require, in addition, for optimal growth a substance contained in the water-insoluble residue of yeast, which was found to be biotin.

4. The optimal requirements of biotin for *Tribolium* are approximately 0.1 µg./g. of the dry diet, and the threshold of action is between 0.003 and 0.006 µg./g.

5. The insects tested do not appear to require any water-insoluble factor present in yeast in addition to a sterol and biotin.

6. The insects used, with the exception of *Sitodrepa*, grow on a mixture of pure B-vitamins as well as on yeast extract. It is concluded that these

insects do not require B-vitamins beyond those already isolated.

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The Sterol Requirements of Several Insects

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It has been shown [Fraenkel & Blewett, 1943a] that six insects which normally feed on flour and other stored food grow satisfactorily on a diet of casein 50, glucose or starch 50, yeast 5, cholesterol 1, salts 1 and water 15 parts. Without cholesterol, growth became slightly (*Tribolium*, *Silvanus*, *Lasioderma*), appreciably (*Ptinus*) or severely (*Sitodrepa*, *Ephestia*) impaired (Table 2 (1)). If the diet con-

tains aqueous yeast extract in place of yeast together with cholesterol, all the insects develop, though at a reduced rate resulting from a deficiency of biotin. Without cholesterol the diet becomes entirely or almost entirely inefficient for all the insects tested (Table 2 (3) and Fraenkel & Blewett [1943a]). In the present work the specificity of the sterol requirements of these insects is investigated.

EXPERIMENTAL

A convenient method of preparing a sterol-deficient diet is Soxhlet extraction of wholemeal flour with chloroform for at least 10 hr. Extraction with ether (Soxhlet) proved unsuccessful in removing the sterols sufficiently. Addition of various sterols fully restored the nutritional qualities of the flour. This effect was used as a means of comparing the nutritional values of sterols and sterol derivatives. In addition to the insects enumerated by Fraenkel & Blewett [1943*b*, p. 686], the flour moth *Ephestia kuehniella* Zell. was also used. The methods of experimenting and assessing results are fully described elsewhere [Fraenkel & Blewett, 1943*a*, *c*].

The following sterols and sterol derivatives were used:

Table 1. Growth on flour extracted with chloroform and with the addition of the extracted material or a number of sterols or sterol derivatives

Addition to basal diet	Growth						With basal diet of yeast and fructose, of <i>Dermestes</i>
	With basal diet of CHCl ₃ -extracted wholemeal flour, of						
	<i>Tribolium</i>	<i>Lasioderma</i>	<i>Silvanus</i>	<i>Pinus</i>	<i>Sitodrepa</i>	<i>Ephestia</i>	
None	+	+	++	±	-	-	-
Chloroform extract	++++	++++	++++	+++	+++	+++	-
Wheat-germ oil	++++	.	.	.	.	++++	.
Cholesterol	++++	++++	++++	++++	++++	+++	++++
Sitosterol	++++	++++	++++	+++	++++	+++	-
Ergosterol	++++	++++	+++	++++	++++	++	-
Cholestanol	++ (+)	++	+++	++	+++	+	-
Δ ⁵ -Cholestene	+ (+)	.	.	-	-	-	-
Zymosterol	+ (+)	++	+++	+ (+)	-	-	-
Calciferol	-	±	++	-	-	-	-
7-Dehydrocholesterol	++++	++++	++++	++++	++++	+++	++++
Cholesteryl-acetate	++++	.	.	.	++++	+++	++++
7-Hydroxycholesterol	±	.	.	.	-	-	-
7-Dehydrocholesteryl monobenzoate	++	.	.	.	-	-	++++
7-Hydroxycholesteryl dibenzoate	±	.	.	.	-	-	-
7-Ketocholesteryl acetate	-	.	.	.	-	-	-

The + and - signs give a rough quantitative estimate of the relative efficiency of diets. Explanation of the symbols in Fraenkel & Blewett [1943*a*].

Fig. 1. *Tribolium confusum*. Growth on wholemeal flour extracted with chloroform, and on extracted flour with the addition of wheat-germ oil, sterols or sterol derivatives.

Fig. 2. *Ephestia kuehniella*. Explanation as for Fig. 1.

The results of adding sterols and sterol derivatives to extracted flour are summarized in Table 1, and are given for *Tribolium* and *Ephestia* in Figs. 1 and 2. Adding cholesterol, ergosterol or sitosterol restored its original efficiency either entirely or almost entirely. Only with *Ephestia* wheat-germ oil proved more efficient than any single sterol (Fig. 2) and from these results, and others not yet published, it seems likely that other fat-soluble factors, possibly unsaturated fatty acids, are required by *Ephestia*. Destruction of such unknown factors during the prolonged chloroform extraction at a high temperature may explain why wheat-germ oil proved more efficient for *Ephestia* than the chloroform extract. We do not yet know whether small differences in the efficiency of cholesterol, ergosterol or sitosterol are significant. Of the other sterols tested, only 7-dehydrocholesterol and cholesterylacetate proved of about the same nutritional value as the three common natural sterols.

In evaluating the effect of sterols and sterol derivatives it has to be borne in mind that extracted flour proved entirely inefficient only for *Sitodrepa* and *Ephestia*, while some growth took place with the other species. This growth was almost certainly only possible through traces of sterols remaining in the flour after extraction. Furthermore, most of the derivatives of cholesterol which we tested were not pure and almost certainly contained cholesterol, while zymosterol was probably not free from ergosterol. Traces of any physiologically active sterol would, therefore, be expected to improve further the extracted flour for those insects which already showed some growth on it by virtue of small amounts of sterol retained. The insects which required large amounts of sterol, and which entirely failed to grow on extracted flour, viz. *Ephestia* and *Sitodrepa*, grew well only on cholesterol, sitosterol,

although inferior to the 'good' sterols. Calciferol (vitamin D₂) was not utilized by any of the insects tested.

DISCUSSION

It has been known for several years that sterols are growth factors for certain insects (literature in Fraenkel, Reid & Blewett [1941]) and this is now shown to be true for six more insects. It is also now certain that the fat-soluble factor necessary for *Ephestia kuehniella*, described by Richardson [1926] as vitamin A, is in fact a sterol. The last column of Table 1 contains the results of a previous investigation of the sterol requirements of another beetle, *Dermestes vulpinus* [Fraenkel *et al.* 1941]. The striking difference in the sterol requirements of *Dermestes* and the flour insects is that *Dermestes* will not grow on ergosterol or sitosterol. This limits its distribution to food of animal origin, while the flour insects grow equally well on a diet of vegetable origin (containing sitosterol), animal origin (containing cholesterol) or micro-organisms (containing ergosterol) [see Fraenkel *et al.* 1941; Fraenkel & Blewett, 1943c].

The present work confirms the observation that certain insects require sterols in the diet, and not calciferol (vitamin D₂), in which the tetracyclic arrangement of the carbon atoms has been changed and which is consequently not a true sterol. 7-Dehydrosterols are utilized as well as sterols with only one double bond in ring B (cholesterol, sitosterol). There is, however, no reason to assume that cholesterol and sitosterol are utilized by virtue of traces of the respective dehydrosterols which are usually associated with them in biological material. Cholesterol, purified via the dibromide and free of dehydrocholesterol, proved just as efficient as unpurified cholesterol. Of the cholesterol derivatives,

Table 2. *Growth on diets slightly (1) or grossly (2 and 3) deficient in sterols*

Diet	Growth of					
	<i>Tribolium</i>	<i>Lasioderma</i>	<i>Silvanus</i>	<i>Ptinus</i>	<i>Sitodrepa</i>	<i>Ephestia</i>
(1) Casein, starch, 5% yeast	+++	+++	+++	++	±	-
(2) Flour extracted with chloroform	+	+	++	±	-	-
(3) Casein, glucose, yeast extract	+	+	+	-	-	-

ergosterol, cholesteryl acetate and 7-dehydrocholesterol, and, to a lesser degree, on cholestanol (Fig. 2). From this it appeared probable that the seemingly positive effects of zymosterol on *Lasioderma* and *Silvanus*, and of Δ^5 -cholestene, dehydrocholesterol monobenzoate and zymosterol on *Tribolium*, were due to impurities. This does not apply to cholestanol, of which a pure sample was obtained on which even *Sitodrepa* and *Ephestia* grew fairly well. Cholestanol is, therefore, of some food value,

only the acetic ester retained full activity, and dihydrocholesterol (cholestanol) appeared of some food value. All the other structural changes, such as loss of the secondary alcohol group in Δ^5 -cholestene, introduction of a second alcohol group in 7-hydroxycholesterol, and esterification of this group in 7-hydroxycholesteryl dibenzoate or introduction of a keto-group on carbon atom 7 in 7-ketocholesteryl-acetate, caused loss of nutritional value.

The requirements for sterols seem, therefore, to be qualitatively the same for the six insect species under investigation, but there are obvious quantitative differences. From a comparison of the growth on three diets relatively deficient in sterols (summarized in Table 2) it can be concluded that *Sitodrepa*, *Ptinus* and *Ephestia kuehniella* require larger quantities of sterols than *Tribolium*, *Lasioderma* and *Silvanus*.

SUMMARY

The sterol requirements of six different insect species have been investigated; all the insects re-

quire a sterol in the diet for growth. The quantitative requirements of sterols by different insects are different, but the qualitative requirements of the insects under investigation are the same. The specificity of the sterol requirements was studied.

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Apparent Vitamin C in Foods

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Titration with 2:6-dichlorophenolindophenol is used to estimate vitamin C in a wide variety of foodstuffs, though the dye may react with substances besides ascorbic acid. Harris [1933] showed that dye-reducing substances can be produced by heating certain sugars with alkali. Johnson [1933] found with germinated peas that dye titrations indicated higher vitamin C contents than was shown by biological tests. In acid extracts part of the dye-reducing power was due to a substance which reduced the dye more slowly than ascorbic acid. Bacharach, Cook & Smith [1934] found an increase in the dye-reducing power of malt extract heated to 100° for $\frac{1}{2}$ –2 hr. McHenry & Graham [1935] obtained increases of 50–170% by heating certain vegetables for a few minutes at 58° or by standing these for a few hours at room temperature in 1% HCl. Ahmad [1935] found that boiling cabbage for 10 min. with 20% CCl_3COOH increased the apparent vitamin C content by nearly 200%. Reedman & McHenry [1938] found that the apparent vitamin C in heated potatoes resembled true vitamin C in both physiological and many chemical properties. Their suggestion that ascorbic acid might exist in combination with protein from which it was set free by heating was discredited by Harris & Olliver [1942], who suggested three possible causes for the

results: (1) incomplete extraction from unheated tissues, (2) variations in sampling, (3) inhibition by heat of the ascorbic acid oxidase in the foods examined. The possible occurrence of non-specific dye-reducing substances in vegetable tissues was considered of little or no practical importance, although it was admitted that germinated seedlings, malt extract, yeast and fermented juices might repay further study.

During the last three years we have been accumulating evidence to show that the apparent vitamin C content of foods can become considerably higher than the true vitamin C value under manufacturing and storage conditions which may occur in practice. Preliminary reference to these findings was made by Wokes, Johnson, Duncan, Organ & Jacoby [1942] and by Wokes, Organ, Duncan & Jacoby [1943a]. The provisional term 'apparent vitamin C' was proposed to describe substances occurring in natural and processed foods which, although not possessing antiscorbutic activity, simulate vitamin C very closely in their chemical and physical properties, so that they are not distinguished from it by the indophenol titration as usually applied, and may therefore cause results to be too high. Methods of estimating true vitamin C in the presence of this apparent vitamin C have