CCLX. GROWTH OF BLOW-FLY LARVAE ON BLOOD AND SERUM.

I. RESPONSE OF ASEPTIC LARVAE TO VITAMIN B.

BY RALPH PERCIVAL HOBSON.

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

(Received November 2nd, 1933.)

THE experiments of Weinland [1906] upon blow-fly larvae are frequently quoted as the classical example of the synthesis of fat from protein. His work, however, is open to criticism as the analytical methods used are no longer considered reliable and the part played by bacteria was not determined. The present investigation arose out of an attempt to repeat Weinland's experiments with larvae reared aseptically on sterile blood. This diet proved inadequate for normal growth, being deficient in accessory food factors which can be supplied by yeast. The present paper deals with the response of blow-fly larvae to these growth factors. The species used was *Lucilia sericata* Meig., the Sheep Maggot Fly. These flies sometimes lay their eggs in the wool of live sheep, the larvae producing extensive sores on the skin. According to recent work in Australia [Mackerras and Freney, 1933], when maggots develop on sheep they feed at first on a serous exudate from the skin and later on blood. The nutritional value of serum and blood is therefore important in relation to the sheep blow-fly problem. Furthermore, blood is the diet of many insects which transmit diseases of man and animals.

The literature on the vitamin requirements of insects has been reviewed by Uvarov [1928] and Imms [1931] and will not be considered in detail here. Several workers have claimed that insects require "vitamin B," on the grounds that the addition of crude yeast preparations to deficient diets improves growth. Since "vitamin B" is now known to be a complex of several substances, evidence of this type does not justify the assumption that insects require the same water-soluble vitamins as higher animals. Sweetman and Palmer [1928] appear to be the only workers who have tested vitamin B₁ concentrates with insects; they reared flour beetles on a synthetic diet and found that brewer's yeast retained part of its activity after fractionation by the method of Levene and van der Hoeven; they concluded that the beetle's vitamin requirements are plural and in part identical with vitamin B₁. With regard to the vitamin requirements of blow-fly larvae, the present author found sterile muscle to be deficient in a growth factor which is present in yeast extract [Hobson, 1932, 2]; bacterial synthesis normally supplies this factor. The active substance was found to be heat-stable and it was suggested that it was not identical with vitamin B₁ or B₂. Experiments with muscle sterilised at a high temperature showed that the larvae also require a heat-labile substance present in yeast extract.

The results of the feeding experiments with blood have confirmed the composite nature of the vitamin requirements of blow-fly larvae. For normal growth

to occur under asepsis with this diet, at least three growth factors must be added: a substance absent from aqueous or alcoholic extracts of yeast, but present in a soluble form in yeast autolysate; a heat-labile factor which can be supplied by Peters's antineuritic concentrate; heat-stable substances present in autoclaved yeast extract.

EXPERIMENTAL.

Since certain micro-organisms synthesise the growth factors under examination, all the experiments were carried out aseptically. The method employed for sterilising blow-fly eggs has already been described [Hobson, 1932, 1]. Sterile horse-blood (Allen and Hanbury) was used; for each test 0.6 cc. of blood was diluted with 0.5 cc. of the test solution and absorbed on muslin bandage in a plugged test-tube. All the solutions and apparatus used were previously sterilised and the mixtures prepared with aseptic precautions. In order to coagulate the blood and ensure sterility, the tubes were then heated for 30 minutes at about 75° on 2 successive days. Three sterilised eggs were placed in each tube and the larvae reared in a moist incubator at 23–24°. In each experiment, a series of 12 to 20 tubes was run together, all the eggs being obtained from a cluster laid by one female.

Fig. 1 illustrates the effect of a deficient diet upon larval growth; these results were obtained with larvae reared without aseptic precautions upon blood absorbed on cotton-wool. With blood supplemented with an aqueous yeast extract (curve A) or marmite (curve B), normal growth occurs. It may be noted that the relative growth rate is greatest in the first 48 hours, during which the weight increases about 100 times (0.05 to 5 mg.); the larvae attain a mean weight of 50 mg. in 4 days, after which little or no further increase occurs. On blood alone (curve C) growth is nearly normal during the first 2 days; subsequently the larvae develop slowly though they will in time reach full size. In the aseptic experiments the larvae were usually weighed on the 4th day, when the maximum difference occurs; growth curves were obtained by weighing larvae from separate tubes at intervals. Sterility was checked by inoculating nutrient media from the food residues.

Fig. 1.

Fig. 2.

Fig. 1. Growth of unsterilised larvae on blood. A, + yeast extract; B, + marmite; C, alone.

Fig. 2. Response of aseptic larvae to marmite and yeast extract. A, blood + marmite; B, blood + yeast extract; C, blood alone. Each point was determined with a separate culture.

Response to a growth factor present in yeast autolysate.

When larvae were reared aseptically on blood alone, they completely failed to grow and weighed less than 1 mg. after 4 days. Tests were then made with blood admixed with aqueous yeast extract, fat and marmite. Yeast extract produced only a slight improvement in growth and no material difference was found when various fats including cod-liver oil were also given. On the other hand, larvae receiving marmite grew at almost the normal rate. Since non-sterile larvae develop readily upon a mixture of blood and yeast extract, it was concluded that blood is deficient in an essential growth factor which is present in marmite and is synthesised by micro-organisms; for convenience this will be referred to as the marmite factor. For studying the properties of this substance, the following basal diet was used:

Diet A. 6 cc. blood.

1 cc. concentrated aqueous extract of yeast (1 cc. $\equiv$ 3 g. fresh D.C.L. yeast).

0.7 cc. of this mixture was added to 0.4 cc. of the test solution.

Fig. 2 shows the growth of aseptic larvae on blood and the effect of adding yeast extract and marmite. The fact that larvae continue to grow slowly upon the basal diet (curve *B*) suggests that traces of the marmite factor are present; this is not supplied by the yeast extract since increasing the amount has no effect (Table I). Horse-blood probably contains a small amount of the active substance since larvae grow better on serum than on whole blood when an excess of yeast extract is supplied; with serum the mean weight of 4-day old larvae varied in different experiments from 20 to 30 mg. and with blood from 6 to 14 mg. If the substance is contained in the plasma, removal of the corpuscles would more than double the ratio of active substance to protein.

Table I gives the results obtained with various substances and extracts.

Table I. *The effect of various substances on the growth of aseptic larvae on a mixture of blood and aqueous yeast extract (diet A).*

Substance tested	Amount expressed as % of fresh material (or its equivalent) on volume of blood	Increase in mean weight (mg.) over negative controls after 4 days' growth
Yeast autolysate	$\equiv$ 30	46, 40
"	$\equiv$ 20	29, 32
	$\equiv$ 45	44
Ditto, autoclaved 4 hours	$\equiv$ 30	28
	$\equiv$ 15	16
Aqueous extract of yeast	$\equiv$ 50	2, 0, -1
70 % alcoholic extract of yeast	$\equiv$ 50	2, 1
Aqueous extract of egg-white	$\equiv$ 50	-1, -2
Alcoholic extract of wheat-germ	$\equiv$ 20	0, -2
Aqueous extract of beef muscle	$\equiv$ 40	2, 1
	25	-3
Commercial meat extract, Bovril	15	-2
	5	0
90% alcohol-soluble fraction from peptic digest of beef	$\equiv$ 50	37, 33
Pepsin	7	14, 12
"	3.5	6, 5
Trypsin	7	30, 33
Yeast nucleic acid	0.3	0, -2
Glycerol	1	-3, -4
Ether-soluble fraction of marmite	$\equiv$ 30	2, 1
Olive oil	5	5, 3

Both muscle and yeast contain the marmite factor, but all attempts to obtain active extracts without digestion failed. Extracts prepared by boiling yeast with 0.1 % acetic acid, 70 % alcohol, 50 % alcohol and 50 % alcohol containing 1 % of strong hydrochloric acid proved inactive. Aqueous extracts of yeast which had been allowed to stand 3 days at about 20° appeared to be slightly active but the differences were scarcely significant; Peters's antineuritic concentrate had no effect in large doses. Active extracts were obtained by mixing yeast with half its weight of water and incubating for 8 days at 35° in the presence of toluene, the mixture being then boiled for 3 minutes and filtered. The presence of the marmite factor in muscle was inferred, since aseptic larvae grow readily on sterile muscle supplemented with aqueous yeast extract [Hobson, 1932, 2]. Successive extraction of cod-muscle with water, 50 % alcohol and 90 % alcohol failed to dissolve the substance, but an active preparation was obtained from beef muscle by the following method. Muscle was digested with pepsin solution for 10 days, the mixture filtered and the filtrate concentrated and poured into 4 volumes of 98 % alcohol. The alcohol-soluble fraction contained the active substance; control tests showed that the activity was not supplied by the pepsin.

Stability to heat. Experiments with alkalis marmite gave unsatisfactory results which could not readily be interpreted; alkaline autoclaving appears to produce substances which are either toxic or repugnant to the larvae. The marmite factor is stable to heat in weakly acid solution since extracts of auto-lysed yeast remained active after 4 hours' autoclaving (22 lbs.; p_H ca. 6.0).

Fractionation from marmite. An attempt was made to concentrate the active substance from marmite, the main object being to obtain preparations free from other growth factors required by the larvae. The method finally adopted was fractionation with alcohol and ether; by this means, although considerable loss of activity occurred, concentrates were obtained rich in the marmite factor but deficient in heat-stable substances essential for larval growth. The procedure varied slightly in different experiments, but the general method was as follows. An alcoholic extract was prepared by stirring 100 g. of marmite into a paste with 20 cc. of water and adding sufficient 98 % alcohol to make the concentration of alcohol 70 %, the precipitate being filtered and washed with a small volume of 70 % alcohol. The filtrate was evaporated *in vacuo* to dryness and extracted with 200 cc. of hot 88 % alcohol. This extract, which contained most of the original activity, was treated with half its volume of ether and the precipitate filtered off; the filtrate proved inactive. The ether precipitate was then extracted successively with 50 cc. of hot 98 and 90 % alcohol; both these extracts and the residue were active.

Table II shows the results of some of the feeding experiments. With crude marmite, normal growth (a mean weight of 40 mg. after 4 days) occurred with 10 % in the diet, but not with 7.5 % (percentage based on the volume of blood). 90 % alcoholic extracts, prepared by pouring 50 % marmite solution into 10 volumes of 98 % alcohol, appeared to be slightly more effective than the original product; normal growth occurred with an amount corresponding to 7.5 % of marmite. The most concentrated preparation was that obtained by extraction of the ether precipitate with 90 % alcohol, the amount of organic matter in the minimum active dose being approximately 0.3 % of the volume of blood, or 1.5 % of the dry weight; this fraction contained about 30 % of the original activity.

Attempts were also made to concentrate the active substance by precipitation and by adsorption on norite charcoal. After precipitation with normal

Table II. *The effect of marmite fractions on the growth of aseptic larvae on diet A.*

Substance	Marmite equivalent as % on blood volume	Series	Mean weight of 4-day old larvae mg.		
			1	2	3
Controls	0	7		10	8
Marmite	15	41		47	44
	10	40		43.5	—
	7.5	34		36	—
	5	19		26.5	—
Crude 90 % alcoholic extract	15	—		50	45
	10	—		53	47
	7.5	—		42	40
	5	—		35	27
90-98 % alcohol- soluble fraction	40	—		45	42
	30	—		43	45
	20	—		36	30
	10	—		17	15

lead acetate, both precipitate and filtrate were active but considerable loss of activity occurred. Adsorption with norite charcoal removed the active substance from extracts prepared by fractionation with alcohol, but the activity could not be recovered in appreciable amount by extracting the charcoal with *N*/10 hydrochloric acid or acid alcohol of different concentrations.

Nature of the marmite factor. The active substance in marmite is readily soluble in water, sparingly soluble in 90 % alcohol and insoluble in ether. It is not therefore a fat-soluble growth factor. Since active extracts could not be prepared from muscle and yeast without digestion, the substance is either a product of digestion, such as an amino-acid, or a soluble compound which is adsorbed by proteins or other substances that are broken down during digestion. It seems unlikely that the substance is an amino-acid since the proteins of blood and muscle do not appear to differ appreciably in their content of essential amino-acids; also, on a diet almost devoid of fat and carbohydrate, only a small part of the amino-acids would be utilised for protein synthesis and protein quality is accordingly less important. The insolubility of the substance in yeast differentiates it from most members of the vitamin B complex. However, Hunt [1928] obtained evidence that an insoluble substance in yeast stimulates the growth of rats. Williams and Lewis [1930] concluded that for optimum growth rats require a substance which is present in yeast residues after extraction and may be identical with Hunt's factor. According to Williams and Lewis, the active substance is heat-stable and insoluble in water and alcohol; in these respects it resembles the "insoluble" growth factor required by blow-fly larvae.

Response to Peters's antineuritic concentrate.

Tests with autoclaved yeast have shown that blow-fly larvae require a heat-labile growth factor which is probably identical with vitamin B₁. It may be noted that Hoagland [1923] found ox-blood deficient in vitamin B₁. Aseptic larvae were found to grow readily on horse-blood admixed with an aqueous extract of autolysed yeast, but with an autoclaved extract no growth occurred after the second day when the larvae had reached only 2 % of the maximum weight; normal growth was restored by supplementing this diet with aqueous yeast extract, alcoholic extract of wheat germ or Peters's antineuritic concentrate.

The basal diet used was prepared as follows:

Diet B. 10 cc. horse-blood.

5 cc. autoclaved yeast autolysate (1 cc. $\equiv$ 0.9 g. fresh D.C.L. yeast).

0.9 cc. of this mixture was diluted with 0.2 cc. of the test solution. The autolysate was prepared as described on p. 1902 and autoclaved 4 hours at 22 lbs. pressure. Fig. 3 shows the growth of aseptic larvae on the basal diet and the effect of adding antineuritic concentrate; the growth rate of larvae receiving antineuritic concentrate was normal and approximately the same as that of larvae reared on meat without aseptic precautions.

Fig. 3.

Fig. 4.

Fig. 3. Response of aseptic larvae to Peters's antineuritic concentrate. Full line, larvae receiving concentrate; broken line, controls. Each point was determined with a separate culture.

Fig. 4. Relationship between growth-promoting effect and antineuritic potency of vitamin B₁ concentrates. Full line, preparation A, pigeon dose activity *ca.* 3 mg.; broken line, preparation B, pigeon dose activity *ca.* 2.5 γ .

Owing to the kindness of Prof. Peters in supplying tested concentrates of different purity, it was possible to apply a critical test as to the identity of the heat-labile larval growth factor and vitamin B₁. Two specimens of antineuritic concentrate were compared for their ability to promote larval growth; preparation A was an acid-alcohol extract having a pigeon dose activity of approximately 3 mg.; B was a purer preparation whose activity was approximately 2.5 γ . The results, given in Table III and Fig. 4, show that the growth-promoting

Table III. *The response of aseptic larvae to Peters's antineuritic concentrate (basal diet B).*

Number of pigeon doses added to 0.6 cc. blood	Mean weight of 4-day old larvae			
	Series 1	Series 2		Series 3
Type of preparation	A	A	B	B
0.08	46	—	—	45
0.06	44	50	42	38
0.05	—	—	—	40
0.04	39	37	29	24
0.02	27	16	10	—
0.01	5	9	5	—
0	1	1.5		1

effect of these concentrates closely followed their antineuritic activity. The amounts required to produce 4-day old larvae weighing 40 mg., when added to 0.6 cc. of blood, were approximately 0.04 to 0.05 pigeon doses of the relatively crude preparation A and 0.05 to 0.06 pigeon doses of the purer material. This agreement is surprisingly good in view of the wide difference in the nature of the biological tests involved and in the purity of the material, preparation B being about 1000 times more concentrated than A.

Kinnersley *et al.* [1933], discussing the purity of their crystalline preparations of vitamin B₁, suggest that the pigeon dose activity of the vitamin itself may be less than 1.0γ. The qualitative evidence that blow-fly larvae respond to a preparation of 2.5γ activity does not therefore prove that the effective substance is vitamin B₁ and not an associated impurity having similar chemical properties. However, the quantitative relationship observed with concentrates of different purity appears to eliminate the latter possibility, since it is unlikely that an impurity would follow the vitamin quantitatively through a process of fractionation which concentrates the activity a thousandfold. It is therefore concluded that the heat-labile growth factor required by blow-fly larvae is identical with vitamin B₁.

Response to heat-stable growth factors.

In addition to the marmite factor and antineuritic concentrate, blow-fly larvae also require heat-stable substances present in aqueous yeast extract but deficient in blood. For studying these growth factors, larvae were reared on blood mixed with antineuritic concentrate and a marmite extract prepared by fractionation with alcohol, the amounts of each being in excess of that required for optimum growth. The method of preparing the marmite concentrate was briefly as follows. An alcoholic extract was made by precipitating a suspension of marmite in water with 10 volumes of 98 % alcohol; this was treated with half its volume of ether and the precipitate extracted successively with 98 and 90 % alcohol. Tests on these extracts when mixed with blood and antineuritic concentrate showed that each produced equally poor growth; the larvae developed normally if autoclaved yeast extract was added to the diet. The basal diet adopted was prepared as follows:

Diet C. 6 cc. blood.

0.5 cc. 90–98 % alcohol-soluble fraction of marmite (1 cc. ≡ 5 g. marmite).

0.5 cc. Peters's antineuritic concentrate (1 cc. ≡ 2 pigeon doses).

0.7 cc. of this mixture was diluted with 0.4 cc. of the test solution. Since larvae grew readily on this diet when infected with a bacillus isolated from blown meat, it was assumed that the food was not deficient in mineral salts. However, later experiments showed that yeast ash stimulates the growth of larvae in the presence of bacteria, the effective ion being phosphate. Further aseptic tests were therefore carried out to determine whether the results obtained with yeast extract were due to phosphate. The following extracts were used in these experiments.

Autoclaved yeast extract. Yeast was extracted by boiling with water and the extract autoclaved 4 hours at 22 lbs. pressure.

Alkalised yeast extract. An aqueous extract was treated with a strong solution of NaOH until the p_H was approximately 10.6 and autoclaved 4 hours at 22 lbs. pressure; the solution was then made faintly acid.

Egg-white extract. An aqueous extract of egg-white was prepared by the method described by Chick, Copping and Roscoe [1930]; the sulphuric acid used to acidify the egg-white was removed by adding barium chloride solution until only traces of sulphate remained in solution.

Tests were made with these preparations to determine whether the deficient substance was vitamin B₂ or an extremely heat-stable substance which stimulates the growth of *Lucilia* larvae on sterile muscle [Hobson, 1932, 2]. Egg-white is rich in vitamin B₂; according to Chick and Copping [1930] this vitamin is destroyed in aqueous yeast extract by 4 hours' autoclaving at p_H 10.6. The larval growth factor deficient in muscle resisted 3 hours' autoclaving at p_H 9.0 in yeast extract.

Table IV. *Growth of aseptic larvae on diet C.*

Amounts refer to the equivalent fresh weight of material extracted.

Substance added	Series	Mean weight of 4-day old larvae (mg.)				
		1	2	3	4	5
Controls		12.5	11.5, 13	8, 10.5	10, 13	12.5, 13.5
Autoclaved yeast extract	10 %	39.5	40, 42.5	39, 44	—	—
Alkalised yeast extract	15 %	—	24.5, 26.5	27, 31	31, 32.5	27, 29.5
Do.	30 %	27	21.5, 23	—	—	—
Alkalised yeast extract + egg-white extract	15 % } 25 % }	34	31, 34	28, 30	—	—
Egg-white extract	25 %	10	11.5, 14.5	10, 13	—	—
Do.	50 %	—	11.5, 12.5	—	—	—
Sodium phosphate	0.5 %	—	—	—	14, 15	10, 12.5
Infected with a bacillus isolated from larvae		—	43, 47	—	—	—

Preliminary tests with the egg-white and alkalised yeast extracts showed that both were toxic in large amount; either extract when given alone depressed growth, but the addition of both produced a slight improvement over the negative controls. The toxic effect of the alkalised yeast extract was largely removed by concentrating and pouring into 9 volumes of 98 % alcohol; the precipitate proved more effective and was used in all the experiments described. Table IV shows that this extract improved growth but could not entirely replace autoclaved yeast extract; doubling the amount of alkalised yeast extract had no further effect. The negative results obtained with sodium phosphate show that the active substance is not phosphate. Egg-white extract given alone had no effect, but appeared to improve the growth of larvae receiving alkalised

Table V. *Growth of aseptic larvae on diet C, supplemented with alkalised yeast extract.*

1.3 cc. serum were taken as equivalent to 0.6 cc. blood.

Substance added	Series	Mean weight of 4-day old larvae (mg.)			
		Blood		Serum	
		1	2	3	4
Controls		30, 33	23, 24	1.3, 1.7	1.5, 1.5
Unsterilised eggs		—	—	37, 39	33, 42
Egg-white extract	15 %	29, 35	31, 32	—	—
	30 %	27, 28	—	—	—
	45 %	39, 44	32, 37	1.5, 2.5	1.5, 2.0
	75 %	—	26, 29	—	—
Autoclaved yeast extract	10 %	40, 42	38, 39.5	44, 48	42, 43

yeast extract (Tables IV and V); however, the differences observed were small and could not always be repeated. This may have been due to the presence of toxic or repugnant substances, since the addition of egg-white extract to an adequate diet depressed the growth rate; also the basal diet contained sufficient of the factors concerned to produce fair growth.

Experiments were also carried out with horse-serum; 1.3 cc. of serum were supplied in each tube in place of 0.6 cc. of whole blood. When larvae were reared aseptically on diet C prepared with serum, very slow growth resulted; the addition of autoclaved yeast extract produced normal development, whereas alkalised yeast and egg-white extract, given either alone or together, had no effect. Table V shows the results obtained with serum, the basal diet used being diet C supplemented with alkalised yeast extract. Since larvae developed readily on this diet in the presence of bacteria, the slow growth found under asepsis is due to lack of an organic substance; this factor appears to be present in blood corpuscles since aseptic larvae grow well on this diet when it is prepared with whole blood.

These experiments show that blow-fly larvae require more than one heat-stable growth factor. The results obtained with blood (Table IV) suggest that the effect of autoclaved yeast extract is due to two substances, one extremely stable to heat and alkali, the other sensitive to alkali under the conditions used. The first substance is probably the heat-stable factor which stimulates the growth of aseptic larvae on muscle and which resembles in some respects factor "Y" [Hobson, 1932, 2]. The second substance, which is stable to autoclaving in acid medium, appears to be present in blood, but the amount is not sufficient for optimum growth; since there was some evidence that egg-white extract supplies this factor, it may be vitamin B₂. Kollath [1930] showed the presence of this vitamin in samples of alkaline haematin prepared from horse-blood. Kollath's results suggested that serum might be poorer in vitamin B₂ than whole blood, but aseptic larvae, although they failed to grow, did not respond to egg-white extract when reared on a basal diet prepared with serum. However, the marked effect of substituting serum for blood in this diet (Table V) shows clearly that the larvae require a heat-stable factor which is present in the corpuscles and is not supplied by the alkalised yeast extract.

DISCUSSION.

The results obtained with sterile blood show that blow-fly larvae resemble higher animals in their "vitamin B" requirements. Thus, the effect of yeast is due to several substances, and normal growth only occurs if each is present in adequate amount. Also, the experiments with Peters's antineuritic concentrate indicate that one of the larval growth factors is identical with vitamin B₁. The other growth factors are probably analogous to members of the vitamin B complex and they may also be identical with vitamins required by higher animals. The chief effect of "vitamin B" deficiency in blow-fly larvae was retarded growth; no evidence was obtained of loss of weight, nor were there any obvious pathological symptoms except that the larvae tended to wander away from the food. "Vitamin B" appears to act as a catalyst of growth, accelerating a reaction which proceeds in its absence at an infinitely slow rate.

It is of interest to note that Wigglesworth [1929] and also Aschner [1931] have suggested on theoretical grounds that blood is deficient in accessory food factors required by insects; this hypothesis was advanced to explain the rôle

of the symbiotic microorganisms present in insects which feed solely on blood throughout their whole life cycle. In most of these insects the alimentary tract is sterile, the symbionts being located in special organs. Aschner and Ries [1933] have recently shown that the symbionts in lice are essential for their host; extirpation of the "stomach-plates" (Magenscheibe), which harbour the symbionts, produced sexual sterility and shorter life in adult females, these effects not occurring if the operation was delayed until the symbionts had escaped from the plates. The present experiments have shown that blow-fly larvae fail to develop on blood unless "vitamin B" is added or living bacteria are present; they therefore help to confirm the hypothesis that the function of the symbionts in blood-sucking insects is vitamin synthesis.

The effect of yeast on growth is less pronounced when blow-fly larvae are reared on blood without aseptic precautions, and under certain conditions growth is approximately normal; further experiments are being carried out upon the effect of bacteria on larval growth.

SUMMARY.

1. Blow-fly larvae are unable to develop aseptically on sterile blood owing to lack of growth factors of the vitamin B type; the presence of bacteria improves growth.

2. Aseptic larvae grow at the normal rate on blood supplemented with yeast autolysate. The effect of yeast is due to an "insoluble" substance and to soluble factors which can be further differentiated by their stability to heat.

3. The "insoluble" growth factor is present in muscle and yeast and is not extracted by water or alcohol; in autolysed yeast, it is soluble in water, sparingly soluble in alcohol and insoluble in ether.

4. Peters's antineuritic concentrate supplies an essential heat-labile factor; since concentrates of different purity showed a correlation between growth-promoting effect and antineuritic potency, it is concluded that the active substance is vitamin B₁.

5. Blow-fly larvae also require at least two heat-stable growth factors, which are present in autoclaved yeast extract.

6. These results support the hypothesis that vitamin synthesis may be the function of the symbiotic micro-organisms present in blood-sucking insects.

I wish to record my thanks to Prof. P. A. Buxton for extending to me the facilities of the Entomological Department. My appreciations are also due to Prof. R. A. Peters for supplying tested samples of antineuritic concentrate and to Dr V. B. Wigglesworth for his active interest and helpful advice. I am indebted to the Agricultural Research Council for a grant which entirely financed this work.

REFERENCES.

- Aschner (1931). *Z. morph. Ökol. Tiere*, **20**, 368.
— and Ries (1933). *Z. morph. Ökol. Tiere*, **26**, 529.
Chick and Copping (1930). *Biochem. J.* **24**, 1764.
— Copping and Roscoe (1930). *Biochem. J.* **24**, 1748.
Hoagland (1923). *U.S. Dept. Agric. Bull.* No. 1138, 1.

- Hobson (1932, 1). *J. Exp. Biol.* **9**, 128.
—— (1932, 2). *J. Exp. Biol.* **9**, 366.
Hunt (1928). *J. Biol. Chem.* **79**, 723.
Imms (1931). Recent advances in entomology. (Churchill, London.)
Kinnersley, O'Brien and Peters (1933). *Biochem. J.* **27**, 232.
Kollath (1930). *Arch. exp. Path. Pharm.* **142**, 86.
Mackerras and Freney (1933). *J. Exp. Biol.* **10**, 237.
Sweetman and Palmer (1928). *J. Biol. Chem.* **77**, 33.
Uvarov (1928). *Trans. Ent. Soc. Lond.* 255.
Weinland (1906). *Z. Biol.* **47**, 232.
Wigglesworth (1929). *Parasitology*, **21**, 288.
Williams and Lewis (1930). *J. Biol. Chem.* **89**, 275.