Backhaus, B., E.Sulkowski and F.W.Schlote. Institute of Biology II (Zoology), RWTH Aachen, FR Germany. A semi-synthetic, general-purpose medium for D.melanogaster.

In the course of long-term experiments on the toxicity, accumulation, and metabolism of heavy metals in Drosophila populations we encountered difficulties in comparing and interpreting results from different laboratories. We understand this as a result of the wide variety of general and specialized culture media that are now available. Most of them are introduced without any comments about pH, amount of living yeast, osmotic values, etc.

For our studies, careful control of nutrition is of great importance. Completely defined, synthetic media are of no use for either population experiments or other long-term studies because they are expensive and laborious to prepare (e.g., Hunt 1970) and can only be turned to good account when sterile conditions are guaranteed. Problems with contaminating microorganisms and low viability stocks grown on complex media (e.g., SPENCER 1950) have often been described. They are not sufficiently defined because they have to be inoculated with live yeast. Semi-synthetic or partly defined media so far published have to be seeded with live yeast in order to warrant reproducible growth of Drosophila (PEARL 1926).

For 4 years, we have worked with a semi-synthetic medium which renders inoculation with live yeast superfluous because an excess of dried brewer's yeast (4.5×10^8 cells/ml medium) and yeast extract. The components are easily stocked and less variable than baker's yeast, maize, cornmeal, etc. This highly reproducible medium is capable of producing from two to three times as many offspring as some standard medium (e.g., SPENCER 1950). The medium is attractive for oviposition and has given good results in cultivating both larvae and adults of Drosophila. Its smooth surface makes it easy to count fertile and non-hatched eggs even after 48 hr from oviposition. No living yeast hinders exact counting, which is when using ordinary agar-baker's yeast media.

Table 1. Ingredients for 1 liter of new medium.

Components	Quantities	Manufacturers
Brewer's yeast (dead, dry)	80 g (4.5×10^8 cells/ml)	DIASANA, Radolfzell[1]
Yeast extract	20 g	DIFCO, Detroit[2]
Peptone	20 g	DIFCO, Detroit[2]
Agar	10 g	FLUKA, Buchs[3]
Sucrose	30 g	MERCK, Darmstadt[1]
Glucose	60 g	MERCK, Darmstadt[1]
$MgSO_4 \times 6H_2O$	0.5 g	FLUKA, Buchs[3]
$CaCl_2 \times 2H_2O$	0.5 g	FLUKA, Buchs[3]
Nipagin mixture (added to food as a 10% solution in 96% ethanol)	10 ml	CAESAR&LORETZ, Hilden[1]
Propionic acid	6 ml	RIEDEL-DE HAENAG, Hanover[1]
aqua bidest.	added to a total of 1000 ml	

1=FRG; 2=USA; 3=Switzerland.

The ingredients required for 1 liter of medium are given in Table 1. For some biometrical characters see Table 3.

Preparation. The ingredients are stirred in one at a time except for propionic acid and Nipagin and brought to a boil. The temperature is then allowed to drop below 60°C and the Nipagin mixture is added, stirring constantly. Propionic acid is stirred into the medium just before pouring it into petri dishes or glass vials with a temperature between 40 and 60°C. It should not be poured too early because brewer's yeast and yeast extract sink down when the agar solution is still too hot and fluid.

When down to room tempera, ture, the vials or dishes are stored at about 8°C until ready to use. No (live) yeast is

Table 2. Some chemical characters of the new medium compared with the standard medium by SPENCER (1950).

Parameter	New Medium	Medium by SPENCER
pE	4.75	5.5
water content	80%	90%
osmotic value	875 mOsmol/l	210 mOsmol/l
Ca-content	400-500 µg/ml	±70 µg/ml
Zn-content	± 10 µg/ml	± 5 µg/ml
(live) yeast	not added	added

added. This medium has been used fresh and after several weeks stored in a refrigerator and was shown to be equally effective in either case.

Table 2 presents some chemical properties of the new medium which are important for the accumulation, metabolism, and toxicity of heavy metals in Drosophila. In comparison, the standard medium of SPENCER (1950) is listed.

Some developmental data of D.melanogaster grown on the medium at 23°C and 75% relative humidity are given in Table 3.

Although no living yeast is added, a certain amount of cells can be regularly found in the presence of D.melanogaster grown on the new medium, even when surface-sterilized eggs are deposited on it (Figure 1). This phenomenon has already been mentioned by BEGON (1974). We could show that on our medium these amounts of yeast are of no nutritional importance for the development of Drosophila: Experiments on the effects of Cd-accumulation, the results of which are described elsewhere (in prep.), showed 5 µg Cd/ml medium to be highly toxic to baker's yeast, but developmental time and survival rates of Drosophila were not effected by this concentration. On standard medium (SPENCER 1950), however, even lower Cd-concentrations provided negative effects not only on yeasts but also on flies. Therefore, live yeast is not a major source of food for developing larvae in our medium. This argument is supported by the fact that we have an excess of brewer's yeast (4.5×10^8 cells/ml medium) compared with live yeast cells.

Conclusions. While this recipe is more expensive than media using baker's yeast, maize, etc., we feel its advantages offset the extra costs: Inoculation with live yeast is rendered superfluous. The medium allows standardization of culture conditions and has shown no contaminations with fungi or bacteria if handled in the described manner. The simple handling of its preparation and the developmental data of D.melanogaster grown on this medium justify its general use.

Table 3. Some cultivation data of D.melanogaster grown on the new medium in an air-conditioned room (23°C, 75% relative humidity).

Parameter	Cultivation Data
Optimal culture conditions	
FLY DENSITY[1] ≦ 25 PAIRS/20 CM^2	MEDIUM:
total egg number	20-50 eggs/female a day
non-hatched eggs	5-10% of total egg number
FLY DENSITY[2] ≦ 50 PAIRS/20 CM^2	MEDIUM:
mean life span of adults	Females: 50% mortality after 30 days Males: no significant mortality before 40 days
EGG DENSITY[3] ≦ 50 EGGS/CM^2	MEDIUM:
mean developmental time from egg to adult	10 days + 8 hr (highly reproducible)
survival rate from egg to adult	80-90%
larvae mortality	± 5%
pupae mortality	± 1%
mean dry weight	Females: 280 µg; Males: 220 µg;
Mass culture conditions[4]	
yield of flies	about 1300 flies/vial=250% of standard medium conditions (e.g. SPENCER 1950)

1= several fly densities from 10 to 25 pairs/20 cm^2 medium with 3-6 days old flies (pre-fed as larvae on standard medium) were created in order to measure the following parameters. Egg numbers and percentage of eggs hatching decline with the age of flies.

2= the medium was renewed every 2 days.

3= 25 pairs of flies aged 3-6 days (pre-fed as larvae on standard medium) were allowed to deposit eggs for 24 hr. The adults were removed and their progeny cultivated for the following parameters to be measured (depth of medium: 1cm).

4= 10 pairs of flies aged 2-4 days were allowed to deposit eggs for 7 days in 1/2 pint bottles containing 35 ml medium. The adults were removed and their total progeny counted.

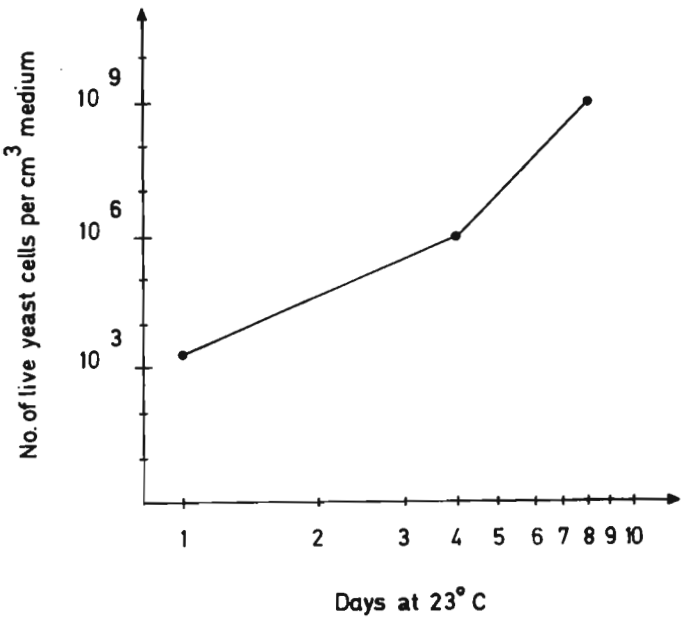

Figure 1. Growth of baker's yeast in the new medium in the presence of D.melanogaster. 25 pairs of flies aged 3-5 days were allowed to lay eggs for 24 hr before day 1. The adults were removed and their progeny cultivated for 8 days. Yeast cells were counted immediately after the removal of adults (day 1), with second instar larvae growing in the medium (day 4), and after all larvae having left the medium for pupation (day 8). From: Köhne, A., A method for determining yeast growth in the medium of D.melanogaster (examination paper, Aachen, 1982, unpubld.).

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References: Begon,M. 1974, DIS 51:106; Hunt,V. 1970, DIS 45:179; Pearl, R. et al. 1926, Am.Nat. 60:357-366; Spencer,W.P. 1950, Collection and laboratory culture, IN: Demerec,M. (ed) Biology of Drosophila, Wiley, New York.

Band, H.T. Michigan State University, East Lansing, Michigan USNA. A high protein medium using soybean protein flour.

The removal of Kellog's Concentrate from the market has created problems for Drosophila workers doing research with species requiring a high protein medium. Two such media used this ingredient (Wheeler & Clayton 1965; Band 1981). In our laboratory we used a high protein diet preparation for a year in place of Kellogg's Concentrate, but this and similar products have been withdrawn from the market. Kellogg's NutriGrain Wheat did not adequately maintain fertility in Chymomyza amoena.

We have found soybean flour to be an acceptable substitute for Kellogg's Concentrate and the high protein diet preparations. The product we use is called Vibrant Health Protein Powder from Michigan Vitamin, Ferndale, MI 48220. We have also continued to use Kellogg's NutriGrain Wheat in our medium since it lists vitamins not specifically mentioned in other ingredients. The following recipe is our current high protein medium:

15 gm Gerber's Hi-Pro	500 ml Spartan applesauce
15 gm Kretschmer's Wheat Germ	650 ml distilled water
5 gm Kellogg's NutriGrain Wheat	45 gm Quick Cream of Wheat
5 gm soybean protein flour	3 ml proprionic acid
7 gm Bacto-agar	9 ml 95% ethyl alcohol

To Prepare: Blend the first 4 ingredients in a Waring Blender for several minutes. Add the applesauce and blend 5 min more. Boil 450 ml of water in a large vessel, add agar and stir to dissolve. Add the applesauce mixture; rinse the blender with 100 ml of water and add to the food mixture. Add the remaining 100 ml of water to the cream of wheat and stir it into the food mixture as it begins to boil. Reduce heat and stir until thickened, usually about