CLXXXVI. A SEMI-SYNTHETIC DIET FOR *HELIX POMATIA*

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IN the course of some preliminary experiments on the calcium metabolism of the edible snail, it became clear that with an animal of this size, and one which contained little blood, many of the problems arising would have to be approached indirectly. One particular investigation necessitated the control of the quantity and nature of the salts of Ca and P eaten by the animal. This would be impossible if the animals were given, for example, cabbage, where the Ca content is known to be very variable, even in the same cabbage [Cowell, 1932]. The only way was to try to make a suitable synthetic diet. Such a diet has been successfully prepared and is described in this paper.

Materials and methods

(a) *Animals.* The animals used in all experiments were reared in the department. When about to lay, *H. pomatia* excavates a hole, about 10 cm. deep, in the soil and later deposits its eggs at the bottom. The site of each clutch of eggs was marked by putting a numbered piece of chalk over the hole. If the eggs were left undisturbed, a day or two after hatching the young snails left the bottom of the hole and formed a cluster on the underside of the chalk. Animals then taken for experiment had a very low mortality indeed, and were the "newly hatched" snails used in all later work.

During the experiments, each batch of animals was confined in a cylinder of perforated zinc, 6 in. in diameter and 5 in. high, standing on one glass plate and closed by another, as described by Howes & Wells [1934]. These cages were kept in a large box, the temperature of which was maintained between 18° and 20°. A constant stream of water dripped on to the heating elements so that there was continual condensation on the sides of the box. The air was stirred by a fan and readings of wet and dry bulb thermometers were usually identical; it is therefore assumed that the air in the box was very nearly saturated with water vapour.

Animals spent roughly 1 hr. in 24 at room temperature and humidity, while they were weighed; they were then transferred to clean cages, given fresh food and water and replaced in the box. Each snail was marked by a number painted on its shell and every animal was weighed individually.

(b) *Diet.* As food for the control animals and also as a basis for the calculation of the synthetic diet described later, the following mixture was used:

Fine oatmeal...	...	5 parts	} Sprinkled on fresh cabbage
Precipitated chalk	...	2 parts	
Ostermilk (dried milk)		1 part	
(1489)			

This is a modification of the diet described for terrestrial pulmonates by Oldham [1934] and was recommended by Prof. R. A. Fisher. Any dried milk may be used.

The synthetic diet was calculated on the assumption that snails eat two parts of cabbage to eight parts of the powder, and accordingly was prepared with the following composition:

Basal diet

	g.
Protein (Glaxo "Ashless" Casein)	17.8
Glucose (Analar)	4.0
Lactose (Analar)	1.2
Starch (potato)	51.6
Dried yeast	1.2
Cystine	0.4
CaCO ₃ (precip. opt., light)	20.0
Salt mixture	3.0

Fat and other substances were added to this before feeding it to the animals. The proportions of these constituents were determined from the results of analyses of oatmeal, dried milk and cabbage given by König [1903], Leach [1920], Heineman [1921] and Sherman [1932].

The salt mixture was derived from the analyses of ash constituents given by the above authors, excepting that the figures for iron, manganese and copper were taken from Lindow *et al.* [1929]. The salts chosen were taken from Mottram's modification of Hopkins's salt mixture used by Hartwell [1922] and as far as possible salts of A.R. purity were used. Where reagents of this standard were unobtainable, "pure" salts were employed.

In deriving the salt mixture from the ash analyses, the following adjustments were made: (a) since part of the P and S in the ash would be derived from protein, an amount of these elements was deducted from the salt mixture equivalent to that in the protein given; (b) since no figures were available for aluminium, this element was included arbitrarily; (c) silica was omitted since it could not be included as a soluble salt; (d) copper was increased arbitrarily to ensure an adequate supply, since snails have the respiratory pigment, haemocyanin, which contains copper, in their blood. The composition of the salt mixture used was:

	g.
MgSO ₄ (anhydr.)	41.88
K ₂ HPO ₄	77.78
NaCl	13.96
NaH ₂ PO ₄ , H ₂ O	11.55
CaH ₄ (PO ₄) ₂ , H ₂ O	52.60
Fe(C ₇ H ₆ O ₇), 1½H ₂ O	1.44
CuSO ₄ , 5H ₂ O	0.717
Al ₂ (SO ₄) ₃ .K ₂ SO ₄ , 24H ₂ O	0.025
KI	0.014
MnCl ₂ , 4H ₂ O	0.022
NaF	0.00027
Total	199.98

Traces of zinc were available for the experimental animals, since the cages were made of this material.

Calculation of the vitamin content of the control diet and hence of the quantities required for the synthetic diet was extremely difficult. Cod liver oil was given in excess of the theoretical value for vitamin A as obtained from the figures of Sherman [1932] and yeast in excess of the figures for vitamin B₂ in cabbage. The quantity of lemon juice was arbitrary.

The proportion of fat in the diet was calculated in the same way as that of the other constituents but while sufficient of the basal diet was made to last several months, fat was added immediately before giving the food to the snails. Thrice-clarified butter and cod liver oil were the fats used. The cod liver oil and dried yeast were obtained from the Glaxo Research laboratory. The oil contained per g.

Vitamin A, 0.050 % (C.P. blue value 30)

Vitamin D, 270 international units.

The cholesterol was the ordinary commercial product obtained from British Drug Houses and, since it was not especially purified, may have contained impurities of dietetic importance to snails.

Whatman No. 44 filter-paper, chosen for its low ash content, was used both as a plate on which to give the diet and as a source of cellulose. The animals usually ate some of it.

The "leaf extract" referred to later was an acetone extract of dried stinging nettle leaves. The first stages in the preparation followed the preliminary steps in the preparation of chlorophyll described by Willstätter & Stoll [1913, p. 133]. 500 g. of dried nettle leaves were placed in a Büchner funnel, sucked tight while still dry and then extracted with 1.6 l. of 80 % by vol. acetone for 30 min. A dark green fluorescent solution was thus obtained. The 80 % acetone was removed by evaporating the extract to dryness at 37° under reduced pressure in a stream of nitrogen. A tarry dark green mass mixed with flakes of a yellow to orange waxy material resulted. This was mixed up together and weighed out as required. It was stored in an exhausted desiccator from which the residual air was washed out with nitrogen and was kept in the dark.

In preparing the food for the animals, sufficient of the basal diet for 2 days was weighed out, together with additional substances necessary and the whole mixed to a thick paste with distilled water. Half of this was given to the appropriate batch of animals on the day of preparation and the remainder stored in covered vessels in a refrigerator overnight and used on the following morning. Thus new food was given each day. It was placed on Whatman No. 44 filter-paper and a small vessel of clean distilled water for drinking was also provided. Each batch of animals was invariably supplied with more food than it could eat. The consistency of a diet very much influences the avidity with which snails will eat it. The animals avoid thin pastes but, while preferring dry powders, will eat thick pastes. Throughout these experiments care was taken to be sure that the snails were actually eating the food supplied and observations on the number and nature of the faeces were made and recorded at each weighing. This is of considerable importance, owing to the tendency of *H. pomatia* to aestivate when environmental conditions are unfavourable.

(c) *Experiments.* Preliminary experiments were made upon yearling snails. These were taken while still hibernating, the epiphragm was removed, they were then weighed and divided into batches of eight. Individuals were allocated in such a way that, as far as possible, each batch contained a series of animals of similar weights. The batches were kept in the thermostat in cages of the type described. They were weighed daily and given fresh food and water and clean glass plates. In all experiments described in detail "newly hatched" snails were used. These were treated in the same manner as the yearlings. In the preliminary experiments, the basal diet was given with the addition of cod liver oil and lemon juice. Controls were fed with the oatmeal-Ostermilk-chalk mixture on cabbage and another set on a protein-free diet.

It was clear that animals fed on the first diet, which was theoretically complete as far as was known, actually grew very little better than those on a protein-free diet, and indeed stopped growing altogether after 6 weeks. The milk-mixture controls, on the other hand, grew steadily. The first diet was manifestly incomplete. The work of Hobson [1935] suggested one possible missing factor, namely cholesterol which appears to be an essential constituent of any diet adequate for growth in insects. Further, it was felt that there might be some factor present in green leaves, e.g. chlorophyll or even carotene as a substitute for vitamin A, which was necessary. Accordingly, experiments were planned as follows:

Table I. *Experiments on "newly hatched" snail chicks*

(1) Controls (26). Diet: oatmeal, 5 parts; precipitated chalk, 2 parts; Ostermilk, 1 part—Sprinkled on fresh cabbage. Tapwater to drink.

(2) Synthetic diets. Diet: basal diet, 1 g.; fat, 0.07 g.; lemon juice, 1 drop; water, 0.3 ml.—Spread on Whatman No. 44 paper. Distilled water to drink.

The following additions or subtractions were made:

No. of exp.	Leaf extract, 13 mg. per g. basal diet	Cholesterol, equivalent to 0.2% basal diet	Cod liver oil, vitamin A 36 γ and vitamin D 19 units per g. basal diet	Yeast	Lemon juice	No. of animals in exp.
IIIb	.	.	+	+	+	8
IIc	.	+	.	+	+	10
IIIc	.	+	+	+	+	10
IIb	+	.	.	+	+	8
VIIb, Xb	+	+	.	+	+	16
Vb, IXb	+	+	+	+	+	16
XIb	+	+	+	.	+	8

(d) *Method of expression of results.* Growing snails undergo the continual fluctuations in water content described in adult animals by Howes & Wells [1934] and the amplitude of these fluctuations is even greater than in adult animals. It is therefore impossible to show the daily weights of several animals together on a single graph with any degree of clarity. Weekly weighings would be equally difficult of interpretation, since any given day of the week may fall irregularly on a point of maximum or minimum hydration or intermediately. The arithmetic mean of each week's weighings has therefore been chosen for comparison between the rates of growth of animals given different diets and is represented by a single point in all figures.

Young snails show very different rates of growth under apparently identical treatments. This is shown in Fig. 1, Controls, which represents the growth of a single batch of eight control animals. Hence, apart from its catholic tastes in foods, *H. pomatia* is by no means an ideal subject for dietetic studies and it is difficult to obtain absolutely clear-cut results. For this reason the following points have been taken into consideration as criteria of an adequate synthetic diet:

(a) there must be few deaths not attributable to causes outside the experimental treatment;

(b) growth must continue steadily and a single animal must not remain stationary in weight for more than three consecutive weeks;

(c) there must be no definite falling-off in the rate of growth between the 4th and 7th weeks, which seems to be a critical period;

(d) at least two out of eight animals should grow more rapidly than the majority of the controls.

RESULTS

(a) *Comparison between growths of experimental and stock animals.* It was important to see whether animals kept under experimental conditions grew as rapidly as the stock kept under approximately normal conditions. Accordingly, in May 1935 the stock cages were searched and the eight largest out of 160 young hibernating snails hatched in 1934 were selected and weighed, after their epiphragms had been removed. In October 1935, cages were again searched and the eight largest out of over 200 young active snails hatched in July 1935 were removed and weighed. The weights so obtained, in descending order of magnitude, are given in columns 1 and 2 of Table II. For comparison, the weights of the eight largest of all the experimental animals are given in column 3, while column 4 indicates the experiments from which these animals were taken.

Table II. *Comparison between weights of experimental and stock animals*

1934 stock hibernating wt. without epiphragm 2 May 1935 g.	1935 stock, wt. on 9 Oct. 1935 g.	Experimental animals, mean wt. for week 7-12 Oct. g.	Experiments from which animals were taken
0.962	0.650	1.491	Control
0.845	0.631	1.239	Control
0.793	0.600	1.085	III c
0.774	0.582	0.999	VII b
0.714	0.577	0.974	III c
0.684	0.547	0.959	Control
0.670	0.505	0.922	Control
0.660	0.428	0.709	VII b

(b) *Survival of animals given different diets.* The mortality of animals given different foods is not a very good criterion of the completeness or otherwise, of a diet, since, generally speaking, young snails can live for a very long time without growing. Such a result follows quantitative vitamin deficiencies, although complete absence of vitamin A and/or vitamin D usually causes death well within 16 weeks. On the other hand, rapidly growing snails, even on a control diet, appear to be slightly less viable than animals not growing at all. This is illustrated in Table III.

Table III. *Survival of animals on different diets*

Diet	No. of animals at beginning of exp.	No. which survived till end of exp.	Notes
Control	26	24	—
Basal + cod liver oil + cholesterol	10	8	1 died 15th week, 1 died 16th week
Basal + cod liver oil	8	8	—
Basal + leaf extract + cholesterol	16	14	1 died 12th week, 1 died 16th week
Basal + leaf extract	8	7	1 died 16th week
Basal + cholesterol	10	1	1 lived to 14th week, 1 lived to 12th week, 2 lived to 10th week
Basal diet without yeast + cod liver oil + cholesterol	8	8	—

(c) *Effects of the different diets on growth.* The experiments were made in two groups and the results are illustrated in Figs. 1 and 2, each group with its set of controls. Of the batches of snails on different diets only two showed a growth rate in any way comparable with that of the controls. The diets in these cases

Fig. 1. Mean weekly weights of experiments with leaf extract, including controls. The numbers assigned to each experiment correspond to those in Table I. The arrows in Exps. IIb and XIb indicate the date when cholesterol and yeast respectively were added to the diets.

Fig. 2. Mean weekly weights of animals given cod liver oil and cholesterol, together and singly. The weight curves of control animals of this group are also included.

consisted of the complete basal diet to which cholesterol had been added, together with either leaf extract or cod liver oil.

The addition of leaf extract alone allowed of slight growth, which was increased towards the end of the experiment when the diet was supplemented with cholesterol. There was scarcely any growth when cod liver oil was added to the basal diet and the addition of cholesterol alone allowed of slight initial growth, but the majority of animals died before the end of the experiment. The removal of dried yeast from the basal diet very considerably suppressed growth, but the animals recovered when it was returned to the diet.

DISCUSSION

The interpretation of the results is made difficult by three important facts.

(1) The cholesterol used had not been carefully purified and may well have contained impurities which would form a source of vitamin D for the snail.

(2) The leaf extract, while probably free from vitamins A and D, since these rarely, if ever, occur in the living plant [Bills, 1936] certainly contained plant sterols which may have a vitamin-like action for snails. It certainly contained carotene (provitamin A).

(3) Cod liver oil usually contains approximately 500 mg./100 ml. cholesterol. Thus the animals given cod liver oil alone obtained about 0.025 mg. of cholesterol, while those given cod liver oil plus cholesterol received 2.025 mg., respectively, per g. of basal diet.

Thus, in the cases where the basal diet, which contained dried yeast and to which lemon juice was added, was given with or without the addition, together or singly, of cod liver oil, cholesterol and leaf extract, the main differences in treatment may be summarized as follows:

Added to basal diet	
Cod liver oil alone	Vitamins A and D plus trace of cholesterol
Cholesterol alone	Possible trace of vitamin D different from that in cod liver oil, plus cholesterol
Cod liver oil plus cholesterol	Vitamins A and D from cod liver oil. Possible second vitamin D from cholesterol, plus cholesterol
Leaf extract alone	Carotene plus plant sterols
Leaf extract plus cholesterol	Carotene plus plant sterols plus cholesterol plus possible trace of vitamin D

From these results it appears that snails require vitamin A for growth. This is available to the animals whether supplied as the vitamin itself or as the provitamin carotene.

The position with regard to vitamin D is not clear since it is confused with the possibility of there being a need for cholesterol as well. In relation to these two substances the results have to be interpreted in the light of three alternative possibilities:

(a) the animals require cholesterol in a concentration of the order of 0.2% of their food, a concentration of 0.0025% being below the threshold value;

(b) the vitamin D of cod liver oil cannot be used by the snail, but another vitamin D present as an impurity in the cholesterol is essential for growth; this may be present as a provitamin;

(c) that both cholesterol and a vitamin D-like impurity in the cholesterol are essential for growth.

A comparison between Exp. II *b*, Fig. 1 and the exps. in Fig. 2 suggests

that cholesterol may be the factor concerned, since some growth takes place when leaf extract alone is added to the basal diet. This result may be compared with those of Hobson [1935] with *Lucilia sericata*, in which he showed that while cholesterol was the chief oil-soluble growth factor for the blow-fly, ergosterol and sitosterol also promoted growth, but were less active than cholesterol.

At this point it is of interest to enquire as to what sterols are likely to be available for *Helix pomatia* under natural conditions, but unfortunately there appears to be no literature whatsoever on the natural food of this snail. I am indebted to Prof. A. E. Boycott for the following information personally communicated to me. Prof. Boycott watched active colonies of *H. pomatia* in Surrey and Hertfordshire. When he has observed them feeding, they have always been on decayed leaves and stalks on the ground, with one exception, which occasionally fed on a plant of burdock. It is likely, therefore, that the snails would eat fungi with decaying plant matter, thus obtaining ergosterol which is characteristically found in fungi.

In view of this, it is impossible to come to any decision with regard to the vitamin D and cholesterol requirements of the snail, especially since the cholesterol used may have contained ergosterol, until further experiments are made with purified sterols.

That at least one factor of the vitamin B complex is necessary for growth in snails, is suggested by Exp. XI *b*, Fig. 1.

There is conclusive evidence that chlorophyll is not an essential constituent in the diet of *H. pomatia* (Exp. III *c*, Fig. 2).

Up to the present, dietetic experiments have largely been confined to animals in three different phyla, namely Chordata (almost exclusively on mammals), Arthropoda, Class Insecta and, in this present case, a Gastropod Mollusc; a brief comparison of results with regard to vitamin requirements is of interest. *H. pomatia* appears to resemble mammals more closely than insects in its vitamin requirements, in that it needs vitamin A, while insects do not. All three groups seem to require several factors of the vitamin B complex [Hobson, 1933, 1935; van't Hoog, 1935; Frost *et al.* 1936]. If snails do require cholesterol, then they resemble insects rather than mammals in being unable to synthesize this sterol themselves.

Finally, unlike the majority of insects, *H. pomatia* appears to be free from symbionts which synthesize and make available vitamins of the B complex.

SUMMARY

1. A technique by which the dietetics of *H. pomatia* can be investigated is described. By this technique young animals may be used in experiments soon after they have hatched.

2. The details of a semi-synthetic diet consisting of purified casein, pure carbohydrates and an artificial salt mixture are given. With the addition of leaf extract or cod liver oil and commercial cholesterol, growth very nearly as rapid as that of control animals was obtained.

3. Snails require vitamin A or the provitamin, carotene.

4. Snails require some or all of the constituents of the vitamin B complex.

5. The requirements of snails in regard to cholesterol and vitamin D are not clear.

6. Snails do not require chlorophyll.

7. There is no evidence of the presence of symbionts of dietetic importance in the alimentary canal of the snail.

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