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

I. DISTRIBUTION AND PROPERTIES.

By RALPH PERCIVAL HOBSON[1].

From the Department of Entomology,
London School of Hygiene and Tropical Medicine.

(*Received April 11th, 1935.*)

Although there is good evidence that insects require vitamin B [see Uvarov, 1928; Hobson, 1933], little is known of their need for fat-soluble growth factors. The previous work will not be reviewed in detail at this stage, except to say that the suggestions of some workers that insects require vitamin A or E have little foundation. The present work arose from the observation that filtration of peptic digests of meat removes a substance which is essential for the growth of blow-fly larvae (*Lucilia sericata* Mg.) and which occurs in certain fats. An investigation was therefore made of the distribution and properties of the active substance.

Methods.

The basal diet used in this work was prepared as follows. Lean beef was digested with pepsin for 4 days at p_H 2, the digest being filtered through filter-

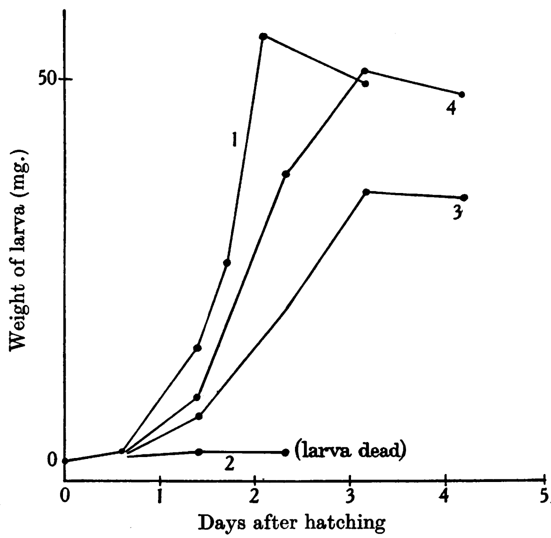

Fig. 1. Growth of blow-fly larvae on meat and peptone. 1, Meat; 2, peptone; 3, peptone + 2 mg. cod-liver oil; 4, peptone + 8 mg. cod-liver oil.

paper and the solution concentrated on a water-bath. After a small amount of aqueous yeast extract had been added and the reaction adjusted to p_H 6 with

[1] Now at the School of Agriculture, University College of North Wales, Bangor.

sodium hydroxide, the mixture was diluted with water to make the concentration of peptone 15 % (based on a dry weight estimation made before the addition of yeast extract and alkali). This solution was stored in a refrigerator with chloroform as an antiseptic; before use samples were heated to remove the chloroform. Attempts to rear larvae on oil emulsions gave unsatisfactory results. Since the peptone solution could not be properly mixed with fat, one larva was used in each test. 0·3 ml. of peptone solution was absorbed on 60 mg. of cotton wool, pressed into a pellet, in a small test-tube. The oil was measured out with a platinum loop and smeared over the cotton wool; under standardised conditions, the loop delivered approximately 2 mg. of oil. One egg was placed in each tube, which was closed with fine muslin gauze, and the larvae were reared at 27° in a moist incubator. The amount of peptone supplied was equivalent to 180 mg. of fresh muscle; this provided sufficient food since a larva can grow to the full size (50 mg.) on 90 mg. of muscle.

Fig. 1 shows the growth of larvae on peptone and on muscle. On peptone alone, larvae fail to grow and usually die on the second day; however, they remain alive under aseptic conditions for 5 or 6 days without growing. When an active oil is given in excess, growth is almost normal and the larva reaches the full size in 70–80 hours; with an insufficient amount of the essential factor, growth is slower and the final weight less. Fig. 1 includes some results obtained with cod-liver oil. Since the rate of growth was not usually so uniform as this, the weight on the fourth day was taken as the criterion of growth.

Table I. *The effect of various substances on the growth of blow-fly larvae on peptone diet.*

Substance	Dose (mg.)	Weights of larvae (mg.) on 4th day
Cod-liver oil	8·0	43, 46, 47, 52
	4·0	30, 32, 42, 43
	2·0	16, 34, 38
	1·0	12, 21, 27
Butter	8·0	27, 37, 42, 51
	4·0	21, 25, 36
	2·0	12, 17, 25
Beef muscle oil	0·8	47, 49, 51
	0·4	32, 47, 48
	0·2	17, 25, 35
Wheat germ oil	4·0	48, 49, 51
	2·0	37, 40, 43, 46
	1·0	33, 39, 40
	0·5	24, 30, 36
Wool wax	0·5	45, 49
	0·25	45, 47
	0·12	33, 47, 48
	0·06	16, 25, 35
Egg yolk	15·0	50, 53
	7·5	44, 48
	3·7	43, 49
	1·9	28, 35
Ovolecithin	3·0	47, 50
	1·5	42, 46
	0·7	29, 36
Lard	8·0	4, 5, 7, 11 } Weights on 3rd day. Larvae usually died before 4th day
Olive oil	8·0	3, 4, 5, 5, 7 } Weights on 3rd day. Larvae usually died before 4th day
Nil	—	Larvae died on 2nd or 3rd day, weight about 1 mg.

Table II. *Distribution of the growth factor.*

Substance	Potency	Active dose mg.
Wool wax	+ + +	0·1
Beef muscle oil	+ + +	0·4
Ovolecithin (technical)	+	2
Wheat germ oil	+	3
Egg yolk	+	4
Cod-liver oil	+	6
Butter	+	8
Lard	–	>8
Olive oil	–	>8

Active dose = smallest amount required for normal growth of 1 larva on 0·3 ml. of peptone solution.

Effect of various fats and oils on growth.

In these experiments the fat-soluble substances were diluted with an inactive oil, olive oil, and tested at different concentrations, each larva receiving 8 mg. of oil. Tests were also made with egg yolk and commercial ovolecithin mixed with the peptone solution as suspensions. The mortality in these trials was high, especially with certain oils and at low concentrations; when the larva was dead or moribund on the fourth day, the experiment was repeated. The results of the successful tests are collected in Table I and summarised in Table II. The active dose was taken to be the smallest amount which produced a larva weighing more than 45 mg. on the fourth day from hatching.

Reference to Table II shows that cod-liver oil and butter fat proved relatively poor sources of the growth factor; wool wax and muscle oil were the most active of the substances tested, wheat germ oil and ovolecithin giving intermediate values. Lard and olive oil were almost inactive. These results show clearly that the active substance is not vitamin A, D or E. Vitamins A and D are present in large amounts in cod-liver oil and butter; also they are absent from wool wax [Drummond and Baker, 1929], the richest source of the larval factor. Since wheat germ oil proved less potent than muscle oil and wool grease, the larval factor is not vitamin E. It should be noted that the muscle oil was obtained from a sample of beef which yielded only 1·3 % of ether-soluble material, the extract being very rich in unsaponifiable matter.

Chemical properties of the growth factor.

In order to test the stability of the factor to oxidation, growth tests were carried out with cod-liver oil, which had been aerated for 24 hours at 90–95°. This product appeared to be toxic as a large proportion of the larvae died. However, since some grew tolerably well, it is concluded that the active substance is resistant to oxidation. It is also stable to alkaline hydrolysis and can be recovered quantitatively in the unsaponifiable residue. Lecithin was used for chemical fractionation since it is rich in the blow-fly factor; it also stimulates the growth of *Drosophila* on a synthetic peptone diet [Guyenot, 1917]. 40 g. of technical ovolecithin, hydrolysed with alcoholic potash on a boiling water-bath for 45 minutes, yielded 1·4 g. of unsaponifiable material. This was fractionated by recrystallisation from alcohol into colourless crystals of cholesterol and a dark-coloured gummy material which consisted largely of cholesterol. Growth tests with olive oil solutions showed that the unsaponifiable residue contained the whole of the original potency. The active dose, for 1 larva on 0·3 ml. of peptone solution, was 30–60γ, equivalent to 1–2 mg. lecithin; the minimum amount of lecithin which produced good growth was about 2 mg. (when emulsified in the same volume of peptone solution). Recrystallisation of the unsaponifiable residue from alcohol did not concentrate the activity, since both fractions proved equally potent. In order to determine whether the active substance is cholesterol itself or an impurity, further growth trials are being made with sterol samples from different sources.

DISCUSSION.

The results of the present experiments show that blow-fly larvae require a fat-soluble factor which is present in the unsaponifiable residue. This substance not only stimulates growth, but seems to possess, also, an "anti-infective" function. Under normal conditions *Lucilia* larvae are highly resistant to bacterial infection and grow readily on meat however badly decomposed. This immunity extends also to diets which are deficient in vitamin B and mineral salts; thus, although larvae grow extremely slowly on infected serum, they show no other ill effects. With the peptone diet alone, death always occurred after two or three days; also, larvae receiving small doses of the fat-soluble factor often died before the end of the experiment. The cause of death was excessive bacterial action since the mortality was reduced by sterile precautions and was not found in aseptic cultures. The fat-soluble factor seems, therefore, to play a part in the immunity of these larvae to infection.

The distribution of the growth factor shows clearly that it is not vitamin A, D or E; thus, wool grease proved more than ten times as potent as cod-liver oil or wheat germ oil. However, the results obtained with blow-fly larvae agree with the findings of previous workers on insects. Guyenot [1917] showed that *Drosophila* larvae require a growth factor which is supplied by yeast fat or egg lecithin, but not by triolein. Bacot and Harden [1922], when studying the response of this insect to vitamin B, found it necessary to include butter in the basal diet. Richardson [1926] showed that *Ephestia* larvae could not grow on wheat flour which had been extracted with fat solvents; wheat oil and egg yolk restored growth, olive oil and lard being ineffective. Although butter gave poor results, Richardson suggested that the active substance might be vitamin A. Moskalenko [1933] concluded that cockroaches require vitamin E and possibly other fat-soluble growth factors. Using an artificial diet, he found wheat-germ oil the most effective in stimulating growth; fish oil (? liver oil)[1] was also active, butter producing slow growth. Zabinski [1926; 1928], working with the same insect, obtained inconclusive results. Michelbacher *et al.* [1932] found that blow-fly larvae could not develop aseptically on a synthetic diet unless cod-liver oil or butter was included. Table III summarises the responses of various insects to fat-soluble growth factors.

The fat-soluble vitamin requirements of higher animals do not apparently differ in the same way as do their needs for water-soluble vitamins. It may be assumed, therefore, in the absence of evidence to the contrary, that the fat-soluble growth factors required by insects do not vary among different types. It will be seen from Table III that the results obtained with different insects bear a general resemblance. Since the blow-fly factor is not vitamin A, D or E, it is inferred that insects do not require the same fat-soluble growth factors as higher animals. Furthermore, the present results suggest a relationship between the blow-fly factor and sterol. Thus, the only substance which compared in activity

[1] I was unable to see the original paper, but was fortunate in borrowing an English translation. The fish oil was probably a liver oil since Moskalenko refers to it as well known to be rich in vitamin A.

Table III. *The response of insects to fat-soluble growth factors.*

Insect	*Lucilia*		*Blatta*	*Ephestia*	*Drosophila*	
Investigator ...	Hobson	Michelbacher *et al.*	Moska-lenko	Richardson	Bacot and Harden	Guyenot
Wool wax	5	—	—	—	—	—
Wheat germ oil	4	—	3	2	—	—
Egg yolk	3	—	—	2	—	+
Cod-liver oil	2	+	2	—	—	—
Butter fat	1	+	1	1	+	—
Lard	0	0	—	0	—	—
Olive oil	0	—	—	0	—	—
Triolein	0	—	—	—	—	0

1, 2, 3 *etc.* (in same vertical column): order of increasing activity.
0 inactive.
+ active (no indication of potency).

with wool wax (mainly composed of sterol esters) was a sample of beef oil (Table II), which was undoubtedly rich in sterol. The other substances tested were relatively poor both in sterol and in the growth factor. Also, a sample of cholesterol obtained from ovolecithin proved highly active. The nature of the growth factor will be considered more fully in a later paper giving the results of tests with sterols from various sources.

Summary.

1. Muscle oil contains a substance which is essential for the growth of blow-fly larvae. Other sources of the growth factor are, in order of decreasing potency: wool wax, wheat germ oil, egg yolk, cod-liver oil, butter. Olive oil and lard have little or no effect on growth.

2. These results agree with the observations of previous workers on insects, but the distribution of the blow-fly factor shows that it is not vitamin A, D or E.

3. The growth-promoting effects of various substances appear to run parallel with their sterol contents.

4. The active substance is present in the unsaponifiable residue after hydrolysis.

5. There was some evidence that the growth factor possesses also an "anti-infective" function.

I wish to record my thanks to the Agricultural Research Council for a grant which entirely financed this work.

REFERENCES.

Bacot and Harden (1922). *Biochem. J.* **16**, 148.
Drummond and Baker (1929). *J. Soc. Chem. Ind.* **48**, 232 T.
Guyenot (1917). *Bull. Biol. Fr. Belg.* **51**, 229.
Hobson (1933). *Biochem. J.* **27**, 1899.
Michelbacher, Hoskins and Herms (1932). *J. Exp. Zool.* **64**, 109.
Moskalenko (1933). *Nutr. U.S.S.R.* **2**, 28.
Richardson (1926). *J. Agric. Res.* **32**, 895.
Uvarov (1928). *Trans. Ent. Soc. London*, 255.
Zabinski (1926). *Compt. Rend. Soc. Biol.* **94**, 545.
—— (1928). *Compt. Rend. Soc. Biol.* **98**, 73, 78.