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Title: Roles of Baker's Yeast and Brewer's Yeast in Supporting Development of Drosophila

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Since the early days of genetic research, Drosophila culture medium has been routinely supplemented with baker's yeast (Saccharomyces cerevisiae) and brewer's yeast (Saccharomyces carlsbergensis) to provide nutrients not provided by other components of the medium. The purpose of this study was to learn more about the specific roles of these two yeasts in supporting the growth of Drosophila.

In the traditional way of preparing fly food, brewer's yeast is mixed in with the other ingredients before cooking and baker's yeast is sprinkled on top of the medium after cooking and cooling. This study investigated 1) the effects of reversing the positions of the two yeasts and of using only one of the yeasts at a time; 2) the effect of varying the amount of baker's yeast added; and 3) the effect of adding chemically well-defined supplements in place of the two yeasts. The chemicals tested included nucleotides, nucleosides, ATP and standard vitamins.

The overall study showed that over a short span, baker's yeast alone was as effective in supporting Drosophila development as the standard supplement which uses both brewer's and baker's yeasts. The best chemically-defined substitute for the two yeasts contained five nucleosides plus ATP plus a standard vitamin mixture. Addition of purine bases alone resulted in delayed reproduction and smaller numbers of offspring.

ROLES OF BAKER'S YEAST AND BREWER'S YEAST IN SUPPORTING DEVELOPMENT OF *DROSOPHILA*

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INTRODUCTION

Drosophila and yeasts are known to be mutally dependent. Great importance has been attributed to insects as bearers of yeasts to fruits and other substrates. Flies of the genus *Drosophila* have been observed carrying yeasts to grapes in French vineyards (Gordon, 1943; Steinhaus, 1946). Several species of *Drosophila* breed in decaying fruits, and yeasts and other microorganisms are found to be present in all cases (Wagner, 1944). Dobzhansky and Dacunna (1955) stated that species of *Drosophila* often showed clear preferences for one or another of the yeast strains that are found among fruits such as banana.

Yeasts contain many nutrients which are essential for insect growth, including minerals (Eddy, 1956), amino acids (Gale and Naguib, 1954), carbohydrates (Trevelyans and Harrison, 1952) and lipids (Klein, 1955). The vitamins important for *Drosophila* development are nicotinic acid (Sang, 1956; Erick and Sang, 1966), pantothenic acid (Hinton et al., 1951a,b), pyridoxine (Schultz et al., 1946; Sang, 1954; Rudkin and Schultz, 1949; Hinton et al., 1951a,b), biotin, D-amino-benzoic acid, folic acid and riboflavin (Hinton et al., 1951a,b) (Table 1).

Yeasts can provide *Drosophila* with not only vitamins, carbohydrates, fats, and proteins, but also with pyrimidines and purines in the form of nucleosides and nucleotides. *Drosophila* needs the purines and pyrimidines to synthesize DNA and RNA during metamorphosis from larva to adult (Hinton et al., 1951a,b; Villee and Bissell, 1948).

A number of purine bases and their analogs (Ho et al., 1984b) and pyrimidine base and nucleoside analogs (Vitt et al., 1982; Adams et al., 1985) have been shown to exert morphogenetic effects and to inhibit development of *Drosophila*. Some information is available on purine

Table 1. Vitamin content of dried Brewer's yeast and dried Baker's yeast (Hinton et al., 1951b).

Vitamin	Dried Brewer's yeast (mg/100g)	Dried Baker's yeast (mg/100g)
Vitamin B_1	5.0-36.0	0.0-4.0
Riboflavin	3.6-4.2	3.9-7.5
Nicotinic acid	32-100	20-70
Pantothenic acid	10	18.0-33.0
Biotin	0.5-0.18	0.05-0.18
p-Aminobenzoic acid	0.9-10.2	2.2-17.5
Vitamin B_6	2.5-10.0	1.6-6.5
Inositol	270-500	400
Folic acid	1.5-8.0	1.5-8.0

metabolism in Drosophila (Ho et al., 1984a; Ho et al., 1985), but little is known about pyrimidine metabolism (Becker, 1974).

Mutations have been observed in the few Drosophila which survive in media containing pyrimidines but no purines. The gene affected is known as "rudimentary". The addition of RNA from an external source restores viability (Sang, 1957; Norby, 1970; Falk and Nash, 1974). The rudimentary mutant or phenotype seems to be a spontaneous mutant when RNA and its derivatives are deleted from the media. This phenotype is caused by a block in the biosynthesis of pyrimidines (Sang and Vyse, 1970; Norby, 1973; Naguib and Nash, 1975).

Other Drosophila mutants affected by the availability of purines and pyrimidines in the diet are the allelic auxotrophic mutants. These mutants respond well to dietary ribonucleosides (uridine, cytidine, adenosine, guanosine and inosine) but less well to bases or pyrimidine precursors. The mutants were originally thought to be deficient in phosphoribosyl pyrophosphate (PRPP), a substance required by both purine and pyrimidine pathways; but when PRPP was monitored, there was no conclusive evidence that PRPP deficiency occurred. It seemed likely that the mutation was a regulatory response to a reduced pyrimidine pool (Norby, 1973; Okata et al., 1974; Rawls and Fristrom, 1975). The females in this mutant case are fertile and do not have obviously defective wings. The eggs produced are somewhat less severely defective (Naguib and Nash, 1976).

Earlier studies suggested that Drosophila can be grown on a nucleic acid-free diet (Villee and Bissell, 1948). In these experiments there was no improvement in the growth rate when the medium was supplemented with DNA. Other experiments (Sang, 1957) showed slower growth rate in Drosophila raised on diets deficient in RNA. Sang (1957) has also shown

that the addition of pyrimidine nucleotides to the diet does not improve growth, unless an adequate source of purine nucleoside is also provided. Drosophila may be unable to use DNA because it cannot digest DNA or because deoxyribonucleotides are not converted into ribonucleotides by the larvae (Burner and Sang, 1963).

Dietary purines, especially adenine, affect growth and development of Drosophila melanogaster as well as the purine-pyrimidine profiles of their larvae and pupae (Ho et al., 1984a). Since Drosophila culture media are supplemented with baker's and brewer's yeasts which contain large amounts of purines and pyrimidines, the purpose of the present research was to observe the effects of these two yeasts both alone and together in supporting the development of Drosophila.

MATERIALS AND METHODS

Animals

The yellow-body mutant strain of *Drosophila melanogaster* was used in all experiments.

Culture conditions

Ten 19.8 cm by 4.95 cm food-containing vials were prepared for each of the different diets utilized in these experiments. One virgin male and one virgin female were placed in each of the vials. The vials were placed in a 25 C incubator with a daily cycle of 12 hours light/12 hours darkness. The food was kept moist with daily additions of small amounts of deionized water solution. Daily records were kept on the numbers of larvae, pupae, and adults emerging in each vial.

Basic diet

The basic diet for these experiments was the following version of Ludwig's (1951) formula for *Drosophila* food which was later modified by Vitt et al., 1982.

Water	935 ml
Agar	7.8 g
Corn syrup	74 ml
Brewer's yeast	14.25 g
Karo	9.5 ml
Ethanol	125 ml
Malt	47 ml
Zephiran (Bactericidal agent)	2 ml
Teg M (Bactericidal agent)	3.17 ml

The amounts listed provide enough food for one tray of 24 vials. The mixture was cooked for 6 to 8 min, then allowed to cool overnight.

Baker's yeast (enough to just cover the food) was sprinkled over the surface.

In some of the experiments either baker's yeast or brewer's yeast or both were omitted. These yeasts are sources of vitamins, known to be requirements for normal development in Drosophila. Vitamins were therefore provided in these diets in a supplement originally developed at the University of California-Davis for rat nutrition experiments. It had the following composition:

Inositol	25 ug/g
Ascorbic acid	5 ug/g
Ca pantothenate	2.5 ug/g
Pyridoxine HCl	1.5 ug/g
Nicotinic acid	1.5 ug/g
Menadione	1.25 ug/g
Riboflavin	0.25 ug/g
Para-aminobenzoic acid	0.5 ug/g
Folic acid	0.03 ug/g
Biotin	0.125 ug/g
Rovamix t:50 %	11.9 ug/g
Rovamix A-250	2.73 ug/g
B12 + mannitol	1.5 ug/g
Rovamix AD3 325/325	0.23 ug/g
Cerelose	895.0 ug/g
Choline chloride (70 %)	71 ml

Just before the food was introduced into the glass vials, the vials and their plastic or cotton stoppers were autoclaved for 20 min. to reduce fungal infections. Five ml of food were placed in each of the vials and left to cool overnight at room temperature. This allowed the

food to settle and solidify and allowed excess moisture to escape.

The yellow body mutant of Drosophila melanogaster has a light yellow-tan body color, rusty eye color and brown bristles. Males are easily distinguished from females. The male has dark sex combs and a dark abdominal tip, while the female has a v-shaped abdomen and lacks pigment on the tip of the abdomen. One male and one female adult were added to each vial. Virgin females were used in all experiments to ensure that mating occurred in the breeding vials rather than in the stock bottles. Ten replicate vials were used in each experiment. The incubator was kept humid throughout these experiments to prevent the food from drying and shrinking. In addition, deionized water solution was dropped into the food frequently, keeping the food mass moist and expanded. This reduced overcrowding in two ways. With no air space developing between the glass vials and the side of the food mass, there was less available surface for egg deposition. Later, when the larvae emerged, they could utilize the whole surface of the food mass, not just those restricted regions which remained moist (Glaser, 1923).

The following five experiments were set up:

Experiment 1. The importance of each of the two yeasts was studied. Four sets of 10 vials were used in the experiment. The first set was the yeast-yeast control prepared in the standard way, with brewer's yeast mixed into the food before cooking and baker's yeast sprinkled on the surface of the medium after cooking. The second set had brewer's yeast only, mixed into food mass before cooking. The third set had baker's yeast only, sprinkled on top of the food. Both yeasts were omitted from the fourth set.

Experiment 2. The purpose of the second experiment was to determine

the optimum quantity of yeast to be added to produce the greatest number of flies. In experiment 2a, the amount of brewer's yeast in the food was kept constant and the amount of baker's yeast sprinkled on top was varied (ten vials each at 10, 20, 30, 50, 75, and 100 mg). In experiment 2b, brewer's yeast was omitted and the amount of baker's yeast varied just as in experiment 2a. The numbers of emerging larvae, pupae, and adults were counted for each of the conditions and a dissecting microscope was used to look for physical abnormalities in the adults.

Experiment 3. In both parts of this experiment baker's yeast was placed in the medium rather than its standard position on top. In experiment 3a, baker's yeast was mixed in the food and cooked with it and brewer's yeast was sprinkled on top of the food after cooking. In 3b, baker's yeast was again mixed in the food before cooking, but this time brewer's yeast was omitted. Larvae, pupae and adult flies were counted.

Experiment 4. This experiment checked the ability of either yeast alone to support fly development when it was sprinkled on top of the medium. Food without either yeast was prepared, then allowed to cool and solidify for one day. Baker's yeast alone was added in 4a and brewer's yeast alone was added in 4b. Numbers of larvae, pupae and adults were counted as before, and the dissecting microscope was used to check for physical abnormalities.

Experiment 5. In this experiment both yeasts were deleted from the food and some of the nutrients ordinarily supplied by the yeasts were added back as mixtures of relatively pure substances. This specific supplement included five purine-pyrimidine bases (adenine, guanine, cytosine, uracil and thymine), the five corresponding nucleosides, the single nucleotide adenosine triphosphate, and rat vitamin mix. The

five bases and their derivatives were chosen because earlier experiments showed that these were predominant in the yeasts (King and Sang, 1961; Hinton et al., 1951a,b). The experiment was divided into five phases described below.

1. Both yeasts were omitted from the fly food and the pure bases, adenine, guanine, cytosine, uracil and thymine were added (Diet #1).

2. The same pure bases as in Diet #1, plus the rat vitamin mix were added to yeast-free medium (Diet #2).

3. Both yeasts were omitted and the nucleosides of the five bases were added. In a parallel series, the supplement consisted of the five nucleosides, one nucleotide, plus the vitamin mix (Diets #3, 4, 7, 8, 9, 10, 13, 14) in the experiment.

4. One nucleotide (adenosine triphosphate) alone was added to the yeast-free food mixture (Diets #5 and 11).

5. ATP and the vitamin mix were added to the yeast-free food mixture (Diets #6 and 12).

In Experiment 5, as in Experiments 1 through 4, replicate sets of 10 vials were used. Larval size and pupal color were recorded, in addition to the number of larvae, pupae, and adults which emerged in these vials.

Experiment 6. The small numbers of emerging adults in the yeast-free diets (Experiment 5) might be due to abnormal amounts of uric acid, urea, or protein in the developing flies. Concentrations of those substances were therefore measured in the larvae, pupae and adults in the seven of the 14 yeast-free diets in which enough flies developed to permit chemical analyses by using the Du Pont Automatic Clinical Analyzer, which operates on the principles developed by LaPorta and Tifford (1974) for urea, Henry et al. (1974) for total protein and uric acid measurement.

RESULTS

In experiment 1, the ability to support development of Drosophila was tested in each of the following four media:

a. yeast-yeast (control medium)

b. baker's yeast only

c. brewer's yeast only

d. no yeast

The results of experiment 1 are shown in Figure 1. The results in the control vials and in baker's yeast vials are very similar. In both, the flies first emerged on the 12th day and in both large numbers of eggs developed to larvae, pupae, and eventually adult flies.

In the brewer's yeast vials only about 39 % as many flies developed as in the control or the baker's yeast media. The adults started emerging one day later than in control or baker's yeast media. Adult weight was 0.132 mg per individual compared to 0.187 mg in the control and 0.169 mg in the baker's yeast vials. This difference was statistically significant. The larvae grown in the brewer's yeast media were transparent instead of white. They also had mean lengths of 1.1 mm compared to 1.3 mm in the controls (Table 2), and this difference in length was significant at the 0.05 level.

In the medium without any yeast, development was greatly slowed and only about 12.5 % as many adults emerged. The first adults emerged on the 16th day, four days later than in the control. Although the number of eggs appeared to be normal in these vials, only a few developed to the pupal stage. Most of those that developed to the pupal stage continued on to develop into adults. The adults were normal but small, (0.084 mg per individual compared to 0.187 mg in the controls). The pupae looked normal and the larvae were transparent and shorter than controls (0.06 mm vs.

Fig. 1. Results of Experiment 1. Number of flies which emerged each day in the media supplemented with yeasts in the following combinations: Brewer's and Baker's yeast (Yeast-Yeast control), Baker's yeast only (on the medium), Brewer's yeast only (in the medium) and no yeast.

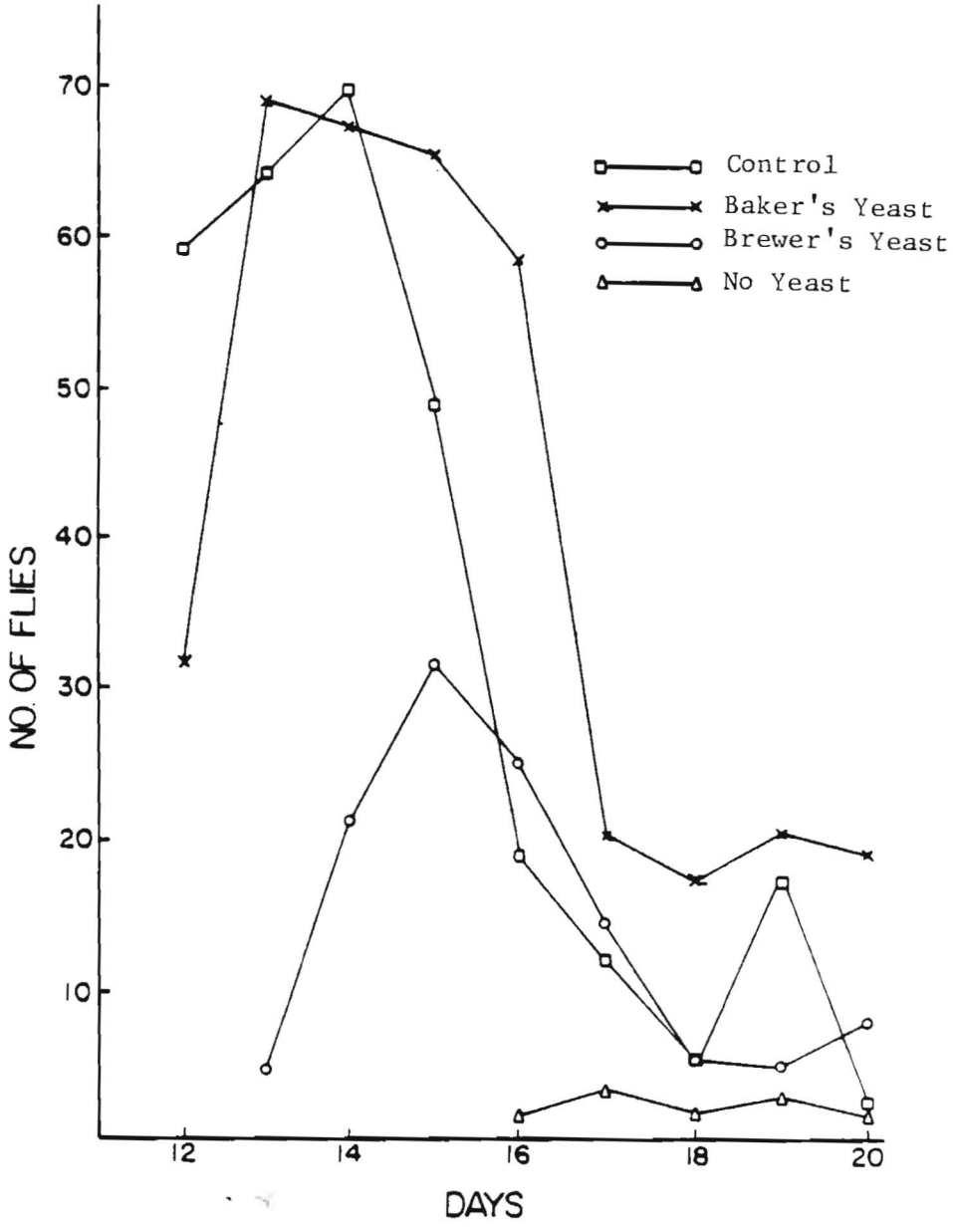

70
60
50
40
30
20
10
NO. OF FLIES
12
14
16
18
20
DAYS
Control
Baker's Yeast
Brewer's Yeast
No Yeast

Table 2. Summary of observations for experiment 1.

Diet Conditions	Appearance of Larvae	Mean Larva Length	Appearance of Pupae	Appearance of Adults and time of Emergence	Mean Adult Weight (N=50)
Control (yeast-yeast)	White normal	1.32 mm	normal and light brown	normal	0.187 mg
Baker's yeast	white normal	1.30	normal and light brown	normal	0.169
Brewer's yeast	white normal	1.10	normal and light brown	normal, but emerged one day later than control	0.132
No Yeast	transparent normal	0.62	normal and light brown	normal, but emerged four days later than control	0.084

Results of T-tests: There were significant differences at the .05 level in weight between the control sample and each of the three other samples. Also there were significant differences in larval length between control sample and the other three samples.

1.32 mm.

In experiment 2 (Fig. 2 and 3), the effect of varying the amount of baker's yeast on top of the media was tested in an attempt to determine the optimum amount of baker's yeast. In experiment 2a, brewer's yeast was included in the medium and in experiment 2b, brewer's yeast was omitted. From Figure 2, it can be seen that the optimum amount of baker's yeast was 50 mg; amounts of baker's yeast either larger or smaller than 50 mg resulted in fewer emerging adults.

A similar result was found in experiment 2 (shown in Fig. 3). When the brewer's yeast was omitted from the medium it was again found that the optimum amount of baker's yeast was 50 mg.

Figure 4 provides an uncluttered comparison of the number of adult flies produced under the two optimum conditions in experiment 2. Surprisingly, more flies were collected, especially on the peak day, from the baker's yeast-only medium than from the yeast-yeast medium; but the difference was not statistically significant.

The vials containing more than 50 mg of baker's yeast were observed to determine why adding larger amounts of yeast resulted in smaller numbers of adults. Numbers of eggs and early larvae appeared to be normal, but a large number of 3rd instar larvae were observed to be dying in the vials. There was a strong odor of alcohol at this time, and it seems highly probable that the extra yeast, instead of providing useful nutrients, produced alcohol in amounts that were toxic to many of the larvae. Larvae normally climb to the sides of the walls to pupate at the third instar, but in these vials, many remained relatively motionless and buried in the medium at the sides of the vials. These turned black as soon as they died.

Fig. 2. The effect of varying the amount of Baker's yeast placed on top of the medium while Brewer's (in the medium) was held constant.

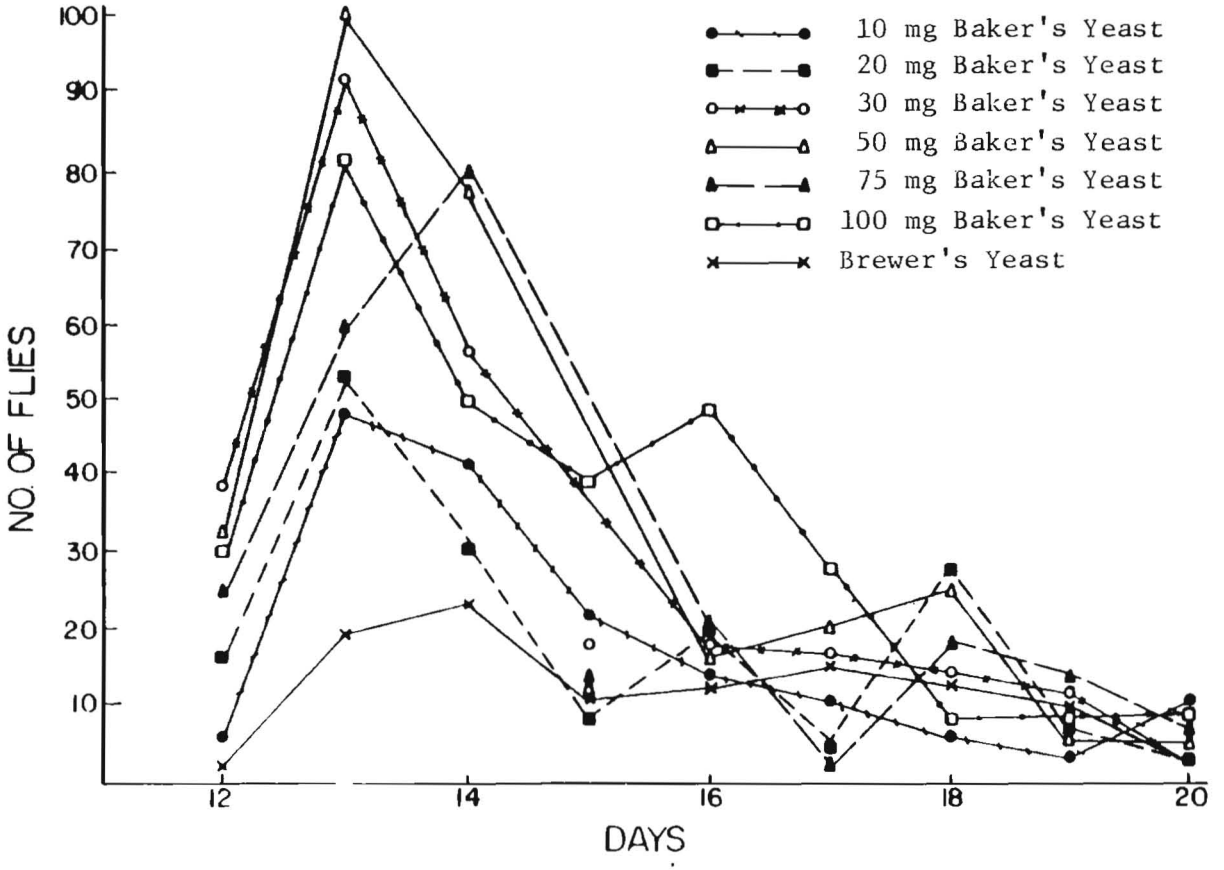

10 mg Baker's Yeast
20 mg Baker's Yeast
30 mg Baker's Yeast
50 mg Baker's Yeast
75 mg Baker's Yeast
100 mg Baker's Yeast
Brewer's Yeast
NO. OF FLIES
100
90
80
70
60
50
40
30
20
10
12
14
16
18
20
DAYS

Fig. 3. The effect of varying the amount of Baker's yeast placed on top of the medium without Brewer's yeast.

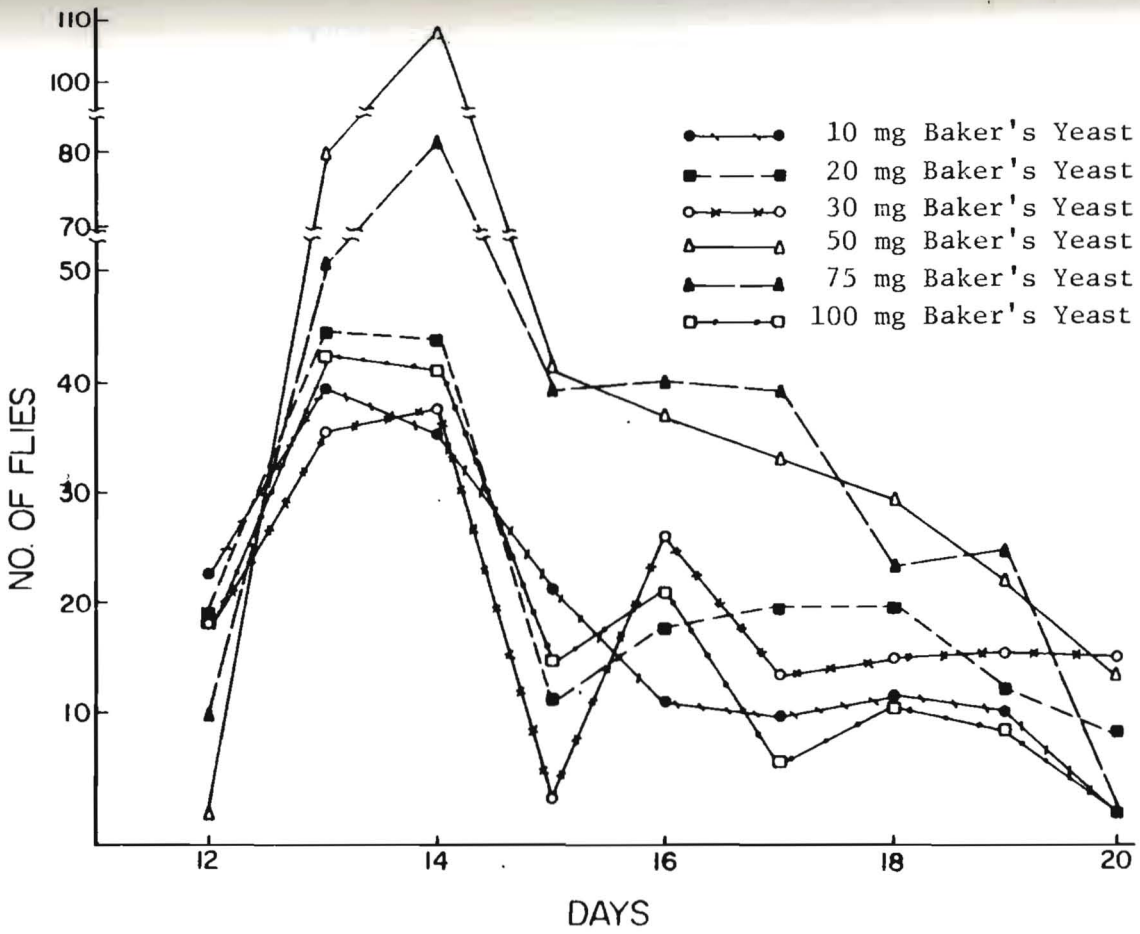
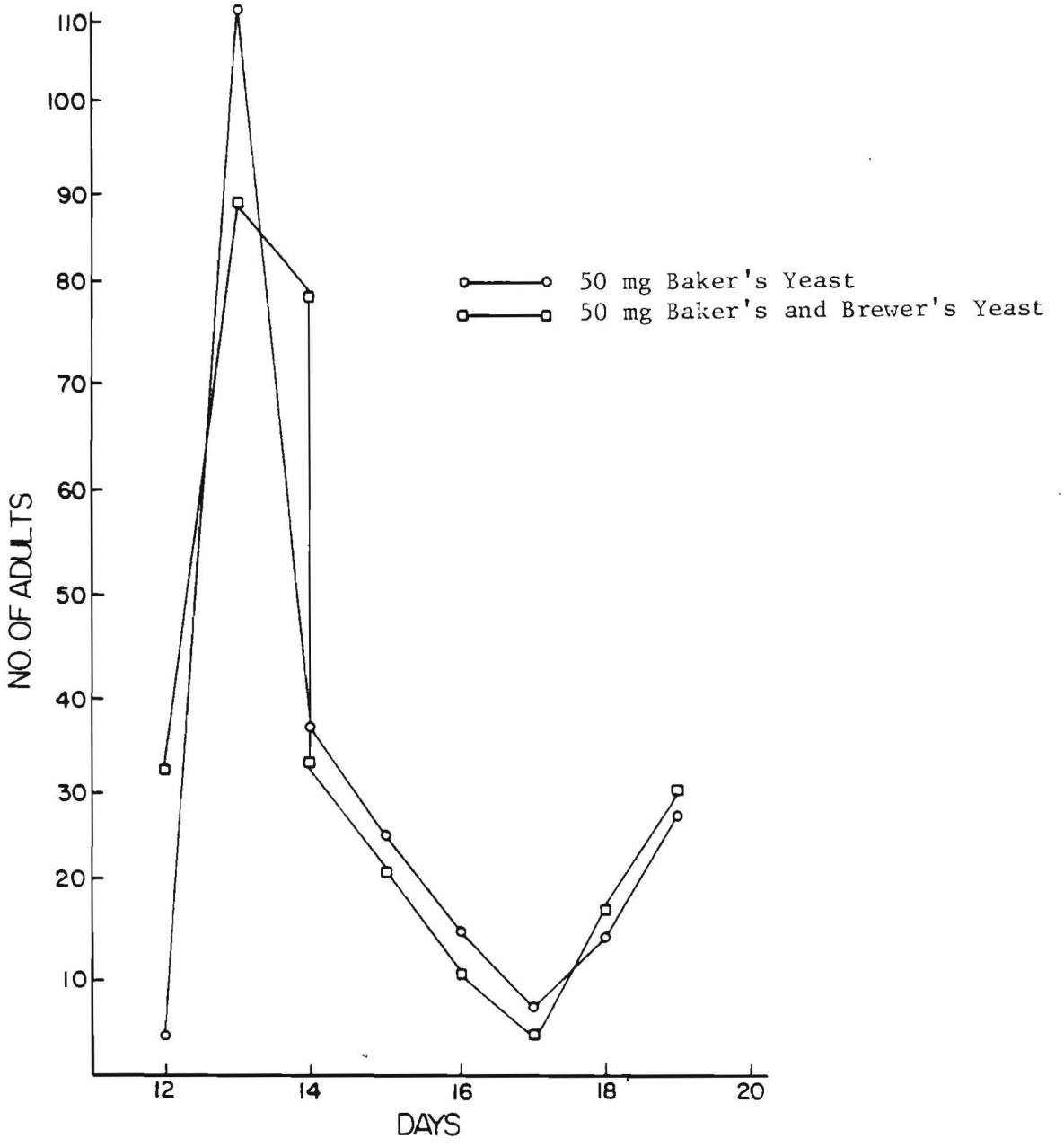

110
100
80
70
50
40
30
20
10
NO. OF FLIES
12
14
16
18
20
DAYS
10 mg Baker's Yeast
20 mg Baker's Yeast
30 mg Baker's Yeast
50 mg Baker's Yeast
75 mg Baker's Yeast
100 mg Baker's Yeast

Fig. 4. Comparison of the optimum amount (50 mg) of Baker's yeast with and without Brewer's yeast.


110
100
90
80
70
60
50
40
30
20
10
NO. OF ADULTS
12
14
16
18
20
DAYS
50 mg Baker's Yeast
50 mg Baker's and Brewer's Yeast

The surviving larvae, pupae and adults were examined in the vials with baker's yeast in amounts of more than 50 mg. The survivors were normal in appearance and were normal in weight and length measurements.

In experiment 3, the positions of the two yeasts were reversed, the baker's yeast now being put in the medium and the brewer's yeast being placed on top. The results are shown in Figure 5. Even with both yeasts present (but in reversed positions) only about one-third as many adults emerged compared to the yeast-yeast control vials with the two yeasts in their usual positions. With baker's yeast only (in the medium), only about one-fourth as many adults emerged and the first emergence was delayed by a day. With brewer's yeast only (on top of the medium), only about one-sixth as many adults emerged as in the yeast-yeast controls, and the first emergence was delayed by a day. As poor as the development was in the three experimental conditions, it was better than that observed in the vials containing no yeast at all, in which the first emergence was delayed for six days and very few adults ever emerged at all.

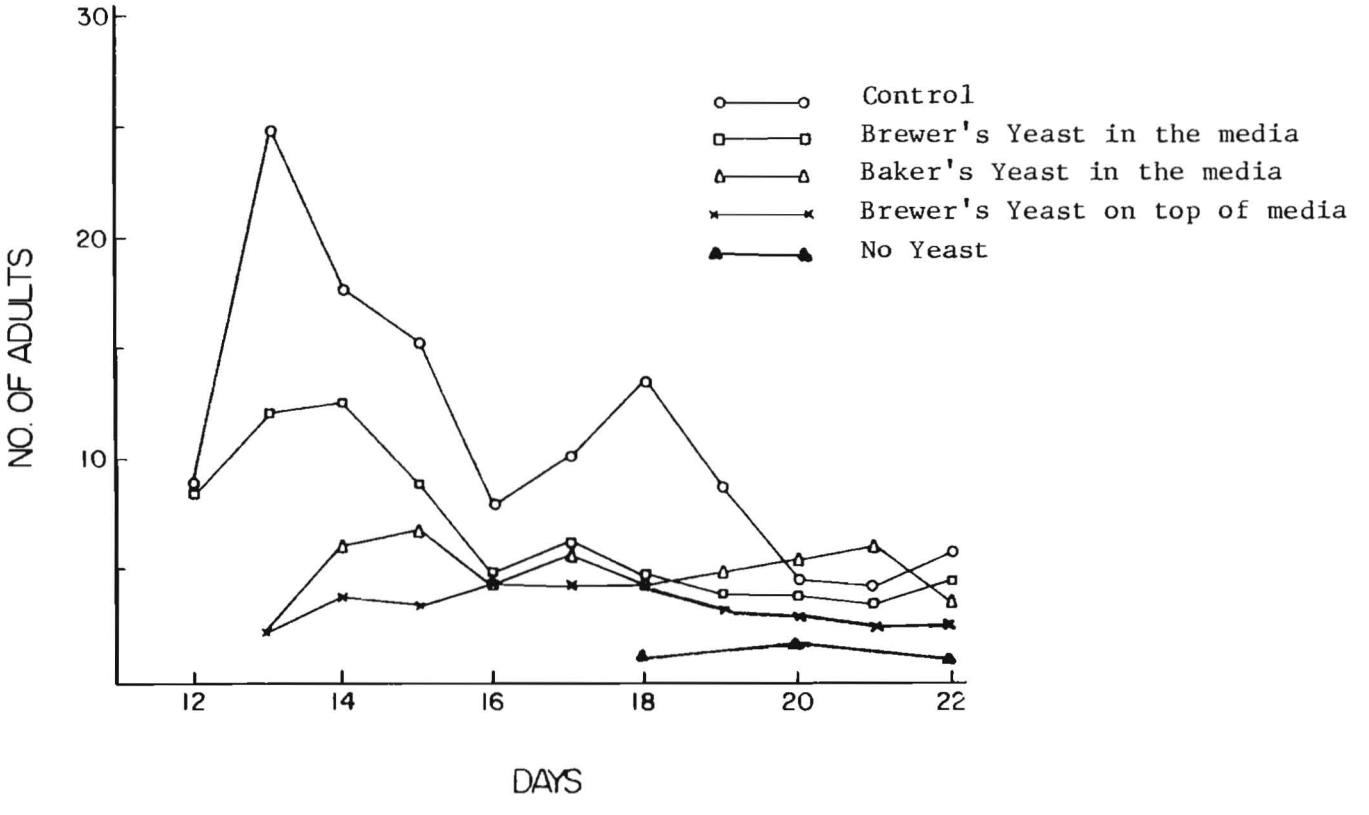
In experiment 4 (Table 3), the objective was to test the effectiveness of either yeast alone when placed on top of the medium. In the medium with baker's yeast alone on top, nearly three times as many adults emerged as in the medium with brewer's yeast alone on top, although the initial number of eggs appeared to be about the same in both media. With brewer's yeast, the emergence of the first adults was delayed one day. As in experiment 1 (Fig. 1), the number of flies emerging with the baker's yeast only on top was almost as great as in the control medium (with both baker's yeast and brewer's yeast in the traditional positions).

Experiments 1 through 4 dealt with the ability of the two yeasts,

Table 3. The ability of either yeast to support fly development alone.

Day	Avg. No. of flies produced in the control (yeast-yeast) (10 vials)	Avg. No. of flies produced with Baker's yeast on top (10 vials)	Avg. No. of flies produced with Brewer's yeast on top (10 vials)
12	8	4	
13	20	17	
14	11	15	2
15	6	8	7
16	8	6	4
17	3	5	3
18	1	2	4
19	2	1	2
20	1	3	1
21	1	1	-
22	1	-	-
Total Avg. No. flies produced	62	62	23

Fig. 5. Effect of reversing the standard positions of the two yeasts. Baker's yeast was mixed into the medium and Brewer's yeast was sprinkled on top of the medium.


30
20
10
NO. OF ADULTS
12
14
16
18
20
22
DAYS
Control
Brewer's Yeast in the media
Baker's Yeast in the media
Brewer's Yeast on top of media
No Yeast

individually and in various combinatins, to support fly development. Experiment 5 (Table 4) was an attempt to replace the yeasts with combinations of relatively pure chemicals. The chemicals were the five purine and pyrimidine bases (adenine, guanine, cytosine, thymine, and uracil), the corresponding nucleosides (adenosine, guanosine, cytidine, thymidine, and uridine), one nucleotide (adenosine triphosphate), and a vitamin mix (the Davis rat vitamin mixture). Fourteen different combinations of the chemicals were tested. The compositions of these media and the results of their testing are summarized on Table 4. Of the 14 combinations, only three produced adults and even in these three only a few adults emerged. The supplement which came closest to replacing the yeast-yeast control diet by the number of adult flies that emerged, was 0.15 % adenosine triphosphate + 0.15 % each of the five nucleosides + 0.15 % rat vitamin mix (Diet #8). The next best supplement, which was almost as effective, was the 0.15 % ATP + 0.15 % of each of the five nucleosides (Diet #7).

In the vials with the vitamins (Diets #2, 4, 6, 10, 12, and 14), the eggs hatched but did not develop further than the pupa stage. The larvae survived longest in the vials which contained ATP alone (Diet #5), ATP mixed with vitamins alone (Diet #6, 12), or ATP with nucleosides alone (Diet #7).

The results could be explained either in terms of the toxicity of the higher concentrations of the purine-pyrimidine bases and of ATP or in terms of taste avoidance by the flies. Normally, the flies were found on top of the food and eating it, but in the experimental vials the flies were most often observed away from the medium near the stoppers. The color of the food changed to a darker brown in the second week, and the flies only then started eating and laying eggs. Of the larvae which

Table 4. Summary of development of flies in 14 different supplemented diets in which an attempt was made to substitute for the two omitted yeasts.

Diet	1st week	2nd week	3rd week	4th week	5th week
Yeast-Yeast Control	Avg. no. of eggs = 30 3-4 days Avg. no. of larvae = 28	(8-9 days) Avg. no. of pupae = 27 Avg. no. of adults = 25			
1. 0.15 % Bases (adenine, guanine, cytosine, thymine, uridine)	Avg. no. of eggs = 25	----------	----------	----------	----------
2. 0.15 % Bases + rat vitamin	Avg. no. of eggs = 30	----------	----------	----------	----------
3. 0.15 % nucleosides	Avg. no. of eggs = 18	----------	----------	----------	----------
4. 0.15 % nucleosides + rat vitamins	Avg. no. of eggs = 19	Larvae Avg. no. = 12	Pupae Avg. no. = 9	----------	----------
5. 0.15 % nucleotide (adenine triphosphate_	Avg. no. of eggs = 25	----------	Larvae Avg. no. = 7	Pupae Avg. no. = 5	Adults Avg. no. = 3
6. 0.15 % nucleotide (ATP) + rat vitamins	Avg. no. of eggs = 23	Larvae Avg. no. = 19	(The food changes to a dark brown color.) Larvae Avg. = 12	Pupae Avg. no. = 9	----------

Table 4. (Continued)

Diet	1st week	2nd week	3rd week	4th week	5th week
7. 0.15 % ATP + (5) nucleosides	Avg. no. of eggs = 27	----------	Few larvae Avg. no. = 5	(1) more larvae (Avg. no. = 11) (2) Developing pupae Avg. no. = 12	(1) mature pupae. Avg. no. = 8 (2) Few adults Avg. no. = 3
8. 0.15 % ATP + (5) nucleosides + rat vitamin	Avg. no. of eggs = 25	Larvae Avg. no. = 18	Larvae Avg. no. = 10	Developing pupae Avg. no. = 10	Mature Pupae Avg. no. = 10 Avg. no. = 5
9. 0.20 % nucleosides (guanosine, thymidine cytosine, uridine, adenosine)	Avg. no. of eggs = 25	----------	----------	----------	----------
10. 0.20 % nucleosides (5) + rat vitamins	Avg. no. of eggs = 30	Few larvae Avg. no. = 7	----------	----------	----------
11. 0.20 % nucleotide (ATP)	Avg. no. of eggs = 42	Larvae Avg. no. = 15	Pupae Avg. no. = 9 Pupae Developing Avg. no. = 2	----------	----------

Table 4. (Continued)

Diet	1st week	2nd week	3rd week	4th week	5th week
12. 0.20 % nucleotide (ATP) + rat vitamin	Avg. no. of eggs = 35	Few Larvae Avg. no. = 17	More Larvae Avg. no. = 11 & Pupae Developing Avg. no. = 3	----------	----------
13. 0.20 % nucleosides + 0.20 % nucleotide (ATP)	Avg. no. of eggs = 27	----------	----------	----------	----------
14. 0.20 % nucleosides + 0.20 % nucleotide (ATP) rat vitamin	Avg. no. of eggs = 32	----------	----------	Few Larvae Avg. no. = 5	----------

developed in these vials, about 20 % died and the rest emerged as pupae. The pupae had a lighter brown color than the normal controls. Of the pupae which developed, most went on to become adults. Most of these adults were normal in size, but had a grayer body color than their typical yellow-bodied parents.

The following additional detailed results can be seen from Table 4. Nucleosides plus vitamins did a better job of supporting fly growth than nucleosides alone (Diet #4 vs. 3 and Diet #10 vs. 9). With the vitamins, the larvae emerged, but two and a half weeks later than in the controls. ATP at lower concentration supported growth better than at higher concentration (Diet #11 and 12 vs. Diet #5 and 6). The purine and pyrimidine bases alone or with vitamins (Diets #1 and 2) did not even support hatching.

In experiment 6, with the aid of the clinical analyser, the tested idea was that the accumulation of nitrogenous products may be the probable cause of the emerging adults.

The concentrations of urea, uric acid, and total protein were measured in the larvae, pupae and adults in each of the supplemented media and in the control.

The data indicated no difference in the concentration level of the three nitrogenous products that developed in the supplemented media vs. the control media. The urea concentration data showed a great deal of scatter, and could not be used to determine whether the various level of urea concentration had played a role in the number of flies produced in some supplemented media.

DISCUSSION

Geneticists have long known that the development and propagation of Drosophila in culture may depend on the biological function of the yeast cells in the medium. However, little research has been done to demonstrate the life-supporting roles played by the different yeasts in the culture medium.

Experiment 1 represented the first step in comparing the individual yeasts alone and together. With baker's yeast alone (sprinkled on top of the medium after it was cooked), almost the same number of adults developed as in yeast-yeast controls. With brewer's yeast only (mixed in the medium before cooking), not as many adults developed. This effect could be due to heat destruction of vitamins in the latter.

Yeasts can secrete into the surrounding medium a variety of nitrogen-containing substances, such as amino acids, peptides (Reindel and Hoppe, 1952), nucleotides and growth stimulants (Higuchi and Uemura, 1959). It should be pointed out that the type of compounds secreted by yeast may be of great practical significance. The occurrence of different growth stimulants from different yeast strains has been reported by Burton-Wright (1952), Hopkins (1945), and Rose (1960a,b). It seems possible that specific substances produced by some yeast strains may trigger Drosophila metamorphosis.

It is possible that the two types of yeasts used in these experiments may selectively secrete different fly growth factors. Also, the two yeasts together would have provided the flies with important nutrients, especially vitamins, which neither yeast could provide by itself (see Table 1). The medium with brewer's yeast only could have lost its vitamins and its fly growth factors to heat, and it may have been this loss which delayed development and reduced dramatically the number of

flies that reached the adult stage, in spite of the fact that numerous eggs hatched in these vials (but developed only as far as the first instar).

Experiment 2 compared numbers of flies developing in media with various amounts of baker's yeast versus yeast-yeast controls, and the optimum amount of baker's yeast was found. This study showed that as the baker's yeast was increased, up to 50 mg, the number of flies also increased. Further increases in baker's yeast did not result in more flies but had an adverse effect on the viability of the flies. The probable reason for the adverse effect was the production of an alcohol, most likely ethanol, as a by-product of yeast metabolism (Minato, 1981). The yeast-produced alcohol has been shown to affect the number of the flies eggs that hatch (Minato, 1981).

Thus, the decreased numbers of adults with increased concentration of yeast could be due to prolonged exposure to alcohol. It, therefore, seems highly probable that the extra yeast, instead of providing useful nutrients, produced alcohol in amounts that were toxic to many of the larvae.

Although the vials with lower concentrations of baker's yeast also had an alcohol scent, this scent was not as strong. The larvae seemed most affected at the third instar when they were about to pupate. At the other stages they seemed to spend most of their time embedded in the food. Future extensions of these experiments should include monitoring of ethanol concentrations as the flies develop and should study the effect of elevated ethanol concentrations on each stage of development.

In experiments 3 and 4, in which the yeasts were reversed in their positions, the data indicated that the yeasts individually play different

roles in the development of the flies. The number of flies produced with brewer's yeast only on top of the food was as low as with brewer's yeast only in the medium. When compared with baker's yeast media, there was a three-day delay in the emergence of the fles. In the brewer's yeast media, many eggs were laid but very few hatched. Many more flies were lost at the larval stage, but most of those which survived to the pupal stage went on to become adults. A look at Table 1 (Hinton et al., 1951b) shows that each yeast has certain deficiencies, but when they are put together each makes up for what the other lacks. The data are also consistent with earlier findings that the different yeast strains selectively secrete different particular growth factors (Burton-Wright, 1952; Hopkins, 1945; Rose, 1950a,b).

Early research on the effects of vitamins on Drosophila development showed that low levels of pantothenic acid (below 0.6 ug/ml) inhibited the larvae from pupating (Schultz et al., 1946; Hinton et al., 1951b). With low levels of the vitamin pyridoxine in the diet, most of the larvae do not survive beyond the second instar and very few adults emerge. When both yeasts were put in the media but with their positions reversed, almost as many flies emerged as in the yeast-yeast controls with the yeasts in their traditional positions (baker's on top and brewer's within the medium). The expectation was that the yeast-yeast controls would produce many more flies than the media with both yeasts in reversed positions. The reason for this prediction was that the vitamins from the baker's yeast, now placed in the medium before it is heated, would be destroyed by heat.

Experiments 1 through 4 dealt with the ability of the two yeasts, individually and in various combinations, to support fly development.

Yeasts are rich in protein, nucleic acids, and vitamins (Matile et al., 1969). An attempt was made to determine whether the flies could survive throughout the life cycle when the yeasts were replaced with a combination of relatively pure chemicals. The chemicals were the purine and pyrimidine bases (adenine, guanine, cytosine, thymine, and uracil), the corresponding nucleosides (adenosine, guanosine, cytidine, thymidine, and uridine), one nucleotide (adenosine triphosphate), and a mixture of rat vitamins (Story, 1977).

In experiment 5, various relatively well-defined supplements were used to replace the two yeasts. The supplements included nucleosides, ATP, and vitamin mix in various combinations. The data (Table 4) showed that the closest substitutes for the yeast-yeast control were the media supplemented with ATP plus five nucleosides and rat vitamin (Diet #7, 8) and ATP plus nucleosides (Diet #5). The data demonstrated that those vials containing the vitamin complex in combination with the nucleosides, the nucleotides, or both, produced a greater number of flies than if the supplements were introduced alone in the diet. This appeared to indicate that the vitamins may play a major role in the viability of the flies, a finding consistent with the earlier results of Burner and Sang (1963). In the present experiments, nucleosides and nucleotides by themselves provide a supplement capable of producing adults, but the numbers of adults were much lower than in the yeast-yeast controls. Also, the rate of development was slower than in the yeast-yeast controls. Similar results have been reported in the literature (Erick and Sang, 1966). Viable adults appeared in the yeast-yeast control media in about twelve days, but it took one week to two and one-half weeks before viable larvae were observed in the nucleotide or nucleoside-supplemented media and

adults were not observed in these latter media until three or three and one-half weeks.

When the rat vitamin mix was added to each of the media (Diets #4 and 8), the first larvae were observed at one to one and one-half weeks. More viable larvae were developed in these media than in the comparable media with nucleosides or nucleotides alone (Diets #4 vs 5 and #8 vs. 7). Large numbers of early stage larvae developed in 0.2 % ATP media (Diets #11 and 12), but only a few late-stage larvae and no adults developed. The number of late-stage larvae averaged only 14 per vial in spite of the fact that a large number of eggs were laid. A few of the larvae developed to pupae, but no adults were produced. With these diets there was a high mortality rate; most eggs did not hatch and the few that hatched did not develop beyond the first instar. This observation provided further evidence for the earlier findings that some of the purines (Ho et al., 1984a), or pyrimidines (Adams et al., unpublished data), or even some part of the "rat vitamin mixture" was toxic to the breeding flies (Kouni and Nash, 1974). The data also may agree with the earlier reported toxic effects of vitamins (Sang, 1954; 1957).

Two potentially significant observations were made in these experiments on the breeding adults which were initially added to the vials. The adult females, though they produced large numbers of eggs, seemed not to feed on the media and these females died earlier than the males.

In the vials with supplemented media, the adult flies introduced at the beginning of an experimental run seemed to avoid feeding and mating, and tended to hover around the stopper, as if trying to escape. Avoidance of mating, rather than slowed development of larvae and pupae, may be the explanation for the observed later emergence of adults in some of

the media. After a longer time with the food, the introduced adults were observed feeding on the medium. There was a delayed increase in mating, but more eggs were laid, and within three to four days after mating, the first instar larvae were observed.

Unhatched eggs in the supplemented vials probably encouraged the mite and fungus infections seen in some of these vials. The data also suggest that pure purine and pyrimidine bases were toxic to the flies. The flies could survive in 0.15 % nucleoside mixture plus vitamins but could not survive in 0.15 % nucleoside mixture alone.

Earlier reports have been confirmed that the addition of purine bases (Ho et al., 1984a), pyrimidine base (Adams et al., 1985) or nucleoside analogs (Riski and Lommen, 1973) to the media inhibits Drosophila development. The supplemented media with nucleotide only or with nucleotide plus vitamin mixture (Diets #5 and 6) produced more viable larvae than did the media with nucleosides only, or nucleosides plus vitamin mixture (Diets #3 and 4). Compared to controls, fewer pupae emerged, but most that survived through pupal stage went on to become adults. In both sets of experiments, many flies were lost between the third instar larval stage and the pupal stage. The data showed a gradual decrease in the number of adults that emerged.

For further research, the effects of varying the amounts of vitamin B concentrations on each stage of development should be studied. Earlier studies showed that omission of B vitamins from their food could be detrimental to the flies, especially at the early stages of development (Hinton et al., 1951b; King and Sang, 1961; Royes and Robertson, 1964).

Experiment 6 was an attempt to find the cause of the die-off in some of the supplemented media. The flies produced in the media nucleosides

(adenosine, guanosine, thymine, uridine and cytosine), nucleotide (adenosine triphosphate), and the rat vitamin mix were found to have no difference in the concentrations of uric acid and total protein when compared to the yeast-yeast controls. Some precursors and intermediates in the formation of uric acid have detrimental effects on Drosophila metamorphosis (Vitt et al., 1982; Ho et al., 1984; Adams et al., 1985 unpublished). The accumulation of these intermediates may have contributed to the fact that in the 0.15 % vials, large numbers of larvae emerged, but few were able to develop into adults. In the 0.2 % vials, no adults emerged at all (Table 4).

Most of the developing individuals were lost at the third larval instar or at the pupal stage. The earliest batches of eggs in these media could not survive after they had hatched. Several weeks later, when the adults started feeding on the media, batches of eggs began developing into larvae. One possibility is that the metabolism of the flies adapted to the food and they were able to use the medium. Another is that the medium itself underwent a chemical change, such as oxidation, or was modified by the action of accidental microbial contaminants.

Toxicity of the media was demonstrated when the second generation was grown on medium with the same composition. A large batch of eggs was laid but few larvae were hatched, and most of these died in a few days. For further research, the first generation (F-1) flies and their progeny should be grown in yeast-yeast control media to see if they could be more productive and to find out how many generations it would take to bring the numbers of hatching larvae back to a normal level. Also, morphological observations should be made on flies grown on the baker's yeast-only media for several generations (up to 20) to see if gene effects, as

well as environmental effects, occur. There seems to be an interaction between the specific environment in which the flies developed and a possible genetic effect since the flies collected from the supplemental media had a grey color rather than yellow-colored body. When the gray colored flies were bred in a yeast-yeast media, no yellow-body flies collected, but there was an increase in fertility.

SUMMARY

The role of baker's yeast (Sacchromyces cerevisiae) and brewer's yeast (Sacchromyces carlsbergenesis), in the breeding of the insect Drosophila yellow-body mutant was studied. Several diet variations were used to determine the role of these yeasts. The yeasts were used together and separately; the quantity of baker's yeast used alone and with brewer's yeast was varied from 10 mg-100 mg. The results showed that baker's yeast was as productive by itself as with control yeast and that brewer's yeast alone was the less productive. When the quantity of baker's yeast was varied, the optimum amount of baker's yeast was 50 mg per vial.

The two yeasts were replaced in the fly food with the following artificial supplements: bases (adenine, guanine, cytosine, thymine, uracil), in the form of nucleosides, one nucleotide (adenosine, triphosphate), and the rat vitamin mix. The bases alone could neither support life separately nor when mixed with other supplement and the rat vitamin. The nucleotide alone, and mixed with other supplemental at 0.15 %, produced the first and most flies. The most productive supplemental set was 0.15 % nucleotide, the five nucleosides and the rat vitamin. Numerous eggs were laid by the parental flies in the various mixed supplemental vials, but very few offsprings were collected. The biochemical analysis showed no difference in uric acid and no difference in the protein levels measured from the three developmental stages when compared to the control. The urea concentration results could not be used for the data interpretation because of the scatter in the data.

The study showed that over a short breeding schedule, baker's yeast was as productive as the control. The replacement of the yeast with artificial supplements was inadequate, although some of the mixed

supplements, e.g. 0.15 % nucleotide, the five nucleosides and the rat vitamin, produced viable flies; most of the flies died in the larva stage. The offspring produced fewer flies than their parental flies, in spite of numerous eggs found in the experimental vials.

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