pensions or supernatant fluid, consisting mainly in a superficial thickening of chorion cells without accompanying cell proliferation or migration; and, (2) preliminary measurements of dry weight increase due to the carbon particle suspension indicate that it is less than that induced by the phosphate buffer/saline solution (about 5–7% increase over untreated membranes).

Growth promoting factors in cytoplasmic granules have been reported in *in vitro* experiments utilizing microsomes from chick embryo extracts[1], and other evidence also links particulate fractions with increased cellular growth *in vitro*[2]. The intervention *in vivo* of cytoplasmic granules in the promotion of cell division has also been demonstrated in the case of the frog egg, where the injection of cytoplasmic granules into unfertilized eggs will induce parthenogenetic cleavage[3]. What role the granules may play in these processes, however, remains unknown. BRACHET has recently[4] pointed out the possible role of microsomes as agents of protein synthesis in the cell, especially emphasizing their ribonucleic acid content in this connection. It will be noted that, under the conditions of the present experiments, the microsomes would be expected to be in the supernatant fluid, which induced greater growth increase than the granules. What relation these facts have to the ribonucleoprotein content and possible protein synthesizing capacities of these fractions can be found only with further analysis.

E. I. SHAW and J. R. SHAVER

Department of Zoology, University of Missouri, Columbia (Mo.), December 1st, 1952.

Résumé

Des membranes chorioallantoïdiennes d'embryons de poule traitées par 0,1 ou 0,2 cm³ d'une suspension de «gros granules» (mitochondries) provenants d'un homogénat de foie de grenouille, augmentent en poids sec de 20% environ (valeur rapportée à la croissance normale, ainsi qu'à l'effet du tampon phosphate/salin). D'autre part, le liquide surnageant, contenant ses microsomes, produit une augmentation de 30%, dans les mêmes conditions. Dans les deux cas, cette croissance s'accompagne d'une prolifération cellulaire et d'un accroissement de la basophilie cytoplasmique et nucléolaire, effets bien différents de ceux produits par une suspension de fines particules de charbon animal, utilisée comme témoin. Ces résultats sont discutés au point de vue du rôle que jouent les particules cytoplasmiques dans la synthèse des protéines et la croissance cellulaire.

[1] R. TENNENT, A. A. LIEBOW, and K. G. STERN, Proc. Soc. Exptl. Biol. Med. *46*, 18 (1941).

[2] G. BARSKI, J. MAURIN, G. WIELGOSZ, and P. LEPINE, Ann. Pasteur *81*, 9 (1951).

[3] J. R. SHAVER, S. SUBTELNY, and A. WANIA, Biol. Bull. *103*, 282 (1952).

[4] J. BRACHET, Actualités biochim. Liège, Paris No. 16 (1952).

Qualitative Vitamin Requirements for Growth of Larvae of *Calliphora erythrocephala* (Meig)

Introduction. Larvae of *Calliphora erythrocephala* were reared from the egg aseptically, on a diet consisting of casein, Marmite, cholesterol, l-cystine, and water. On this medium the larvae developed into normal imagines. We also obtained good growth if the Marmite in this medium was replaced by the salt mixture according to OSBORNE and MENDEL[1] and a mixture of 9 vitamins based on the composition of Marmite[2], plus vitamin B_{12}.

With the aid of this semi-synthetic diet we determined which of these 10 vitamins are required for normal growth of the larva.

Material and methods. The components of the medium, casein, cholesterol, salts, l-cystine, vitamins, and water were homogenised in an "atomix". The pH was adjusted to 7·0 with some drops of 33% NaOH solution. 40 g portions of this mixture were brought into 200 ml ERLENMEYER flasks with a wide neck. After this the vitamins were added to the flasks, by pipetting them from stock solutions containing all the vitamins or all the vitamins except one. The contents of the flasks were made into a rather solid mass with the aid of cotton-wool. Finally the flasks were plugged with cotton-wool and autoclaved for an hour at 120°C. The following medium was used as a basal diet (Tab. I):

Table I

Vitamin-free casein (Labco or H.L.R.*)		100 mg
l-Cystine** (British Drug House)		4 mg
Salt mixture (Osborne and Mendel)		6 mg
Cholesterol (Comm.)		10 mg
Thiaminchloride (H.L.R.)	3 µg	
Riboflavin (H.L.R.)	6 µg	
Nicotinic acid (H.L.R.)	60 µg	
Ca pantothenate (H.L.R.)	6 µg	
Pyridoxin (H.L.R.)	4 µg	
Folic acid (H.L.R.)	6 µg	
Cholinechloride (H.L.R.)	460 µg	
Biotin (Organon)	0·1 µg	
Inositol (H.L.R.)	180 µg	
Vitamin B_{12} (Organon)	0·015 µg	
Cotton-wool (Comm.)		–
Distilled water up to		1000 mg

* Hoffmann La Roche.

** The omission of l-cystin results in a growth retardation, considerable mortality and abnormal imagines. 2–4 mg l-cystine per gram diet was found to be the minimum quantity for optimal growth stimulation of *Calliphora* larvae. This is in good agreement with the findings of MICHELBACHER *et al.*[3] for larvae of *Lucilia*.

Eggs were obtained by placing fresh meat into a cage, containing the adult flies. About 4–6 h after oviposition the eggs were collected and transferred to a tube containing a solution of 0·9% NaCl and some Rogipon-T[4] in order to decrease the surface tension. With a sterile marten hair brush the clusters of eggs were separated and mixed (since the eggs are laid by different flies, mixing is necessary in order to obtain a homogenous material). The eggs were divided into as many equal groups as there were diets to be investigated in one experiment. After this each group of eggs was poured into a sterile tube and sterilized for 40 min with a mixture of chloramine-T and alcohol according to BLEWETT and FRAENKEL[5]. The sterilizing mixture was removed and the eggs were rinsed with sterile water. After some minutes, the eggs were aseptically transferred into the flasks containing the media to be investigated. In each flask about

[1] T. B. OSBORNE and L. B. MENDEL, J. Biol. Chem. *32*, 309 (1917).

[2] 1 g Marmite from the Marmite Food Extract Company, Ltd., London, contains 30 µg thiamin, 60 µg riboflavin, 600 µg nicotinic acid, 40 µg pyridoxin, 60 µg pantothenic acid, 60 µg folic acid, 4400 µg choline, 1 µg biotin, and 1800 µg inositol.

[3] A. E. MICHELBACHER, W. M. HOPKINS, and W. B. HERMS, J. Exp. Zool. *64*, 109 (1932).

[4] Fa. R. Bosman, Rotterdam.

[5] M. BLEWETT and G. FRAENKEL, Proc. Roy. Soc. B. *132*, 212 (1944).

50 larvae were reared. The cultures were kept in the dark in an incubator at 25°C. These larvae reared on the basal diet show a tendency to pupate after seven days. After this period the larvae were tested for contamination, killed with chloroform, dried for two days at 80°C and weighed. The nitrogen content was determined on samples of dried larvae, according to the method of KJELDAHL[1]. Tests for contamination were performed aerobically and anaerobically according to the usual methods on seven days old larvae. The results obtained from experiments in which contamination occurred were discarded.

This method for rearing sterile larvae proved to be successful; the contamination was only 5·5% and the eggs used to hatch about 20 h after oviposition.

Table II
Nitrogen content in mg per larva reared on a complete diet and on diets deficient in one of the vitamins tested.

Diet	Labco casein		H.L.R. casein	
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Basal diet (see Table I)	2·82	2·74	2·46	2·30
Basal diet without thiaminchloride	0·16	0·14	0·15	+
Basal diet without riboflavin	+*	+	+	+
Basal diet without nicotinic acid	+	0·11	+	+
Basal diet without pyridoxin	–**	0·03	+	+
Basal diet without Ca pantothenate	+	0·06	0·22	+
Basal diet without folic acid	2·53	1·68	1·81	1·30
Basal diet without cholinechloride	0·61	0·69	0·42	+
Basal diet without biotin	2·52	0·92	0·73	+
Basal diet without inositol	2·68	2·47	2·56	2·58
Basal diet without vitamin B_{12}	2·71	2·48	2·57	2·54

* All larvae died in an early stage. ** Contaminated.

Results and discussion. Table II comprises the results of 4 series of experiments in which vitamin-free casein served as protein component of the diet, two series with Labco casein, the other two with vitamin-free casein Hoffmann La Roche. The figures represent the nitrogen content in mg per larva after seven days, reared on a complete diet and on diets in which each of the vitamins was omitted in turn (the Figures of the dry weight, showing essentially the same results, are omitted).

From this Table it is clear that the omission of thiamin, riboflavin, nicotinic acid, pyridoxin, pantothenic acid or choline results in very slow growth. In many cases even death occurs in an early stage of larval development. From these results we are justified in concluding that these vitamins are required for normal growth.

On replacing nicotinic acid by nicotinic amide, growth is not retarded. So it is obvious that these vitamins may replace each other.

If folic acid or biotin is omitted, growth is retarded considerably and mortality is high as compared with the complete diet. In subsequent experiments it could be shown that this growth retardation on diets deficient in either folic acid or biotin is significant (resp. $P = 0{\cdot}01$ and $P = 0{\cdot}02$). So it appears that besides the vitamins mentioned above, folic acid and biotin are likewise required for normal growth.

On diets from which inositol or vitamin B_{12} was omitted, growth was about equal to that on the complete diet. However it is probable that the other components of the diet may contain these vitamins in sufficient quantity to meet the needs for normal growth. Therefore, any conclusions regarding these two vitamins must await further experiments.

Comparing the above results with investigations from other authors as reviewed by TRAGER[1], the requirements of the *Calliphora* larva for B vitamins are in good agreement with those of other insects. Especially is this the case for *Drosophila*[2] and *Attagenus*[3], which require all the vitamins which proved to be likewise essential for normal growth of the larva of *Calliphora.*

Acknowledgement. I wish to thank Professor K. C. WINKLER for his advice about the sterile technique; Mr. A. P. DE GROOT for his help and for the correction of the manuscript; the Netherlands Organisation for Pure Research (Z.W.O.) for a grant which partly made this investigation possible; and the N.V. Organon, Oss, for providing me with biotin and vitamin B_{12}.

PH. D. J. W. SEDEE

Laboratory of Comparative Physiology, University of Utrecht, January 10, 1953.

Zusammenfassung

Larven von *Calliphora erythrocephala* wurden bei semisynthetischer Diät aus Kasein, Cholesterol, Salzen, l-Zystin, Vitaminen und Wasser steril aufgezogen. Die Verpuppung erfolgte normal, und es schlüpften normale Imagines. Für gutes Wachstum sind an Vitaminen notwendig: Aneurin, Riboflavin, Nikotinsäure oder Nikotinsäureamid, Pantothensäure, Adermin, Folsäure, Biotin und Cholin. Für gutes Wachstum und für die vollständige Entwicklung benötigt die Larve ausserdem l-Zystin.

[1] W. TRAGER, Biol. Rev. *22*, 148 (1947).
[2] E. L. TATUM, Proc. Nat. Acad. Sci. *27*, 193 (1941). – T. HINTON, D. THEIRAULT NOYES, and J. ELLIS, Physiol. Zool. *24*, 335 (1951).
[3] W. MOORE, Ann. Entom. Soc. Amer. *39*, 513 (1946).

[1] A. P. DE GROOT and J. C. A. MIGHORST, Chem. Weekbl. *47*, 219 (1951).

Modifications dans la figure chromatographique des acides aminés libres et des substances fluorescentes de l'hémolymphe du *Bombyx mori* L., consécutives à une paralysie d'origine microbienne

L'étude physiologique des paralysies d'origine microbienne est rendue difficile par le fait que l'on se trouve en face de complexes d'effets indirects inconnus. Il semble que la nature de ces derniers doive être recherchée surtout avec l'aide de la pathologie comparée.

C'est dans cet ordre d'idées que nous rapportons ici un effet que nous avons observé dans le bilan des acides aminés et des substances fluorescentes du sang au cours d'une paralysie provoquée chez le lépidoptère *Bombyx mori* L. par l'action d'une bactérie flachérigène, le *Bacillus cereus* var. *alesti* TOUMANOFF et VAGO[1].

[1] C. TOUMANOFF et C. VAGO, C. r. Acad. Sci. *233*, 1504 (1951). – C. VAGO, C. r. Acad. Agric. *37*, 593 (1951).