A SYNTHETIC FOOD MEDIUM FOR THE CULTIVATION OF DROSOPHILA.

PRELIMINARY NOTE.

BY RAYMOND PEARL.

(From the Institute for Biological Research, Johns Hopkins University, Baltimore.)

(Accepted for publication, January 28, 1926.)

I.

For a number of years past the writer has been studying the duration of life of the fruit-fly, *Drosophila melanogaster*, and the factors which influence it.[1] As this work has progressed it has become more and more evident that the degree of quantitative precision desirable in experimental actuarial work was not attainable when the flies were grown upon a medium which has as its chief ingredient so variable a product, seasonally and otherwise, as the banana. *Drosophila* is now widely used as a laboratory animal, especially in genetic investigations, following the lead of Morgan and his students. It is generally cultivated upon the following medium, which originated in Morgan's laboratory.

H_2O	500 cc.
Agar-agar	10 gm.
Banana pulp	500 gm.

Boil agar until dissolved—about 10 minutes. Mash bananas and add to agar and water and boil for 5 minutes. Bananas must be ripe but not rotten. Pour into bottles for use. Allow to cool and sprinkle lightly with yeast.

The work of Guyénot,[2] Loeb and Northrop,[3] and Baumberger[4]

[1] See a series of papers under the general title, Experimental studies on the duration of life, *Am. Naturalist*, 1921–24, lv–lviii.

[2] Guyénot, E., *Compt. rend. Soc. biol.*, 1913, lxxiv, 97, 178, 223, 270, 332, 389, 443; *Bull. biol. France et Belgique*, 1917, li, 1.

[3] Loeb, J., and Northrop, J. H., *Proc. Nat. Acad. Sc.*, 1916, ii, 456; *J. Biol. Chem.*, 1916, xxvii, 309; 1917, xxxii, 103. Northrop, J. H., *J. Biol. Chem.*, 1917, xxxii, 123; 1917, xxx, 181.

[4] Baumberger, J. P., *J. Exp. Zool.*, 1919, xxviii, 1.

has shown that any notion that fruit in any form is in anyway necessary for any biological process in *Drosophila* is not true. Baumberger found that the flies "can develop normally on yeast nucleoprotein, sugars, and salts."

In the preliminary experiments undertaken to determine the essential conditions for the making of a satisfactory synthetic food medium it was found that the acidity of any medium on which *Drosophila* is grown increases during the life of the culture, to a point where it is stabilized by buffering. In the banana medium this buffering action presumably is produced by chemical compounds and reaction prod-

TABLE I.

pH and Total Acid in Banana and Synthetic Media.

Duration of culture.	pH		Cc. N/10 NaOH equivalent to total acid per gm.	
	SA	BA	SA	BA
days				
Start.	4.1	5.3	0.30	0.24
1	3.9	5.3	0.41	0.28
2	3.5	5.15	0.71	0.29
3	3.5	4.85	0.77	0.41
4	3.5	4.7	1.24	0.67
5	3.5	4.6	0.88	1.27
6	3.5	4.8	1.05	1.66
7	3.5	4.7	1.26	2.20
8	3.5	4.8	1.68	3.33
9	3.5	4.8	1.08	2.45

ucts from the banana itself. In the synthetic media buffer salts were included for the purpose.

Table I shows the mean changes in pH and in total acid (expressed as cc. N/10 NaOH) in a 9 day run of cultures on standard banana (BA series) and one of our trial synthetic media (SA series), the composition of which it is not necessary to detail here, as it was subsequently discarded in favor of a more satisfactory medium.

The general biological results on fertility and mortality of the flies in these experiments showed that *Drosophila* could be successfully cultivated on an entirely artificial medium, in which no natural fruit product was present, and which had a much higher degree of acidity,

as indicated by the pH, than has the standard banana medium in common use. There are certain obvious practical advantages in carrying the acidity of the culture medium to as high a point as possible, having regard to normal behavior and vitality of the flies themselves, because if the pH of the medium can be brought below the limit for the growth of moulds and bacteria which occasionally contaminate cultures of *Drosophila*, the deleterious effects of these other organisms in experimental work will be automatically avoided.

On the basis of these and other experiments there was devised a synthetic medium, called in the laboratory records S-101, which has proved after exhaustive tests to be extremely satisfactory.

The composition and mode of preparation of this new synthetic medium are as follows:

Solution A.	Cane-sugar	500	gm.
	$KNaC_4H_4O_64H_2O$	50	"
	$(NH_4)_2SO_4$	12	"
	$MgSO_47H_2O$	3	"
	$CaCl_2$	1.5	"
	H_2O to make 3000 cc. of solution.		
Solution B.	Agar-agar	135	gm.
	Tartaric acid ($C_4H_6O_6$)	30	"
	KH_2PO_4	6	"
	H_2O to make 3000 cc. of solution.		

Melt the agar thoroughly in the water with heat, add the salts, and for the medium to be used in the fly bottles, mix equal parts of Solutions A and B. For some kinds of work it has proved desirable to have the final food a little less stiff, in which case a smaller amount of agar is used, without changing the composition otherwise.

This medium has a pH when freshly made, cooled, and the agar set, of approximately 3.7. As the flies live upon it the pH falls to a value of 3.0, or in some cases even lower.

II.

This new medium has been tested in a great variety of ways in the laboratory. It has proven so satisfactory that all of our *Drosophila* stocks are now carried on it, as a routine. On account of its high acidity there is practically never any contamination of the cultures by troublesome bacteria. In particular *Bacillus subtilis*, which can make

a great deal of trouble on the standard banana medium, never gets a foothold on this medium S-101. Some moulds will grow on it, but the trouble from this source in routine *Drosophila* work is greatly reduced by the use of this medium.

In order to test the influence of this medium on the fertility of *Drosophila* a series of experiments was performed, according to the following plan.

Using wild type flies of a line-bred strain, half pint milk bottles were set up according to the scheme which follows:

No. of bottles.	No. of parent flies put in each bottle at start.	Initial density (flies per bottle).	Medium.
4	1 pair.	2	Two bottles at each specified density were filled to the depth of ¾ in. with standard banana medium, and lightly seeded with yeast.
4	2 pairs.	4	
4	4 "	8	
4	8 "	16	The other two bottles at each specified density were filled to the same depth with S-101 medium, lightly seeded with yeast, and incubated at room temperature 2 days before the flies were put in.
4	16 "	32	
4	32 "	64	

From these conditions it is seen that volume of food and surface area of food were the same in all bottles. Density of population in the strict sense was selected as the variable in these experiments.

All of the bottles were placed into the same incubator operating at 25°C. and carried at that temperature throughout the experiment. Each day each bottle was examined, any dead flies removed, and a record made of the date of death and the sex of the fly. At the end of 8 days the parent flies were removed from the bottles, before any of their progeny had emerged as imagoes. The bottles themselves were then continued in the incubator and counts of the progeny emerging as imagoes made each day for a period of 8 days after the first progeny fly emerged.

III.

The results respecting fertility in these experiments are set forth in Tables II and III. In these tables are recorded the initial population densities at which each bottle started (number of flies per bottle, all

bottles being the same size and containing the same volume and surface area of food, the volume of air space above food); the mean density of population over the 8 day period, which figure takes into account the number and time of the death of all the parent flies; the number of female days, being the sum over 8 days of the number of female parent flies in each bottle each day; the absolute number of progeny flies produced in 8 days forward from the time of emergence of the first

TABLE II.

The Production of Drosophila melanogaster on the Synthetic Medium S-101. Averages.

Initial density of population.	Mean density over 8 day period.	Total female days.	Total progeny in 8 days.	Progeny per female per day.	Total deaths in 8 days.	Death rate over 8 day period.
2	2.00	16	551	34.44	0	0
4	3.90	28	703	25.11	1	12.5
8	7.80	64	710	11.09	2	12.5
16	15.75	124	612	4.94	2	6.25
32	31.40	247	468	1.89	5	7.81
64	61.66	493	499	1.01	12	9.38

TABLE III.

The Production of Drosophila melanogaster on Standard Banana Medium. Averages.

Initial density of population.	Mean density over 8 day period.	Total female days.	Total progeny in 8 days.	Progeny per female per day.	Total deaths in 8 days.	Death rate over 8 day period.
2	2.00	16	373	23.31	0	0
4	3.65	26	380	14.62	1	12.5
8	7.65	66	435	6.59	5	31.25
16	15.60	120	353	2.94	8	25.0
32	31.00	226	263	1.16	23	35.9
64	62.53	494	250	0.55	31	24.2

progeny fly; the number of progeny produced per female per day over the 8 day period, got by dividing the figures in the 4th column by those in the 3rd column; the total number of deaths among the parent flies in the 8 day period; the death rate per 100 exposed to risk over the 8 day period, got by dividing the total deaths ($\times$ 100) by the number of flies exposed to risk of dying at the beginning of the period.

It is at once obvious from the data in Tables II and III that many

more progeny flies (imagoes) per bottle were produced on the synthetic, S-101, food than on the standard banana medium, the total volume and surface area of food being the same in the two series. This was true of all population densities. The absolute progeny productivity curves rise rather sharply from initial density 2, to a high point at initial density 8. They then fall off rapidly until the bottles of initial density 32 are reached. On the synthetic medium (S-101) the total absolute progeny produced per bottle is a little higher at initial density 64 than at initial density 32, whereas in the case of the banana series the absolute productivity value at initial density 64 is slightly lower than at initial density 32.

The same thing is shown if the more precise method of expressing fertility in terms of progeny per female per day is adopted. The relative amount of this excess is shown by the following percentage figures, which are the percentages which the differences between the two series are of the banana figures.

Percentage Increase in Fertility (Progeny Produced per Female per Day) on the Synthetic Medium S-101, as Compared with Standard Banana Medium.

Initial density.	Percentage increase.
2	47.7
4	71.8
8	68.3
16	68.0
32	62.9
64	98.0

There can be no doubt that the production of progeny, however measured, is much higher on the synthetic medium than on standard banana.

It may be noted, although it is not our purpose to discuss this point especially in this paper, that the results of these experiments agree closely with those obtained by Pearl and Parker[5] in their earlier study of the effect of density of population upon fertility in *Drosophila*, in which work standard banana medium was used.

[5] Pearl, R., and Parker, S. L., *Proc. Nat. Acad. Sc.*, 1922, viii, 212. See also Pearl, R., The biology of population growth, New York, 1925.

The difference between the two series in respect of mortality is quite as striking as that just shown in fertility. Whereas in the 8 days only 8.73 per cent died of the 252 flies exposed to risk of dying in the S-101 bottles, 26.98 per cent of the 252 flies exposed to risk over the 8 days in the banana bottles died. The mortality was relatively three times as great on the banana medium as on the synthetic. The mortality on the banana medium was heavier in this experiment than is usual in our work, so that it would be unwarranted to conclude that generally the new synthetic medium will show as great a superiority in respect of mortality as it did in this particular case. Yet, in spite of this necessary reservation, we feel reasonably certain from other experience with this new medium that there will generally be found to be a smaller mortality of the flies kept on S-101 medium than of those kept on standard banana medium. Experiments are now in progress from which we expect to be able to present much more detailed figures covering this question of relative mortality on the two media.

SUMMARY.

In this paper is described the composition and method of making a standard synthetic medium for the laboratory cultivation of *Drosophila melanogaster*.

It is shown that this medium is greatly superior to the banana medium commonly used for this purpose in respect of both the fertility and the mortality of flies kept on it. The range of superiority in respect of fertility is at different densities of population from about 48 per cent at the lowest, to 98 per cent at the highest densities experimentally reported here. The general experience of the laboratory with this medium, which frees experimental work on *Drosophila* from the incubus of the highly variable banana, shows it to have other points of superiority besides those discussed here.

A detailed account of this investigation, in which Dr. W. B. D. Penniman, Dr. Mary Gover, and Miss Agnes Allen shared, will be published elsewhere.