be drawn with confidence on the basis of experiments showing positive results.

This argument can equally be applied to the failure to apparently improve the diet by including the Osbourne and Mendel salt mixture.

It has been shown in Chapter I that although nutritional studies have been carried out on a number of phytophagous insects, yet there are only a few successful examples in which purely synthetic diets have been used, all the rest of the findings being based on semi-synthetic diets. In the present investigations when all efforts to make a suitable synthetic diet had failed, the inclusion of cabbage leaf powder seemed the only possible way in which the diet could be modified at this stage in order that the larvae could feed and grow. Though it had certain disadvantages, and it was difficult to establish the role of certain materials which may be present in the leaf powder and which, if required by the larvae at all, are only required in very small quantities, nevertheless, it has made possible a comparison of the growth and development of larvae on different diets. The results for sugars, casein and cystine are conclusive. These substances proved necessary even in the presence of cabbage leaf powder, since an agar medium containing only leaf powder failed to support life. On the other hand, attempts to replace cabbage leaf powder have failed, although further investigations may ultimately lead to the total exclusion of this material from the diet. Analysis of the cabbage leaf might be helpful although this has its difficulties as outlined in Chapter I and at

this stage we do not know what vital role is performed by the leaf powder or its various constituents.

Apart from other considerations the importance of the physical suitability of the diet, particularly for phytophagous insects, has been stressed by various authors. In the present studies one of the materials used to give a suitable texture to the medium was nutrient agar. It was included to make a cohesive diet which was easy to handle. Tanton (1965) showed that the consumption of food increased with increased agar concentration in the diet of the mustard beetle, the highest concentration used being 6 per cent. In the present investigation no such correlation could be demonstrated. Other constituents of the diet were shown to have a far more important and over riding effect on consumption, and the insects showed little ability to discriminate between agar concentrations over the range tested. The concentration of 2 per cent used in these studies was selected for the advantages gained in handling the material rather than for any nutritional advantage to the insect.

The other substance used as a major influence on physical texture was cellulose which was used in two forms. One form was cellophas, which was incorporated into the diet to give it a leaf like structure as suggested by Stride (1953), but it proved a total failure as it absorbed too much water and the medium dried up very quickly. The other form, which proved of great value, was cellulose

powder. The consumption of food on the 'normal' diet was low compared with that on cabbage, and the addition of cellulose proved highly beneficial in increasing this consumption to the level of that on cabbage. The significantly lower consumption on the 'normal' diet was perhaps due to the lack of 'bulk' or 'roughage', a deficiency which was made up by the cellulose powder used at 5 grams per 25 grams of medium.

The food consumption appears, from the data given in Tables 32 and 33, to be inversely related to the nutrient value of the diet i.e. the 'richer' the diet, the less the animal consumes, and this is a situation with parallels in the field of vertebrate nutrition (for rats see Piekarska 1964; for cattle see Meyer et al. 1956). The relationship is not a matter of direct proportionality, however, this being well illustrated by the very marked increase in consumption when the cellulose content is raised from 4 grams to 5 grams. There is however an exactly parallel rise in faecal production, and the total weight of food utilised (i.e. weight of food consumed - weight of faeces) by larvae fed on the diet containing 5 grams cellulose does not differ significantly from that utilised by larvae fed on the medium containing 4 grams cellulose, or in fact by those fed on the normal medium.

The fact that the coefficient of utilisation falls in value when cellulose is added to the diet is thus apparently due solely to the change in the weight of food consumed, not to any change in

the weight of food utilised, and this serves to emphasise the wholly inert role of cellulose nutritionally. In other words, it appears to control only the amount of food passing through the gut, and does not affect the amount absorbed by the insect from the gut.

Some of the observations, however, suggest that the affect of adding cellulose is a little more complicated than has so far been implied. If the amount of food passing through the gut is controlled solely by the animals' need for a given weight of nutrients, the very sudden increase in food consumption resulting from adding 5 grams of cellulose to the diet rather than 4 grams is difficult to explain. This additional quantity of cellulose produced only a small (2.5 per cent) decrease in nutrient concentration, but an extremely large increase in consumption. One possible explanation might be that it is the concentration of individual nutrients which is important rather than the total weight of nutrients. As the medium is diluted with cellulose the concentration of some of the nutrients, present in small amounts initially, may drop to a point at which the animal is unable to absorb them efficiently. On this basis one might speculate that the greatly increased food consumption is the animal's response to this apparent scarcity of essential chemicals. Data concerning both the nutrient content of the faeces, and the effect of cellulose on the rate of passage of food through the gut would be of interest in this connection.

This, of course, implies a quite different role for cellulose. Although nutritionally an inert compound in the strict sense it would be playing a nutritional role in influencing the relative availability of compounds required by the insect. One might expect that as a result the composition of that proportion of the food utilised by the larvae would also be affected by the cellulose dilution, and in this connection it is interesting to note the greatly increased weight gain of the larvae reared on the diet containing 5 grams cellulose. Since the amount of food utilised has not changed as a result of adding cellulose, it seems a reasonable assumption that this increased weight gain must result from some change in the composition of the food utilised. In other words one effect of adding 5 grams cellulose appears to have been to increase the coefficient of growth, with a consequent lowering of the coefficient of metabolism.

If these speculations have any validity then the mechanism by which cellulose controls the uptake of food by the caterpillar is not, as suggested originally, a purely physical one, but is partly at least chemical, the mechanism involving not the concentration of cellulose itself, but the effect which it has on the concentration of other dietary constituents.

Thus it has been found possible to adjust the diet in order to achieve a larval intake comparable to that on cabbage even though our understanding of the underlying mechanisms is very incomplete.

A good deal of progress has yet to be made before this diet could be considered comparable to cabbage in other respects. The coefficient of utilisation of cabbage is 40.5 per cent for the fifth instar caterpillars, a figure which compares closely with the published value of 41.4 per cent for fifth instar larvae of Phalera bucephala L. reared on hornbeam. In the case of the diet containing 5 grams cellulose, only 17.5 per cent of the diet was utilised and any attempt to improve this figure had a detrimental effect on food consumption.

The major requirement here is to increase the total weight of food utilised in the diet. The problem, therefore, becomes much more complicated since one moves a stage further in attempting to control not the ingestion of food, but its absorption by the larvae. The coefficient of growth is very much higher on the cellulose diet than on cabbage and the coefficient of metabolism, therefore, much lower. The central problem to be pursued in these nutritional studies has, therefore, become that of discovering the adjustments which must be made to a diet in order to control the use to which the larva puts the food ingested.

A much greater amount of detailed information on the quantitative inter relationships of the materials utilised by the cabbage white larvae is obviously required. The related problem of the role of leaf powder remains unsolved and the author is acutely aware of the need for further study of these problems together with

clarification of many points of detail. Many of the limitations of this work have been imposed by limitations of time and it is hoped to pursue these investigations in the near future.

SUMMARY

1) The literature on nutritional studies on insects has been reviewed and the need for devising a suitable synthetic diet for these studies has been stressed. Attempts to produce a wholly synthetic diet for Pieris brassicae L. larvae all proved a failure.

2) After a series of experiments, a semi-synthetic diet containing cabbage leaf powder was evolved, which could support the life and maintain the growth of the caterpillars. A leaf powder concentration of 8 per cent or 2 grams per 25 grams of medium was the most suitable, as the concentrations lower than this produced a very high mortality and the higher ones did not show any improved effect.

3) Sugars were found to be nutritionally essential in the diet and out of 18 sugars tried sucrose was found to be the most suitable as it had a comparatively higher nutritive value in addition to acting as a phagostimulant. The optimum concentration of sucrose in the diet was 500 mgm. per 25 grams of medium.

4) In contrast to the previously published data of other authors, sinigrin - a mustard oil glucoside - failed to evoke any feeding response in the larvae fed on the semi-synthetic diet in these investigations.

5) Casein was found to be valuable as a nitrogen source. Its omission produced larvae with comparatively low final weights in the early instars, and resulted in loss of weight in the fifth instar caterpillars. A concentration of 0.87 grams per 25 grams of medium was found to be adequate.

6) The addition of cystine to the diet resulted in a greater proportion of the larval population moulting and its omission from the diet of the fifth instar caterpillars caused failures in the subsequent emergence of adults from the pupae. The adults which were produced frequently showed various deformities. A concentration of 50 mgm. of cystine per 25 grams of medium was found to be adequate for the larvae.

7) The addition of β-carotene to the diet retarded the growth of the larvae.

8) B vitamins did not produce any observed effect on larvae reared on the semi-synthetic diet.

9) The addition of ascorbic acid (vitamin C) to the diet had an adverse effect on the larvae, producing a high mortality.

10) A concentration of 500 mgm. per 25 grams of medium of cholesterol or β-sitosterol adversely affected the growth and development of the caterpillars, whereas, a concentration of 50 mgm. per 25 grams of medium did not produce any observed effects.

11) Minerals added in the form of the Osbourne and Mendle salt mixture did not produce any detectable effects.

12) A concentration of 2 per cent of nutrient agar helped in making the diet cohesive and manageable and perhaps provided a better feeding surface for the larvae as well.

13) Attempts to give a leaf like structure to the diet by adding cellophas were a failure since the diet absorbed enormous quantities of water and dried up overnight.

14) Cellulose powder proved very useful in providing 'bulk' and it increased the consumption of food when incorporated into the 'normal' medium although it lowered the coefficient of utilisation of food.

15) The absolute weight gains and percentage weight gains of larvae fed on diets containing 5 grams of cellulose powder per 25 grams of medium, were comparable with those feeding on cabbage.

16) The coefficient of utilisation of food and that of metabolism on the cellulose diet were significantly lower than those on cabbage but the coefficient of growth was higher on the cellulose diet.

ACKNOWLEDGEMENTS

The author is greatly indebted to Dr. J.B. Ford under whose kind supervision and inspiring guidance these investigations have been conducted. My grateful thanks are due to Mr. J. Hobart, head of the Department of Agricultural and Forest Zoology, U.C.N.W., Bangor, for his valuable and useful criticism during the preparation of the manuscript, and also for the provision of all the facilities required for research in his department.

I am indebted to all members of the Department of Agricultural and Forest Zoology, U.C.N.W., Bangor, for their cooperation and help during the course of this research, and especially to Mr. R.G. Hickson, Mrs. M. Bentley and Miss Valmai Hughes for their invaluable technical assistance.

I would like to acknowledge with gratitude the gifts of eggs of Pieris brassicae L. from Dr. W.A.L. David of the Agricultural Research Council's Unit of Insect Physiology, Cambridge, and I am thankful to Miss Arlene McMorran of the Insect Pathology Research Institute in Sault Ste. Marie, Ontario, Canada, for supplying the fomrula of the semi-synthetic diet used by her for rearing pine shoot moth larvae.

My profound thanks are also due to the trustees of the Sir William Roberts Scholarship, for their generous grant which made this research possible.

The help rendered by the staff of the Zoology Department and their cooperation in allowing the use of their dark room is gratefully acknowledged.

Acknowledgement must also be made to Mrs. G. Marshall who typed the manuscript.

BIBLIOGRAPHY

Akov, S.	1962	A qualitative and quantitative study of the nutritional requirements of *Aedes aegypti* L. larvae. J. Insect Physiol., 8 (3): 319-335.
Albritton, E.C.	1955	Standard values in nutrition and metabolism. W.P. Saunders Co., Philadelphia,Pa., 380 pp.
Allen, M.D. and Selman, I.W.	1957	The response of larvae of the large white butterfly (*Pieris brassicae* L.) to diets of mineral-deficient leaves. Bull. ent. Res., 48 (1): 229-242.
Baker, A.C., Stone, W.E., Plummer, C.C. and McPhail, M.	1944	A review of studies on the Mexican fruit fly and related Mexican species. U.S.D.A. Misc. Publ. No. 531: 155 pp.
Beck, S.D.	1950	Nutrition of the European corn borer, *Pyrausta nubilalis* (Hbn.). II. Some effects of diet on larval growth characteristics. Physiol. Zool., 23 (4): 353-361.
Beck, S.D.	1956a	Nutrition of the European corn borer, *Pyrausta nubilalis* (Hbn.). IV. Feeding reactions of first instar larvae. Ann. ent. Soc. Am., 49 (4): 399-405.
Beck, S.D.	1956b	The European corn borer, Pyrausta *nubilalis* (Hbn.), and its principal host plant. II. The influence of nutritional factors on larval establishment and development on the corn plant. Ann. ent. Soc. Am., 49 (6): 582-588.
Beck, S.D.	1956c	A bimodal response to dietary sugars by an insect. Biol. Bull., 110 (3): 219-228,

Beck, S.D.	1957	The European corn borer, Pyrausta nubilalis (Hbn.), and its principal host plant. IV. Larval saccharotrophism and host plant resistance. Ann. ent. Soc. Am., 50 (3): 247-250.
Beck, S.D., Lilly, J.H., and Stauffer, J.F.	1949	Nutrition of the European corn borer, Pyrausta nubilalis (Hbn.). I. Development of a satisfactory purified diet for larval growth. Ann. ent. Soc. Am., 42 (4): 483-496.
Beck, S.D., and Stauffer, J.F.	1950	An aseptic method for rearing European corn borer larvae. J. econ. Ent., 43 (1): 4-6.
Bertholf, L.M.	1927	The utilisation of carbohydrates as food by honeybee larvae. J. agric. Res., 35 (5): 429-452.
Blewett, M., and Fraenkel, G.	1944	Intracellular symbiosis and vitamin requirements of two insects, Lasioderma serricorne and Sitodrepa panicea. Proc. R. soc. Lond. Ser. B., 132: 212-221.
Bottger, G.T.	1942	Development of synthetic food media for use in nutrition studies of the European corn borer. J. agric. Res., 65 (10): 493-500.
Brookes, V.J., and Fraenkel, G.	1958	The nutrition of the larvae of the housefly, Musca domestica L. Physiol. Zool., 31 (3): 208-223.
Brown, A.W.A.	1937	A note on the utilisation of polysaccharides by a grasshopper. Bull. ent. Res., 28: 333-336.
Buddington, A.R.	1941	The nutrition of mosquito larvae. J. econ. Ent., 34 (2): 275-280.
Chippendale, G.M., and Beck, S.D.	1964	Nutrition of the European corn borer, Ostrinia nubilalis (Hbn.). V. Ascorbic acid as the corn leaf factor Entomologia exp. appl., 7 (3): 241-248

Chippendale, G.M., Beck, S.D., and Strong, F.M.	1965	Nutrition of the cabbage looper, Trichoplusia ni (Hbn.). I. Some requirements for larval growth and wing development. J. Insect Physiol., 11 (2): 211-223.
Chiu, S.F. and McCay, C.M.	1939	Nutritional studies of the confused flour beetle (Tribolium confusum Duval) and the bean weevil (Acanthoscelides obtectus Say). Ann. ent. Soc. Am., 32 (1): 164-170.
Clayton, R.B., Edwards, A.M., and Bloch, K.	1962	Biosynthesis of cholesterol in an insect, silverfish (Cetenolepisma sp. Nature, 195: 1125-1126.
Cooper, M.I., and Fraenkel, G.	1952	Nutritive requirements of the small eyed flour beetle, Palorus ratzeburgi Wissman (Tenebrionidae: Coleoptera) Physiol. Zool., 25: 20-28.
Crowell, H.H.	1941	The utilisation of certain nitrogenous and carbohydrate substances by the southern armyworm, Prodenia eridenia Cram. Ann. ent. Soc. Am., 34 (2): 503-512.
Dadd, R.H.	1957	Ascorbic acid and carotene in the nutrition of the desert locust, Schistocerca gregaria Forsk. Nature Lond., 179: 427-428.
Dadd, R.H.	1960a	The nutritional requirements of locust I Development of synthetic diets and lipid requirements. J. Insect Physiol., 4 (4): 319-347.
Dadd, R.H.	1960b	The nutritional requirements of locust II Utilization of sterols. J. Insect Physiol., 5 (3,4): 161-168.
Dadd, R.H.	1960c	The nutritional requirements of locust III Carbohydrate requirements and utilization. J. Insect Physiol., 5 (3,4): 301-316.

Dadd, R.H.	1960d	Some effects of dietary ascorbic acid on locusts. Proc. R. Soc. Lond. Ser. B., 153: 128-143.
Dadd, R.H.	1960e	Observations on the palatability and utilization of food by locusts, with particular reference to the interpretation of performances in growth trials using synthetic diets. Entomologia exp. appl., 3: 283-304.
Dadd, R.H.	1961a	The nutritional requirements of locust IV Requirements for vitamins of the B complex. J. Insect Physiol., 6 (1): 1-12.
Dadd, R.H.	1961b	The nutritional requirements of locust V Observations on essential fatty acids, chlorophyll, nutritional salt mixtures, and the protein or amino acid components of synthetic diets. J. Insect Physiol., 6 (2): 126-145.
Dadd, R.H.	1964	A study of carbohydrate and lipid nutrition in the wax moth, Galleria mellonella (L.), using partially synthetic diets. J. Insect Physiol., 10 (2): 161-178.
Dadd, R.H., and Mittler, T.E.	1965	Studies on the artificial feeding of the aphid, Myzus persicae (Sulzer) - III. Some major nutritional requirements. J. Insect Physiol., 11 (6): 717-743.
Davey, P.M.	1955	Quantities of food eaten by the desert locust, Schistocerca gregaria Forsk., in relation to growth. Bull. ent. Res., 45 (3): 539-551.
David, W.A.L., and Gardiner, B.O.C.	1952	Laboratory breeding of Pieris brassica L. and Apanteles glomeratus L. Proc. R. ent. Soc. Lond. (A), 27 (4-6) 54-56.

David, W.A.L., and Gardiner, B.O.C. 1961a The mating behaviour of Pieris brassicae L. in a laboratory culture. Bull. ent. Res., 52 (2): 263-280.

David, W.A.L., and Gardiner, B.O.C. 1961b Feeding behaviour of adults of Pieris brassicae L. in a laboratory culture. Bull. ent. Res., 52 (4): 741-762.

David, W.A.L., and Gardiner, B.O.C. 1962 Oviposition and the hatching of the eggs of Pieris brassicae L. in a laboratory culture. Bull. ent. Res., 53 (1): 91-109.

David, W.A.L., and Gardiner, B.O.C. 1965 Rearing Pieris brassicae L. larvae on a semi-synthetic diet. Nature, 207: 882-883.

Evans, A.C. 1939 The utilisation of food by certain lepidopterous larvae. Trans. R. ent. Soc. Lond., 89 (2): 13-22.

Evans, A.C., and Dethier, V.G. 1957 The regulation of taste thresholds for sugars in the blowfly. J. Insect Physiology., 1 (1): 3-17.

Evans, A.C., and Goodliffe, E.R. 1939 The utilisation of food by the larvae of the mealworm, Tenebrio molitor L. (Coleop.). Proc. R. ent. Soc. Lond. (A), 14 (4-6) 57-62.

Fraenkel, G. 1936 Utilisation of sugars and polyhydric alcohols by the adult blowfly. Nature, 137: 237-238.

Fraenkel, G. 1953 The nutritional value of green plants for insects. Trans. 9th Int. Congr. Ent. Amsterdam, 2: 90-100.

Fraenkel, G.S. 1958 The effect of zinc and potassium in the nutrition of Tenebrio molitor, with observations on the expression of a carnitine deficiency. J. Nutr., 65 (3): 361-395.

Fraenkel, G., and Blewett, M.	1943a	The natural foods and the food requirements of several species of stored products insects. Trans. R. ent. Soc. Lond., 93 (2): 457-490.
Fraenkel, G., and Blewett, M.	1943b	The basic food requirements of several insects. J. exp. Biol., 20: 28-34.
Fraenkel, G., and Blewett, M.	1943c	The vitamin B-complex requirements of several insects. Biochem. J., 37: 686-692.
Fraenkel, G., and Blewett, M.	1943d	The sterol requirements of several insects. Biochem. J., 37: 692-695.
Fraenkel, G., and Blewett, M.	1946a	The dietetics of the clothes moth, Tineola bisselliella Hum. J. exp. Biol., 22: 156-161.
Fraenkel, G., and Blewett, M.	1946b	The dietetics of the caterpillars of three Ephestia species, E. kuehniella, E. elutella and E. cautella and of a closely related species, Plodia interpunctella. J. exp. Biol., 22: 162-171.
Fraenkel, G., and Blewett, M.	1946c	Folic acid in the nutrition of certain insects. Nature, 157: 697.
Fraenkel, G., and Blewett, M.	1946d	Linoleic acid, vitamin E and other fat-soluble substances in the nutritio of certain insects, Ephestia kuehniell E. elutella, E. cautella and Plodia interpunctella (Lep.). J. exp. Biol., 22: 172-190.
Fraenkel, G., and Blewett, M.	1947a	The importance of folic acid and unidentified members of the vitamin B-complex in the nutrition of certain insects. Biochem. J., 41 (3): 469-475.

Fraenkel, G., and Blewett, M.	1947b	Linoleic acid and arachidonic acid in the metabolism of two insects, Ephestia kuehniella (Lep.) and Tenebrio molitor (Col.). Biochem. J., 41 (3): 475-478.
Fraenkel, G., Blewett, M., and Coles, M.	1950	The nutrition of the mealworm, Tenebrio molitor L. (Tenebrionidae: Coleoptera). Physiol. Zool., 23 (2): 92-108.
Friend, W.G.	1955	Problems in nutritional studies on phytophagous insects. Ann. Rep. ent. Soc. Ont., 86: 13-16.
Friend, W.G.	1956	The nutrition of phytophagous insects with special reference to Hylemya antiqua (Mg.). Proc. 10th Int. Congr. Ent., 2: 145-149.
Friend, W.G.	1958	Nutritional requirements of phytophagous insects. A. Rev. Ent., 3: 57-74.
Friend, W.G., and Patton, R.L.	1956	Studies on vitamin requirements of larvae of the onion maggot, Hylemya antiqua (Mg.) under aseptic conditions Can. J. Zool., 34 (2): 152-162.
Gay, F.J.	1938	A nutritional study of the larva of Dermestes vulpinus F. J. exp. Zool., 79 (1): 93-107.
Gilmour, D.	1961	The biochemistry of insects. Academic Press, New York and London, 343 pp.
Golberg, L., and De Meillon, B.	1948	The nutrition of the larvae of Aedes aegypti L. 4. Protein and amino acid requirements. Biochem. J., 43: 379-387.
Haydak, M.H.	1949	Causes of deficiency of soybean flour as a pollen substitute for honeybees. J. econ. Ent., 42 (4): 573-579.

Haydak, M.H. 1953 Influence of the protein level of the diet on the longevity of cockroaches. Ann. ent. Soc. Am., 46 (4): 547-560.

House, H.L. 1949a Nutritional studies with Blatella germanica (L.) reared under aseptic conditions. II. A chemically defined diet. Can. Ent., 81 (5): 105-112.

House, H.L. 1949b Nutritional studies with Blatella germanica (L.) reared under aseptic conditions. III. Five essential amino acids. Can. Ent., 81 (6): 133-139.

House, H.L. 1954 Nutritional studies with Pseudosarcophaga affinis (Fall.), a dipterous parasite of the spruce budworm, Choristoneura fumiferana (Clem. I. A chemically defined medium and aseptic-culture technique. Can. J. Zool., 32 (5): 331-341.

House, H.L. 1956 The nutrition of insects with particular reference to entomophagous parasites. Proc. 10th Int. Congr. Ent., 2:139-143

House, H.L. 1961 Insect nutrition. A. Rev. Ent., 6: 13-26.

House, H.L. 1965a Insect nutrition. The Physiology of Insecta vol. II, pp. 769-813 (Morris Rockstein ed.) Academi Press, New York and London, 905 pp.

House, H.L. 1965b Effects of low levels of the nutrient content of a food and of nutrient imbalance on the feeding and the nutrition of a phytophagous larva, Celerio euphorbiae (Linnaeus)(Lepidoptera: Sphingidae). Can. Ent., 97 (1): 62-68.

House, H.L. 1965c Effects of vitamin A acetate and structurally related substances on growth and reproduction of Agria affinis (Fallen) (Diptera:Sarcophagidae).
J. Insect Physiol., 11 (7): 1039-1045.

House, H.L., and Barlow, J.S. 1956 Nutritional studies with Pseudosarcophaga affinis (Fall.), a dipterou parasite of the spruce budworm, Choristoneura fumiferana (Clem.). V. Effects of various concentrations of the amino acid mixture, dextrose, potassium ion, the salt mixture, and lard on growth and development; and a substitute for lard.
Can. J. Zool., 34 (3): 182-189.

House, H.L., and Barlow, J.S. 1958 Vitamin requirements of the house fly, Musca domestica L. (Diptera: Muscidae)
Ann. ent. Soc. Am., 51 (3): 299-302.

House, H.L., and Barlow, J.S. 1960 Effects of oleic and other fatty acids on the growth rate of Agria affinis (Fall.) (Diptera: Sarcophagidae).
J. Nutr., 72: 409-414.

House, H.L., and Barlow, J.S. 1965 The effects of a new salt mixture developed for Agria affinis (Fallen) (Diptera: Sarcophagidae) on the growth rate, body weight, and protein content of the larvae.
J. Insect Physiol., 11 (7): 915-918.

Hussain, M.Z. 1963 The experimental study of the chemotactic basis of host acceptance in a phytophagous insect, Pieris brassicae Pieridae: Lepidoptera.
A thesis submitted to the West Pakista Agricultural University, Lyallpur, West Pakistan. (Unpublished).

Ishii, S., Azim, A., and Hirano, C.	1959	A further experiment on the effect of dietary levels of protein and carbohydrate on the growth of the rice stem borer, Chilo suppressalis, larvae. Jap. J. appl. Ent. Zool., 3: 143-145.
Ishii, S., and Urushibara, H.	1954	On fat and water soluble growth factors required by the rice stem borer, Chilo simplex Butler. Bull. natn. Inst. agric. Sci. Tokyo Ser. C, 4: 109-133.
Ito, T.	1960	Effect of sugars on feeding of larvae of the silkworm, Bombyx mori. J. Insect Physiol., 5 (2): 95-107.
Ito, T., and Horie, Y.	1962	Nutrition of the silkworm, Bombyx mori - VII. An aseptic culture of larvae on semi-synthetic diets. J. Insect Physiol., 8 (5): 569-578.
Ito, T., Kawashima, K., Nakahara, M., Nakanishi, K., and Terahara, A.	1964	Effects of sterols on feeding and nutrition of the silkworm, Bombyx mori L. J. Insect Physiol., 10 (2): 225-238.
Kamano, S.	1961	On the successive rearing of rice stem borer on the artificial diets under aseptic conditions. I. The effect of choline contained in the artificial diets on the oviposition and the hatching potency. (In Japanese Jap. J. appl. Ent. Zool., 5: 254-259.
Kamano, S.	1964	On the successive rearing of rice stem borer on the artificial diets under aseptic conditions. II. (In Japanese). Jap. J. appl. Ent. Zool., 8: 101-105. (Rev. appl. Ent., 1965, Ser. A., 53: 62)

Kasting, R., and McGinnis, A.J.	1962	Nutrition of the pale western cutworm, Agrotis orthogonia Morr. (Lepidoptera, Noctuidae) - IV Amino acid requirements determined with glucose-U-C^{14}. J. Insect Physiol., 8 (1): 97-103.
Kasting, R., and McGinnis, A.J.	1965	Measuring consumption of food by an insect with carbon-14 labelled compounds. J. Insect Physiol., 11 (9): 1253-1260.
Leclercq, J.	1948	Sur les besoins nutritifs de la larve de Tenebrio molitor L. Biochem. biophys. Acta, 2: 329-332.
Lemonde, Par A., and Bernard, R.	1951a	Nutrition des larves de Tribolium confusum Duval. I. Recherche d'un regime synthetique basal satisfaisant leurs besoins nutritifs. Can. J. Zool., 29 (2): 71-79.
Lemonde, Par A., and Bernard, R.	1951b	Nutrition des larves de Tribolium confusum Duval. II. Importance des acides amines. Can. J. Zool., 29 (2): 80-83.
Levinson, Z.H.	1962	The function of dietary sterols in phytophagous insects. J. Insect Physiol., 8 (2): 191-198.
Lipke, H., and Fraenkel, G.	1956	Insect nutrition. A. Rev. Ent., 1: 17-44.
McGinnis, A.J., and Kasting, R.	1964a	Digestion in insetcs - Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. J. agric. Fd. Chem., 12 (3): 259-262.
McGinnis, A.J., and Kasting, R.	1964b	Comparison of gravimetric and chromic oxide methods for measuring percentage utilization and consumption of food by phytophagous insects. J. Insect Physiol., 10 (6): 989-995.

McGinnis, A.J., Newburgh, R.W., and Cheldelin, V.H. 1956 Nutritional studies on the blowfly, Phormia regina (Meig.). J. Nutr., 58 (3): 309-323.

McMorran, A. 1964 Personal communication.

McMorran, A.R., and Grisdale, D.G. 1964 Insect rearing. Ann. rep. Forest ent. path. branch, Deptt. of Forestry, Canada, pp. 9-10.

Meyer, J.H., Lofgreen, G.P., and Ittner, N.R. 1956 Further studies on the utilisation of alfalfa by beef steers. J. anim. Sci., 15: 64-75.

Michelbacher, A.E., Hoskins, W.M., and Herms, W.B. 1932 The nutrition of flesh-fly larvae, Lucilia sericata Meig. J. exp. Zool., 64 (1): 109-128.

Mittler, T.E., and Dadd, R.H. 1962 Artificial feeding and rearing of the aphid, Myzus persicae (Sulzer), on a completely defined synthetic diet. Nature, 195: 404.

Mittler, T.E., and Dadd, R.H. 1963 Studies on the artificial feeding of the aphid Myzus persicae (Sulzer) - I. Relative uptake of water and sucrose solutions. J. Insect Physiol., 9 (5): 623-645.

Monroe, R.E. 1960 Effect of dietary cholesterol on house fly reproduction. Ann. ent. Soc. Am., 53 (6): 821-824.

Monroe, R.E. 1962 A method for rearing house fly larvae aseptically on a synthetic medium. Ann. ent. Soc. Am., 55 (1): 140.

Moore, W. 1943 Some vitamin requirements of black carpet beetles, Attagenus (?) spp. (Coleoptera: Dermestidae). Ann. ent. Soc. Am., 36 (3): 483-485.

Moore, W. 1946 Nutrition of Attagenus (?) sp. II. Ann. ent. Soc. Am., 39 (3): 513-521.

Niimura, M., and Ito, T.	1964	Feeding on filter paper by larvae of the silkworm, Bombyx mori L. J. Insect Physiol., 10 (3): 425-430.
Noland, J.L., and Baumann, C.A.	1949	Requirement of the German cockroach for choline and related compounds. Proc. Soc. exp. Biol. Med., 70: 198-20
Noland, J.L., Lilly, J.H., and Baumann, C.A.	1949	Vitamin requirements of the cockroach Blatella germanica (L.) Ann. ent. Soc. Am., 42 (2): 154-164.
Ouye, M.T., and Vanderzant, E.S.	1964	B-vitamin requirements of the pink bollworm. J. econ. Ent., 57 (4): 427-430.
Parkin, E.A.	1936	A study of the food relation of the Lyctus powder-post beetles. Ann. appl. Biol., 23 (2): 369-400.
Perry, A.S., and Miller, S.	1965	The essential role of folic acid and the effect of antimetabolites on growth and metamorphosis of housefly larvae Musca domestica L. J. Insect Physiol., 11 (9): 1277-1287.
Piekarska, J.	1964	Wplyw skladu chemicznego blonnika na niektore funkcje i ciezar przewodu pokarmowego. Rocz. panstowowego Zakt. Hig. 15: 471-480.
Rasso, S.C., and Fraenkel, G.	1954	The food requirements of the adult female blow-fly, Phormia regina (Meige in relation to ovarian development. Ann. ent. Soc. Am., 47 (4): 636-645.
Sang, J.H.	1956	The quantitative nutritional requirements of Drosophila melanogaster. J. exp., Biol., 33: 45-72.
Sedee, Ph.D.J.W.	1956	Dietetic requirements and inter_mediar protein metabolism of the larvae of Calliphora erythrocephala (Meig.) Van Gorcum and Corp. N.V., Assen.

Singh, K.R.P., and Brown, A.W.A.	1957	Nutritional requirements of Aedes aegypti L. J. Insect Physiol., 1: 199-220.
Smith, D.S.	1959	Utilisation of food plants by the migratory grasshopper, Melanoplus bilituratus (Walker) (Orthoptera: Acrididae) with some observations on the nutritional value of the plants. Ann. ent. Soc. Am., 52 (6): 674-680.
Stride, G.O.	1953	On the nutrition of Carpophilus hemipterus L. (Coleoptera: Nitidulidae Trans. R. ent. Soc. Lond., 104 (6): 171-194.
Tanton, M.T.	1965	Agar and chemostimulant concentrations and their effect on intake of syntheti food by larvae of the mustard beetle, Phaedon cochleariae Fab. Entomologia exp. appl., 8 (1): 74-82.
Thorsteinson, A.J.	1953	The chemotactic responses that determine host specificity in an oligophagous insect (Plutella maculipennis (Curt.) Lepidoptera). Can. J. Zool., 31 (1): 52-72.
Thorsteinson, A.J.	1955	The experimental study of the chemotactic basis of host specificity in phytophagous insects. Can. Ent., 87 (2): 49-57.
Thorsteinson, A.J.	1956	Acceptability of plants for phytophagous insects. Proc. 10th Int.Congr. Ent., 2: 599-602
Thorsteinson, A.J.	1960	Host selection in phytophagous insects A. Rev. Ent., 5: 193-218.
Trager, W.	1953	Nutrition. Insect Physiology pp. 350-386 (Roeder, K. Ed.) John Wiley and Sons, Inc. New York, N.Y., 1100 pp.

Uvarov, B.P.	1928	Insect nutrition and metabolism. Trans. ent. Soc. Lond., 76 (2): 255-343.
Vanderzant, E.S.	1957	Growth and reproduction of the pink bollworm on an amino acid medium. J. econ. Ent., 50 (2): 219-221.
Vanderzant, E.S.	1959	Inositol: An indispensable dietary requirement for the boll weevil. J. econ. Ent., 52 (5): 1018-1019.
Vanderzant, E.S.	1965	Axenis rearing of the boll weevil on defined diets: amino acid, carbohydrat and mineral requirements. J. Insect Physiol., 11 (5): 659-670.
Vanderzant, E.S., Pool, M.C., and Richardson, C.D.	1962	The role of ascorbic acid in the nutrition of three cotton insects. J. Insect Physiol., 8 (3): 287-297.
Vanderzant, E.S., and Reiser, R.	1956a	Aseptic rearing of the pink bollworm on synthetic media. J. econ. Ent., 49 (1): 7-10.
Vanderzant, E.S. and Reiser, R.	1956b	Studies of the nutrition of the pink bollworm using purified casein media. J. econ. Ent., 49 (4): 454-458.
Verschaffelt, E.	1911	The cause determining the selection of food in some herbivorous insects. Proc. K. ned. Akad. Wet. Amsterdam Sec. Sci., 13: 536-542.
Waldbauer, G.P.	1964	The consumption, digestion and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco hornworm, Protoparce sexta (Johan.)(Lepidoptera: Sphingidae). Entomologia exp. appl., 7 (3): 253-269.

APPENDIX

APPENDIX I

Procedure for estimation of chromic oxide concentration.

Digestion mixture:

Dissolve 10 grams of sodium molybdate in 150 ml. of distilled water; slowly add 150 ml. of concentrated sulphuric acid. Slowly add with stirring 200 ml. of 70 per cent perchloric acid.

Colour-developing reagent:

A freshly prepared solution of diphenylcarbazide, 0.25 per cent (W/V), in 50 per cent acetone.

Procedure:

1) Weigh a sample containing 150 to 1,200 μg. of Cr_2O_3 into a 100 ml. kjeldahl flask.

2) Add 10 ml. of digestion mixture and heat for 30 minutes on a micro-kjeldahl digestion rack. In the first 15 minutes the colour changes from green to yellow or orange.

3) Remove from heat and cool to room temperature.

4) Dilute the digest to 500 ml. with distilled water.

5) Transfer 5 ml. of the diluted digest to a 12.5 ml. colorimeter tube and add 4.5 ml. of 0.25 N sulphuric acid.

6) Add 0.5 ml. of colour-developing reagent with a hypodermic syringe (1 ml. capacity).

7) Allow at least 3 minutes for colour development, and measure the absorbance at 540 mμ against a blank consisting of 9.5 ml. of 0.25 N sulphuric acid and 0.5 ml. of colour-developing reagent.