

Detailed accounts of the experiments will be reported elsewhere.

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Nutritional Requirements of the Common Housefly, *Musca domestica vicina* Macq

THE housefly *Musca domestica vicina* Macq., which has been reared in our laboratory for four years with a modified NAIDM medium¹, was used in these experiments. Eggs were collected from oviposition dishes, washed and sterilized. The most satisfactory sterilizing solution and technique were as follows. Modified Vanderzant's solution² was prepared, with the omission of sodium glycocholate and less mercuric chloride (0.15 gm. instead of 0.25 gm.). Instead of a single treatment, the eggs were submerged in the solution twice, each for 10 min., with an interval of 1 hr. between the two treatments. After each treatment, the eggs were rinsed with bacteriologically sterile water. Plating on common bacteriological medium revealed that the percentage of contamination was less than 10 per cent and the percentage of eggs hatched was more than 50 per cent³.

The sterilized eggs were transferred to cotton-stoppered vials with sterile media, one egg to each vial. Observations on the growth and development of the larvæ were made at frequent intervals and growth-rates recorded. Vials contaminated with micro-organisms were discarded.

The composition of the medium was based on the medium for *Hylemyia antiqua*⁴ and *Calliphora erythrocephala*⁵, since it is reasonable to assume that the nutritional requirements of these flies might be similar. The ingredients of the medium per ml. were as follows:

Agar	20.0 mgm.
Ribonucleic acid	1.0 "
Cholesterol	0.1 "
Dextrose	15.0 "
Mineral mixture (U.S.P. XIII, No. 2)	2.0 "
Casein	11.0 "
Vitamin mixture (per 100 ml.)	
Vitamin A	5,000 U.S.P. units
Rachitosterol D	1,000 "
Thiamine	2.0 mgm. "
Riboflavin	2.5 "
Ascorbic acid	30.0 "
Niacin amide	20.0 "
Pyridoxine hydrochloride	0.2 "
Calcium pantothenate	1.0 "

Solutions of casein and ribonucleic acid were prepared separately by dissolving in distilled water, the former with 2 N sodium hydroxide. The mineral mixture was dissolved in hot distilled water. These solutions were mixed and agar added. When agar was dissolved by heating, the whole mixture was autoclaved at 15 lb. for two minutes. Cholesterol was first dissolved in 95 per cent ethyl alcohol, and emulsified with 5 per cent gum arabic solution. This emulsion, together with dextrose solution and vitamin mixture, were then added. The final mixture was adjusted to pH 6.4 with 2 N sodium hydroxide. 2 ml. of this

mixture was put in each food vial, a cotton stopper was used, and the vials were again sterilized at 15 lb. for 15 min.

Larvæ developed normally in the basic medium except that the larval period was somewhat prolonged and the pupal weight decreased (Table 1). This was probably due to the deficiency of glycine and cystine in casein. Further retardation of growth-rates resulted when ribonucleic acid or vitamins A, C and D were omitted. Non-sterile eggs on the same basic medium showed no advantages, suggesting that bacteria or other micro-organisms were not necessary symbionts for the housefly larvæ, at least when all other nutritional requirements were fulfilled.

Table 1. GROWTH-RATES AND PUPAL WEIGHT OF HOUSEFLIES REARED ON BASIC MEDIUM AND MODIFIED MEDIUM

Medium	Larval period			Pupal period (days)	Pupal weight (mgm.)
	First instar (days)	Second instar (days)	Third instar (days)		
Basic medium	1	1	3-6*	3-5	12-15
Basic medium less ribonucleic acid	3	3	3-4	4	11-15
Basic medium less vitamins A, C and D	1-3	1-2	3-5	3-8	12-15
Basic medium non-sterile eggs	1-2	1	5-11	3-4	11-13
Casein hydrochloride hydrolysate	1-3	3-5	—	—	—
Casein pancreatin hydrolysate	1-2	2-4	2-4	6	11-12
Ten amino-acids	3-6	3	7	4	11
Modified NAIDM	1	1	3-4	3	15-18

* One larva had an extremely long third instar of 10 days.

Preliminary experiments on the amino-acid requirements of the housefly larvæ had been studied by using different hydrolysates of casein. The casein hydrochloride hydrolysate failed to support growth due to the lack of tryptophan, while pancreatin hydrolysate of casein (tryptophan not destroyed during hydrolysis) will support normal growth. Preliminary studies by varying the contents of amino-acid mixture to substitute for casein had also revealed that the essential amino-acids were probably the same for other dipterous insects such as *Drosophila melanogaster*⁶ and *Pseudosarcophaga affinis*⁷. They are arginine, lysine, leucine, isoleucine, tryptophan, histidine, phenylalanine, methionine, valine and threonine; but the rate of growth with only these ten acids was very much retarded. Further studies are now in progress.

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Occurrence and Filterability of Protoplast-like Elements in Aged Bacterial Cultures

CELLULAR or subcellular units of certain bacteria that have been deprived of a rigid cell wall have been regarded in the recent literature as protoplasts. The conversion of rods into spherical protoplasts was observed mostly in media containing penicillin and