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Influence of Nutrition upon Appearance of Tumors in Tu^{50j} Stock of *D. melanogaster*

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Tu^{50j} is a recessive mutation which appeared upon inbreeding Chicago wild *D. melanogaster,* and it contributes to the production of melanotic growths in the abdomen of the fly. Recently Burdette (*1*) has shown that tu^{48} reared on a medium deficient in yeast produced fewer tumors than those raised on re-yeasted medium, and since then Begg and Robertson (*2*), in an attempt to produce a completely synthetic medium for *D. melanogaster,* have reported that an unknown alkali-soluble factor from yeast must be present for growth. Hence, to determine the penetrance of tu^{50j} the influence of nutrition must be controlled. To be certain that all the nutritive factors (*2*)—chiefly amino acids and vitamins—came from the yeast and not the medium (usually cornmeal-molasses), a survey was made of yeasts that could live on a vitamin- and amino acid-free medium, as shown in Table 1. Included in this medium are the essential elements with which some of the yeasts are able to synthesize their own protoplasm. It is primarily a glucose and ammonium sulfate medium, plus several trace elements.

Twenty-seven species of yeasts from 14 genera were

TABLE 1

VITAMIN- AND AMINO ACID-FREE MEDIUM

Ingredient	Amount
Agar	15 g
$C_6H_{12}O_6$	30 "
KH_2PO_4	1 "
$NaKC_4H_4O_6$	8 "
$(NH_4)_2SO_4$	2 "
$CaCl_2$	0.5 "
NaCl	.5 "
$MnSO_4$	.5 "
$MgSO_4$	.5 "
$FeSO_4$	0.5 "
H_2O	1000 ml

TABLE 2

ABILITY OF YEASTS TO LIVE ON COMPLETE MEDIUM AND ON VITAMIN- AND AMINO ACID-FREE MEDIUM

Yeast	Vitamin- and amino acid-free	M–Y Complete medium
Candida monosa	−	+
C. pseudotropicalis	−	+
C. mesenterica	−	+
C. sorbosa	+	+
Endomyces magnusii	+	+
Hansenula anomala	+	+
H. saturnus	+	+
Rhodotorula sanniei	−	+
R. aurantica	−	+
R. gracilis	+	+
R. glutinis	+	+
R. suganii	−	+
Pichia membranaefaciens	+	+
Debaryomyces globosus	+	+
D. membranaefaciens	+	+
D. disporus	−	+
D. matruchoti	−	+
D. guilliermondii	−	+
Kloeckera apiculata	−	+
Zygosaccharomyces lactose	−	+
Schizosaccharomyces versatilis	−	+
S. fragilis	−	+
Torulopsis utilis	+	+
Nadsonea fulvescens	+	+
Endomycopsis fibuliger	−	+
Geotrichium	+	+
Saccharomyces cerevisiae	−	+

tested for growth on the vitamin- and amino acid-free medium (Table 2); 12 were found capable of living on the minimal medium. The yeasts were transferred twice onto vitamin- and amino acid-free medium to make sure that none of the nutritive ingredients were carried over from the complete medium in which the yeasts were originally grown. To prevent overcrowding and to maintain a standard of comparison, a pair of flies from a highly inbred stock of tu^{50j} were placed on the minimal medium inoculated with yeast. It is believed that practically all the nourishment that the larvae obtained came from the yeast on the medium. The penetrance of tu^{50j} reared on yeasts that do require vitamins and amino acids on a minimal medium is presented in Table 3. It is quite evident from the results that tu^{50j} reared on yeasts growing in the minimal medium produced fewer tumors than those in tu^{50j} reared on *S. cerevisiae,* the yeast most commonly used in Drosophila work, grown on the cornmeal-molasses medium, for *S. cerevisiae* cannot grow on the minimal medium (Table 2). Hence, the larvae in the control series obtained some nourishment from the medium, for the substances essential for the growth of Drosophila are present both in the yeast and in the medium. There are probably as many variations in medium used to rear *D. melanogaster* as there are research workers who study them. It is difficult to separate the effect of the medium and *S. cerevisiae* upon the penetrance of tu^{50j}. However, one can compare the penetrance of the tumor gene when the flies

TABLE 3

PENETRANCE OF tu^{50j} WHEN REARED ON VITAMIN- AND AMINO ACID-FREE MEDIUM INOCULATED WITH VARIOUS YEASTS AND INCUBATED AT 24° C

Yeast	Percentage of *tu*	Total No. counted
Saccharomyces cerevisiae (Control on agar-cornmeal media)	4.7	12,521
Hansenula anomala	4.3	2246
Pichia membranaefaciens	2.1	2381
Candida sorbosa	1.9	2976
Nadsonia fulvescens	1.4	1443
Debaromyces globosus	1.3	1394
Hansenula saturnus	1.2	1603
Torulopsis utilis	1.1	1675
Rhodotorula gracilis	0	490
R. glutinis	0	310
Geotrichium	0	5

are raised on various yeasts that do not require vitamins or amino acids. One can state with assurance that tu^{50j} reared on *Torulopsis utilis* has less penetrance than when reared on *Hansenula anomala*. There is a significant difference between two yeasts in the same genus, *H. anomala* and *H. saturnus,* with respect to the penetrance of tu^{50j}. It may be the presence or absence of certain chemical factors in the yeast that influences tu^{50j} in production of tumors.

The number of flies produced per vial was greater with *S. cerevisiae* than with other yeasts on the minimal medium. Among the yeasts that can grow in the absence of vitamins and amino acids there is little difference with respect to total number of flies produced, except for *Rhodotorula* and *Geotrichium.* On these 2 yeasts the total number of flies was reduced, and *D. melanogaster* had difficulty in reaching adulthood.

Although wild *D. melanogaster* are attracted to the odor of fermenting substances, they can live on the nonfermenting yeast *Pichia membranaefaciens,* and also exclusively on any one of 8 other yeasts.

The above-described method of rearing *D. melanogaster* on a minimal medium, in which the yeast supplies all the essential nutritive substances, is useful in study of gene action and nutrition. The penetrance of tu^{50j} varies with the yeast used for nutrition. What are the chemical differences between the yeasts that give rise to this variation? Will other genes show variable gene penetrance and expressivity when the fly is grown exclusively on the minimal medium and with yeasts which require no vitamins or amino acids?

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Prolongation of Blood Clotting Time in the Dormant Hamster[1]

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It has been shown (*1*) that in ground squirrels of the species *Citellus columbianus* and *C. parryi ablusis* the time necessary for the blood to clot is prolonged when they are in a dormant state. These squirrels both hibernate during the cold winter months and estivate during the summer months. It was considered desirable to know whether prolongation of blood clotting time was peculiar to them or whether it occurred in other mammals, particularly those that hibernate but do not estivate.

TABLE 1

Active				Dormant			
No. animals tested	Clotting times			No. animals tested	Clotting times		
	Max	Min	Av		Max	Min	Av
11	10′17″	2′03″	4′52″	4	156′00″	14′00″*	50′45″

* Hamster was breathing rapidly and was not in "deep" hibernation.

[1] Acknowledgment is made to the Air Force under contract AF33(038)-18509 for aid and assistance in making this study.

The golden hamster of the species *Mesocricetus* (*Cricetus*) *auratus* was chosen as a convenient animal for experimentation, since it can be induced to assume dormancy in the laboratory if the temperature of its surroundings is lowered. The clotting times of the blood of both active and dormant hamsters were determined by using Lee and White's technique. Since superficial blood vessels are small in these mammals, it was necessary to obtain blood samples by cardiac punctures. The results of the experiment are shown in Table 1.

It is apparent from these data that prolongation of blood clotting time is not restricted to dormant ground squirrels but also occurs in dormant hamsters.

It was pointed out in (*1*) that the prolongation of clotting time in ground squirrels is apparently an adaptation to the dormant state, for then the blood flows very slowly and some mechanism is therefore necessary to prevent the formation of thromboses. This same interpretation may also apply in the case of the torpid hamster, whose heart rate and blood flow are similarly reduced It may very well be that this lengthening of the clotting time of the blood is characteristic of those mammals that periodically assume a state of dormancy.

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