XXVII. DIETETIC EXPERIMENTS WITH FROGS.

BY ARTHUR HARDEN AND SYLVESTER SOLOMON ZILVA.

From the Biochemical Department, Lister Institute.

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THE preliminary experiments of which the results are recorded in the present paper were instituted with the object of ascertaining whether the accessory food factors had any influence on the development and growth of the frog. Both the larval and adult forms of *Rana temporaria* were investigated.

1. *Experiments with Tadpoles.*

These experiments were not entirely satisfactory and are therefore not described in detail. Sets of 6 tadpoles were kept in 200 cc. of boiled tap-water in Erlenmeyer flasks, and were transferred into fresh water every day. The diets were added to the water on five days during the week. They consisted of a basal diet of purified caseinogen (20 %), starch (75 %) and salts (5 %), to which the accessory factors were added in the form of fat-soluble *A*, 4 drops of butter-fat; water-soluble *B*, 0.5 cc. of 1 % marmite; anti-scorbutic *C*, 1 cc. of lemon juice freed from citric acid. For comparison a set of 11 tadpoles was kept in a small aquarium, to which small pieces of raw meat were occasionally added. These control animals thrived well, passed through the normal stages and emerged as frogs in the course of 6–9 weeks (with the exception of three which were accidentally lost). On the other hand the experimental animals on the complete diet (Basal + *A* + *B* + *C*) did not succeed nearly so well—only 2 out of 6 reaching the mature state, the others dying on the 15th, 40th, 81st and 155th day in various stages. Similar results were obtained with the diets Basal + *A* and Basal + *A* + *B*, two frogs being produced in the former and one in the latter case.

It is obvious that under these circumstances observations on rates of growth are of no great value and it is proposed to repeat the experiments when a thoroughly satisfactory form of experimental diet has been found. It may however be pointed out that no tadpole reached the mature state which had not had butter-fat in its diet.

The experiments of Emmett and Allen [1919] which were published while our experiments were in progress show that the problem is a very difficult one as the rate of growth and development is greatly affected by the amount and nature of the fat, carbohydrate and protein as well as by the presence or absence of accessory food factors.

2. *Experiments with adult Frogs.*

The frogs were kept in glass basins covered by book muslin and containing a shallow layer of water. They were fed by hand two or three times a week, a bolus of about 0.5–1 g. of the diet being administered. The diets employed were identical with those commonly used for dietetic experiments. The basal mixture consisted of a mixture of caseinogen (20 %), starch (75 %) and salts (5 %), and the accessory factors were added in the form of clarified butter-fat (*A*), autolysed yeast (*B*) and lemon juice freed from citric acid (*C*).

The following diets were used, the composition being in every case adjusted to give a mass of suitable consistency.

	Control	<i>ABC</i>	<i>AB</i>	<i>AC</i>	<i>BC</i>	<i>A</i>	<i>B</i>	<i>C</i>
Basal mixture, g. ...	10	10	10	10	10	10	10	10
Butter-fat, g. ...	—	1.6	1.85	1.8	—	1.3	—	—
Autolysed yeast, cc. ...	—	4.7	5	—	4	—	4	—
Anti-scorbutic equivalent to lemon juice, cc. ...	—	4.7	—	5	4	—	—	4
Olive oil, cc. ...	3.1	0.24	2.5	1.6	0.6	—	3.1	3.1
Water, cc. ...	5	—	—	—	—	4	2	2

A disturbing factor was introduced by the fact that some of the animals were attacked by a fungoid disease which caused ulceration of the flesh. The animals which suffered in this way are noted in the tables of results. The frogs were weighed weekly, but it was found that a considerable casual variation occurred. Most of them gained weight during the course of the experiment.

	Diet	No.	Orig. wt. g.	Final wt. g.	Duration of experiment days	Result
<i>ABC</i>	...	5	18.8	26.4	371	Survived
		10	10	26.8	369	"
		11	10.2	14	54	Disease; chloroformed
		27	10	21.4	348	Survived
<i>BC</i>	...	2	38	47.5	361	"
		4	23.1	35.5	339	Disease; died
		23	10.7	14.5	267	" "
<i>AC</i>	...	15	17.3	21	82	Died
		16	12.7	18	74	"
		13*	17	23.2	155	"
		3	17	30.8	42	"
<i>AB</i>	...	17	15.9	32.1	369	Survived
<i>A</i>	...	21	17.5	24.5	56	Died
		25	9.8	11.8	37	"
<i>B</i>	...	19	14.6	20	70	"
		20	15	27.6	369	Survived
<i>C</i>	...	22*	18.2	25.3	91	Died
		26*	13.9	20.6	73	"
Control	...	6	10.2	15.1	52	"
		7	27.1	31	112	Disease; died
		12*	9.2	19.1	26	Died
		9*	13.2	12.8	22	"
		28	12.4	16.8	111	"

In the cases marked * (Nos. 13, 22, 26, 12 and 9) the animals took up a characteristic attitude before death, which is often adopted by moribund frogs, with the hind legs extended and the fore part of the body raised on the fore limbs.

In several cases (Nos. 6, 15, 16, 22, 25, 26) a nerve was removed and submitted to Miss E. M. Tozer to whom we are indebted for their microscopic examination. In only one case (No. 6) was any abnormality observable. The nerve (the sciatic) had very few nodes and the sheath had an eroded appearance.

In spite of the small number of animals on some of the diets, the following conclusions appear to be justified:

1. All the 7 frogs deprived of both *A* and *B* died within four months. Of these animals 4 showed the "extended legs" symptom; one suffered from the fungus disease.

2. Of the 5 animals receiving both *A* and *B*, with or without *C*, only one died during a whole year and this one was attacked by the fungus.

3. Of the 6 animals receiving *A* (with or without *C*) but not *B*, all died within 155 days, one showing the extended legs symptom.

4. Of the 5 animals receiving *B* (with or without *C*) but not *A*, 4 survived for 250 days. Two of these subsequently died (at 267 and 339 days), both having shown signs of external haemorrhage.

5. Considering the whole set of animals, 11 received *A* in their diet and 12 did not. After 6 months 7 of those receiving *A* and 9 of those not receiving *A* were dead. On the other hand 10 received *B* in their diet and 13 did not. After 6 months all those not receiving *B* and only 2 of those receiving *B* were dead.

This affords clear evidence that *B* is necessary for the continued existence of adult frogs. On the other hand the effect of absence of *A* does not show itself definitely within 6 months. The absence of *C* in these experiments did not produce any definite effect.

In order to obtain definite confirmation of this result 6 frogs were kept on a complete *ABC* diet and 6 on an *AC* diet, with the following results:

Diet		No.	Orig. wt.	Final wt.	Duration of	Result
					experiment	
<i>ABC</i>	...	29	26.9	28.7	273	Disease; survived
"	...	30	26.9	28.3	273	Survived
"	...	31	24	20.8	154	Disease; died
"	...	32	30.2	30.9	273	Survived
"	...	33	23.2	23.9	273	"
"	...	34	21.2	21.4	272	Disease; survived
<i>AC</i>	...	35	26.7	28.5	255	Died
"	...	36	26.7	26.5	241	"
"	...	37	27.3	27.3	229	"
"	...	38	24.7	22.2	146	Disease; chloroformed
"	...	39	20.7	19.8	149	Died
"	...	40	18.6	19.8	219	"

In this set, not considering two frogs (31 and 38), one on each diet, which died while suffering from the fungus, we have 5 on *ABC* diet, all of which survived 272 days, and 5 on *AC* diet, all of which succumbed within 255 days, the actual duration of life being 255, 241, 229, 149 and 219 days respectively.

Characteristic symptoms were only noticed in two cases. No. 39 adopted the "extended legs" position 3 days before death. No. 40 became rigid after being fed on the 14th July, gradually recovered, but had a second similar attack, preceded by a series of convulsions on the 17th July, and was found dead on the 18th. The sciatic nerve was examined in both these cases with the result that the nerve of No. 39 was normal, whereas that of No. 40 contained several degenerating fibres.

These results show that the addition of autolysed yeast juice to the *AC* diet employed in these experiments greatly prolongs the life of the frog. This is most probably due to the presence of the water-soluble *B* factor, but it must be remembered that the autolysed yeast juice also contains a considerable proportion of easily absorbable nitrogen which may also have some effect. Further experiments in which a basal diet richer in protein is being employed are however yielding precisely similar results to those described above.

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SUMMARY.

A series of feeding experiments with synthetic diets indicates that the water-soluble *B* factor is necessary for the continued life of the adult frog.

REFERENCE.

Emmett and Allen (1919). *J. Biol. Chem.* **38**, 325.