XXI. VITAMIN REQUIREMENTS OF *DROSOPHILA*. I. VITAMINS B AND C.

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LOEB [1915] ascertained the fact that flies of the genus *Drosophila* could be reared on a culture medium containing as mineral constituents only K, Mg, PO_4 and SO_4 ions. In these experiments micro-organisms were not excluded, and the growing larvae undoubtedly lived at the expense of these. Loeb also found that when Na was substituted for K no growth took place and concluded that K was essential for the development of the insect. It is however possible that the absence of K stopped the growth of the micro-organisms and thus deprived the larvae of nitrogen in an available form. Loeb's experiments therefore only prove that the mineral constituents named above are adequate, but not that they are essential.

Subsequently Loeb and Northrop [1916] succeeded in sterilising the eggs of *Drosophila* by exposure for 6–7 minutes to 0.1 % aqueous HgCl_2 or a saturated alcoholic solution of the same salt and found that the larvae hatched out from the few eggs which survived the disinfection were sterile. They ascertained that these sterile larvae could not be reared on sterile media consisting only of purified proteins, carbohydrates, fats and salts, but that when yeast killed by heat was added, normal development occurred. Alcoholic extracts of yeast were however inactive. Northrop [1917] pursued the subject further and found that the addition of caseinogen and similar substances along with cane sugar to yeast greatly increased the number of flies which could be reared at the expense of a definite amount of yeast and concluded that they acted as foodstuffs when properly supplemented by yeast. Similar results were obtained by the addition of kidney, liver and pancreas from the dog, but many other organs of the dog were inactive. The author was inclined to interpret these results in the sense that yeast supplies one or more accessory substances, necessary for the growth of the organism.

The question was again investigated by Baumberger [1919] who found that the pupae and eggs of flies reared in presence of a pure culture of yeast could readily be sterilised by immersion for 20 minutes in 85 % alcohol. The sterile larvae obtained from such eggs could easily be reared in sterile culture media provided the latter contained killed yeast or nucleo-protein prepared from yeast. Baumberger came to the conclusion that the essential requisite

was a sufficient concentration of yeast nucleo-protein and that accessory food factors were not concerned.

The following experiments were instituted with the object of ascertaining more definitely whether any of the recognised vitamins are essential for the nutrition of these flies. The subject is of general interest as regards the extent to which the need for accessory food factors is distributed in the animal kingdom. Special interest attaches to the possibility of introducing a simplified technique by which results could be obtained more rapidly and economically than by the tedious and costly feeding experiments which are at present necessary.

TECHNIQUE OF THE EXPERIMENTS.

It was soon found that the eggs could be sterilised much more readily and certainly than the pupae and this method was always employed.

Following Baumberger's instructions the flies were reared in banana-agar (approximately equal parts of mashed banana and 1.5 % water-agar) which had been autoclaved and subsequently inoculated with yeast (*S. cerevisiae*). After a few sub-cultures on this medium the flies were found to be free from bacteria, both anaerobic and aerobic, and the pure stock was readily maintained by occasionally passing a few flies into a fresh banana-agar-yeast tube.

When eggs were desired for experimental purposes, freshly emerged flies were transferred to a special apparatus. This consisted of an inverted wide necked bottle, standing in a Petri dish and having a strip of moistened filter paper on the wall. A small circular glass dish with vertical walls was also placed on the Petri dish, so as to be covered by the inverted bottle. The walls of this dish were lined with hardened filter paper moistened with water. The bottle was in turn covered by an inverted cylindrical tinned iron can and the whole apparatus was autoclaved at 120° for half-an-hour. A few cc. of a 24-48 hour culture of yeast in yeast extract containing glucose were then placed in the small dish, the flies introduced into the inverted bottle and the whole apparatus placed in an incubator at 30°. Under these conditions the eggs were laid within 24-48 hours almost exclusively on the hardened filter paper lining the small circular glass dish containing the yeast culture. The strips of paper were removed, placed on a sterile slide under a dissecting microscope and the eggs picked off singly by means of sterilised dissecting needles and placed in 85 % alcohol as directed by Baumberger. They were then divided up into collections of the number required for each tube by means of a sterile pipette provided with a rubber teat, and were finally, after half-an-hour's exposure to the alcohol, sucked up into the pipette and delivered into the appropriate tube of medium. It was found most convenient to place 20 eggs in each test tube (6" × $\frac{3}{4}$ ") containing 5-5.5 cc. of medium in the form of an agar slope.

The agar jelly in the medium tube must be so soft that the larvae can readily penetrate it in their search for food. As a rule 3 cc. of 1½ % agar

were added to 2 cc. of medium and the whole then steamed for 20 minutes on three successive days, and cooled in the form of slopes.

The inoculated tubes were incubated at 30° and examined daily, note being made of the number hatched, the general progress and the time of appearance of pupae and flies.

At the close of each experiment the tubes were tested for sterility by making cultures on beef broth-peptone-agar and incubating at 37°. Contamination by yeast usually showed itself in the experimental tube, but was when necessary tested for by culture on beer wort agar at 25°.

DIET.

A basal diet consisting of

Caseinogen (purified)	0.15 g. per tube
Starch and salts ¹ (94 % starch and 6 % salts)	0.05 g. „
Cane sugar	0.1 g. „

was employed, to which various additions of butter-fat (vitamin *A*) yeast-extract (vitamin *B*) and lemon juice freed from citric acid (vitamin *C*) were made as required, 2 cc. of liquid in all being added, and then 3 cc. of 1½ % agar. The caseinogen had been extracted with alcohol and light petroleum or in some cases heated at 120° for many hours and repeatedly stirred.

RESULTS.

The experiments were directed in the first instance to ascertaining which, if any, of the three recognised accessory food factors are necessary for the growth and metamorphoses of these insects.

The results, which are summarised below, showed definitely that:

- (1) in the presence of butter-fat and yeast extract (vitamins *A* and *B*) growth occurred irrespective of the presence or absence of 1 cc. of lemon juice (vitamin *C*);
- (2) in the presence of butter-fat (vitamin *A*) growth occurred in presence of 1 cc. of yeast extract (vitamin *B*), but not in its absence, this result being independent of the presence or absence of lemon juice;
- (3) in the presence of 1 cc. of yeast extract (vitamin *B*), with or without 1 cc. of lemon juice (vitamin *C*), growth occurred in the presence of two drops of butter-fat, but not in its absence.

In the following summary only results of experiments in which the tubes remained sterile are quoted.

	Additions to basal diet			Tubes inoculated	Eggs hatched	Pupae formed	Flies emerged
	Butter-fat	Yeast extract	Lemon juice				
1	-	+	+	10	100	0	0
2	+	+	-	11	115	92	63
3	+	-	+	10	57	0	0
4	+	+	+	10	68	48	42
5	-	+	-	2	19	0	0
6	-	-	-	2	16	0	0

¹ As used in this laboratory for rats.

Control experiments were carried out to ascertain whether the yeast extract alone was capable of supplying sufficient nitrogen for the growth of the larvae, but with entirely negative results. This was done by omitting the caseinogen from mixtures 2 and 4, which gave positive results in its presence. Even the addition of 2 cc. of the yeast extract gave entirely negative results in the absence of the caseinogen.

Other sources of vitamin B.

As flies of this group are specially associated with yeast, which probably forms the chief food of the larvae in their natural condition, it was thought that in order to prove the necessity for vitamin *B*, it would be essential to show that it could be supplied from other sources.

The first experiments were made with milk, but without success. Recourse was then had to wheat germ, the alcoholic (80 %) extract of which is known to contain a considerable amount of vitamin *B*.

50 g. of wheat germ were heated on the water-bath for one hour with 200 cc. of 80 % alcohol, pressed out and the clear liquid evaporated to dryness at 50°. The residue was shaken up with 50 cc. of water and filtered, the clear filtrate being used. This contained 10.47 g. total solids, 0.21 g. mineral matter, and 0.196 g. N per 100 cc.

Tubes were made up with 2, 1 and 0.5 cc. of this extract in addition to the usual basal diet of caseinogen, starch, salts, cane sugar and butter-fat. Some difficulty was experienced in adjusting the stiffness of the agar jelly, but finally 0.5 cc. H₂O and 3 cc. of 1.25 % agar were added to each tube, so that the total volume was 5.5 cc. as shown below.

	Caseinogen, starch and salts	Cane sugar	Butter- fat	Wheat germ extract	H ₂ O	1.25 % agar
	g.	g.		cc.	cc.	cc.
7	0.2	0.1	2 drops	2	0.5	3
8	0.2	0.1	"	1	1.5	3
9	0.2	0.1	"	0.5	2.0	3
	Starch and salts					
10	0.05	0.1	"	2	0.5	3

The following are the summarised results of three quite independent and consistent experiments:

	Wheat germ extract	Eggs known to have hatched	Pupae	Flies
11	2	34	18	9
12	1	56	24	13
13	0.5	39	0	0
14	2 cc. as sole source of N	9	0	0

The experiments were somewhat hindered by the hot weather prevailing at the time, which caused unduly rapid drying of the tubes, but they show quite conclusively that, like yeast extract, wheat germ extract is capable of supplying some factor necessary for the growth and metamorphosis of the fly.

It is at the same time obvious that the wheat germ extract (1 cc. of which corresponds with 1 g. of wheat germ) is much less efficacious than the yeast

extract (1 cc. of which corresponds with 0.5 g. yeast) and this is in accordance with what is known of the vitamin *B* content of these two materials, 1 g. of yeast being approximately equivalent to 3.6 of wheat germ.

Taken together the experiments with yeast extract and wheat germ extract leave little doubt that these insects require vitamin *B* for their development, thus confirming Northrop's view. Experiments are in progress on the amount of yeast extract required and the effect of varying concentrations on the duration of the period of development.

THE FAT-SOLUBLE FACTOR (VITAMIN *A*).

The striking fact that in presence of 1 cc. yeast extract growth occurred in the presence of two drops of clarified butter-fat, but not in its absence, may be taken to show either that a fat of some kind is necessary or that the fat-soluble factor is required or that both are required. To decide between these possibilities it is necessary to ascertain whether or not growth occurs in presence of a fat devoid of vitamin *A*. Attempts of this kind are being made, but no definite result has yet been obtained. The preliminary results however indicate that the fly is certainly able to develop in the presence of very small amounts of the vitamin if not in its entire absence.

SUMMARY.

The complete development of *Drosophila* requires the presence of vitamin *B* but not of vitamin *C*.

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