CCXXXVI. ON A FAT-SOLUBLE GROWTH FACTOR REQUIRED BY BLOW-FLY LARVAE.

II. IDENTITY OF THE GROWTH FACTOR WITH CHOLESTEROL.

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IN the previous paper of this series [Hobson, 1935], it was shown that blow-fly larvae (*Lucilia sericata* Mg.) respond to a fat-soluble growth factor. Since there was evidence that the active substance might be cholesterol, further growth tests have been carried out with samples from various sources. The results of these experiments leave little doubt that the larval growth factor is cholesterol.

METHODS.

The basal diet was the same as that used in the earlier experiments [Hobson, 1935]. It consisted essentially of a 15% peptone solution prepared from meat by digestion with pepsin and subsequent filtration. In addition, the preparations used in these experiments were extracted with ether to remove the last traces of fat. A small amount of aqueous yeast extract was added to the peptone solution so as to ensure a sufficiency of vitamin B. Cholesterol samples were tested in the following way. An emulsion was prepared by pouring an alcoholic solution into water and removing the alcohol by evaporation on a boiling water-bath. The emulsion was then filtered through cotton-wool and its sterol content determined by a dry weight estimation. Suitable mixtures of peptone solution and emulsion were prepared and 1 ml. lots absorbed on pieces of bandage in small test-tubes; three larvae were reared in each tube. Since fine emulsions could not be obtained with the other sterols, they were supplied as triolein solutions. The procedure adopted in this case was the same as that used in earlier experiments with different fats [Hobson, 1935]. In the present growth trials, the larvae were reared for 2½ days at 35°, then starved for 4 hours and weighed. A weight of 40 mg. was taken to represent satisfactory growth under these conditions.

Materials used.

Table I describes the origin of the samples tested. This work was considerably facilitated by gifts of sterols. I take this opportunity of thanking Prof. J. C. Drummond, Mr A. L. Bacharach and Mr A. R. Freney for specimens respectively of purified cholesterol, sitosterol and wool sterols.

Table I. *Description of the sterol samples.*

Material	Method of preparation and purification
Purified cholesterol	Regenerated from dibromide
Brain cholesterol	Extracted with acetone; recrystallised from alcohol
Egg cholesterol	Non-sap. from technical ovoidlecithin; recrystallised from alcohol
Wool cholesterol	Separated from wool wax [Freney, 1934]
Lanosterol	
Sitosterol	Crude sterol from wheat germ oil non-sap.; recrystallised from alcohol
Ergosterol	Presumably derived from yeast

RESULTS.

Preliminary trials with an emulsion of egg cholesterol showed that good growth occurred with 0.01 % of added sterol. The four samples were compared in two series, egg cholesterol being included in each. The results, given in Table II, show that the growth-promoting effect of cholesterol does not vary with the source and is not altered by chemical purification (conversion into bromide). It is, therefore, concluded that the active substance is not an impurity, but the sterol itself. It will be shown also that the potency of various fats can be correlated with their sterol content if allowance is made for the type of sterol. Other sterols stimulate growth but are less active than cholesterol (Table III).

Table II. *Effect of cholesterol on the growth of larvae on peptone diet.*

Sample	Mean weight of 3-day old larvae (mg.).					
	% sterol on volume of peptone solution					
	0.03	0.01	0.005	0.0025	0.001	0
Wool	—	43, 45	32, 34	20, 22	—	Died ca. 1 mg.
Brain	—	39, 43	35, 36	18, 24	—	
Egg	41, 44	39, 44	36, 37	25, 27	—	
Egg	41, 47	38, 48	37, 40	22, 27	9, 11	Died ca. 1 mg.
Purified	44, 49	44, 40	32, 38	24, 28	7, 10	

Table III. *Effect of various sterols on the growth of larvae on peptone diet.*

	Dose mg.	Weight of 3-day old larvae mg.
Cholesterol	0.06	39, 41, 44
	0.03	33, 40, 44
	0.015	x, 26, 29
	0.007	x, x, 15
Sitosterol	0.12	41, 46, 48
	0.06	x, 26, 33, 37
	0.015	x, x, x
Ergosterol	0.12	31, 35, 45
	0.06	x, 25, 28
	0.015	x, x, x
Lanosterol	0.12	23, 32, 39
	0.06	x, x, x
Controls	0	x, x, x, x

x indicates larva dead on 3rd day.

The sterol was given in triolein.

Solutions of sterol in triolein were used in these tests; the amount of oil given was the same in each case, 6 mg., except for the higher doses of ergosterol and lanosterol which required more solvent. Since blow-fly larvae normally breed only in animal tissues, it is not surprising to find that they utilise cholesterol more readily than sterols from other sources.

Growth under asepsis.

The experiments already described were carried out with larvae from unsterilised eggs. It is, therefore, possible that the sterol is not the growth factor itself, but the precursor of an active substance produced by bacterial action. Aseptic growth trials showed that this is not the case. Larvae grew readily under aseptic conditions on the peptone diet supplemented only with cholesterol (sterility being confirmed by inoculation of nutrient media); also, they pupated and produced normal adults. Since the diet contained no fat whatever, these results suggest that blow-fly larvae can synthesise fat but not sterol. Analyses are being carried out to confirm this.

Correlation between the sterol content and potency of natural fats and oils.

Table IV shows that the growth-promoting effect of various fats and oils can be correlated with their sterol content. The potencies were calculated from the inverse of minimum active dose [Hobson, 1935], cholesterol being assigned

Table IV. *Correlation between the sterol content and potency of various substances.*

	% non-sap.	% sterol	Potency
Cholesterol	—	100	100
Wool wax	40	27	25
Wheat germ oil	5.8	3.5	1
Egg yolk	—	1.3	0.8
Cod-liver oil	0.8	0.4	0.5
Butter	0.3	—	0.4
Lard	0.3	—	<0.4
Olive oil	0.9	—	<0.4

the arbitrary value of 100. Since the samples used in the growth tests were no longer available, the figures for the contents of unsaponifiable matter and sterols were taken from published analyses: wool wax [Dorée and Garratt, 1933]; wheat germ oil [Drummond *et al.*, 1935]; cod-liver oil [Drummond *et al.*, 1925]; egg yolk, mean value from various sources; the remainder, average figures from analyses quoted by Lewkowitsch [1922]. It must be realised that these values are subject to variation and that the error in assaying the potency was probably not less than $\pm 50\%$.

When expressed in this way, the potency should equal the percentage of the sterol, if this is the active substance. It will be seen that the animal fats and oils show good agreement. The potency of the two vegetable oils tested is considerably less than their sterol content (assuming that sterols form about half of the unsaponifiable matter of olive oil, *i.e.* 0.4–0.5%); this is to be expected since the activity of the typical plant sterol, sitosterol, proved to be about a quarter of that of cholesterol.

The effect of sterol on the immunity of larvae to bacterial infection.

The absence of sterols from the diet, besides affecting the growth rate directly, also renders larvae susceptible to bacterial infection [Hobson, 1935]. The natural immunity of larvae seems to be due to an acid secretion in the intestine; normally, the gut contents are strongly acid in the middle part of the mid-gut, the function of the acidity probably being bactericidal action [Hobson, 1931]. It has been found that the acidity in the mid-gut is dependent upon the presence of cholesterol in the food. This observation explains the "anti-infective" action of cholesterol and also confirms the view that the acid serves as an antiseptic. An account of the actual experiments will be given in a later communication dealing with the nature of the acid secretion. It is of interest to note that the alleged bactericidal action of blow-fly larvae has led to their being used therapeutically in the treatment of chronic osteomyelitis. This question is also of importance in relation to the development of septicaemia in sheep infested with blow-fly larvae.

DISCUSSION.

The results of the feeding tests show that the growth-promoting effect of cholesterol is the same for various samples, including one subjected to chemical purification. Also, the potency of natural fats and oils can be explained by their content of cholesterol or sitosterol. The sterol is not the precursor of a

vitamin formed by bacterial action, since the absence of bacteria does not affect its potency. It is, therefore, concluded that the fat-soluble growth factor required by blow-fly larvae is a sterol. Furthermore, a sterol is the only fat-soluble substance essential for larval growth, since the preparation of the basal diet was such as to remove all lipid matter. Blow-fly larvae, therefore, differ from higher animals; these can apparently dispense with sterols but need certain fatty acids in addition to vitamins occurring in the non-sterol fraction of the unsaponifiable residue.

It seems not improbable that a sterol may be an essential growth factor for insects generally; however, further investigation is clearly necessary before this question can be decided. The earlier work on the fat requirements of insects, reviewed in a previous paper [Hobson, 1935], shows no discrepancies between blow-fly larvae (Diptera) and other insects. In addition to *Drosophila* (Diptera), both *Blatta* (Orthoptera) and *Ephestia* (Lepidoptera) have been shown to respond to a fat-soluble growth factor (other than fat itself). If this substance is not a sterol, it follows that these insects require some "vitamin" which is not needed by blow-fly larvae, a hypothesis which seems very unlikely.

Since blow-fly larvae fail to grow without cholesterol, they presumably cannot synthesise this substance. However, this does not necessarily follow; yeast, for example, can synthesise bios and yet cannot develop in a medium completely lacking in bios. It is possible that blow-fly larvae can synthesise a part but not all of their sterol requirements. This question can only be settled by investigating the sterol balance. Bloor [1934], discussing the question of cholesterol synthesis, says: "It is now generally admitted that cholesterol can be synthesized by the animal organism." The evidence for this statement is based on experiments with higher animals; little appears to be known about the sterol metabolism of invertebrates.

SUMMARY.

1. Purified cholesterol supplies an essential growth factor required by blow-fly larvae.
2. The potency of cholesterol samples of different origin is the same, the minimum active dose being about 0.07 % of the dry weight of the diet.
3. Ergosterol, lanosterol and sitosterol also promote larval growth, but are less active than cholesterol.
4. The potency of natural fats and oils can be correlated with their content of cholesterol or sitosterol.
5. It is concluded that the fat-soluble growth factor is the sterol itself.
6. Blow-fly larvae do not require fatty acids or fat-soluble vitamins present in the non-sterol fraction of the unsaponifiable residue.

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REFERENCES.

- Bloor (1934). *Ann. Rev. Biochem.* **3**, 187.
Dorée and Garratt (1933). *J. Soc. Chem. Ind.* **52**, 141 T.
Drummond, Channon and Coward (1925). *Biochem. J.* **19**, 1047.
— Singer and Macwalter (1935). *Biochem. J.* **29**, 456.
Freney (1934). *J. Soc. Chem. Ind.* **53**, 289 T.
Hobson (1931). *J. Exp. Biol.* **8**, 109.
— (1935). *Biochem. J.* **29**, 1292.
Lewkowitsch (1922). *Chemical technology of oils, fats and waxes*, **2**. (Macmillan, London.)