Table 1. Response of a tumor-free strain (wild 51–52) to larval-extract injections of various tumor and tumor-free strains.

Donor strain	Larval hosts	Emerged adults	Tumorous adults	Tumor induction (%)
bw tu	647	68	48	71
tu-vg	770	96	61	64
tu-C-S	700	67	39	58
tu-w	697	41	22	54
+/f	400	49	11	22
tu^{36a}	400	54	0	0
C-S	414	53	0	0

produced in a tumor-free strain of *Drosophila* after the injection of hemolymph obtained from a tumorous strain of *Drosophila* (*4, 5*). A tumor-inducing factor, present in acellular *tu-e* larval extracts, when injected into hosts of a tumor-free strain, induced the formation of melanotic tumors which were histologically similar to the tumors present in the *tu-e* strain (*6, 7*). Recent research has been concerned with the activity, properties, and nature of the *tu-e* tumor factor (*8*). This survey was conducted to determine the prevalence of comparable tumor-inducing factors in seven other strains of *Drosophila melanogaster* (*9*).

The technique for the preparation of acellular 96- to 120-hr larval donor extracts, the injection apparatus and procedure, and the preoperative and postoperative care of the 96- to 120-hr host animals was essentially similar to that described by Harnly *et al.* (*6*) and Burton (*10*).

The host strain (wild 51–52) is a tumor-free strain from which no active tumor-inducing factor could be extracted (*6*). However, this strain is highly reactive to the injected tumor factor extracted from *tu-e* larval donors (*6, 10*). Repeated tests have conclusively demonstrated that neither operational injury (incurred in the insertion of the needle into the hosts or in the preoperative and postoperative procedures) nor the injection of diluting medium (Waddington's salt solution) induced tumors in the wild 51–52 hosts (*6, 10*). Barigozzi has also indicated that the trauma of the injection is not causal in the induction of melanotic masses (*4*). A histological study confirmed that injury or the injected diluting medium, or both, did not induce the formation of tumors in the wild 51–52 hosts (*7*).

Four known tumor strains, *bw tu, tu-C-S, tu-w,* and *tu-vg* were tested. Two other strains, *+/f* and *st sr e^s ro ca;* tu^{36a} (hereafter abbreviated tu^{36a}), which were considered to be possible tumor strains (not one tumorous imago was observed in a random sample of more than 500 animals of each of these two strains reared at 25°C on standard food, dissected, and examined) were also tested. The *C-S* strain was a tumor-free strain.

Only adult hosts were dissected and examined for the presence of melanotic tumors because those hosts that failed to reach the imago stage of development might have died before a possible tumor-inducing factor could have operated (Table 1).

Larval extracts of the four tumor strains and one of the purported tumor strains induced melanotic tumor formation in the wild 51–52 hosts, ranging from 22 to 71 percent. Injections of larval extracts of *C-S* and tu^{36a} strains failed to induce the formation of melanotic tumors in host animals.

These results demonstrate that extractable tumor-inducing factors may be obtained from tumor strains other than the *tu-e* tumor strain. However, not all tumorous strains, at least from donors of the age used, provide extracts that contain an active tumor-inducing factor. Extracts of one tumor-free strain (*C-S*) did not possess an active tumor-inducing factor.

Since tumor strains other than *tu-e* contain active tumor-inducing factors, it is very likely that these tumor strains also possess genes involved in the synthesis or activation of tumor-inducing factors or both. Nevertheless, a tumor strain of low incidence that does not possess an active tumor-inducing factor may contain genes that modify tumor gene activity or tumor-inducing factor activity or both (*11*). Indeed, under the proper conditions (ages of donors and hosts) active tumor-inducing factors may possibly be extracted from all tumor strains of *Drosophila melanogaster.*

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Osmotic Gradients across Cellular Membranes

In a recent article [*Science* **121**, 302 (1955)] W. A. Brodsky and others present arguments to prove that the Franck-Mayer hypothesis for the maintenance of osmotic gradients across cellular membranes is untenable for mammalian tissues. They state that the hypothesis would require a minimal energy expenditure of 21,000 kcal/kg hr to maintain the osmotic gradient obtained when mammalian kidney tissue produces a hypertonic urine. Since this value is about 1000 times the maximal rate observed for mammalian tissues, they conclude that the proposed mechanism is inadequate for the maintenance of the observed osmotic gradients.

It would appear that the authors have overlooked an important point in their analysis—namely, that the energy expenditure required is proportional to the tubular surface area *available* for water transport. The authors calculate the total tubular surface area to be 5×10^5 cm²/kg. This is 2 or 3 times larger than is generally assumed. However, such a discrepancy is of minor importance. Of crucial importance is the fact that the fraction of this total area which is actually available for water permeation may be quite small—for example, water may pass only through "pores" that occupy a small part of the surface area of tubular cells, or possibly only through a small percentage of the total number of cells. Much recent work indicates that flux rates across many living membranes as measured with isotopes can be easily accounted for by extremely small fractions of the total surface area of the membrane and that the area actually available for transport may, in many cases, be only a fraction of 1 percent. If the same is true for kidney tubules, the energy required to maintain the osmotic gradient obtained during production of hypertonic urine would also be only a fraction of 1 percent of that calculated by the authors' methods. Thus, a mechanism of the type proposed by Franck and Mayer does not necessarily demand an impossibly high energy expenditure.

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Since Eugene Grim's comment raises some questions on the problem of intracellular osmotic gradients, it is important to restate the problem and to define some of the terms used.

The analysis considered the solute flux and intensity of respiration required for the steady-state maintenance of an os-

motic gradient within a cell under conditions of "idling"—that is, zero water transport. The term *solute flux* means the rate of diffusion of solutes per unit time, per unit area of cell, or per unit area of gradient-maintaining tissue. Intensity of respiration means the rate of energy expenditure per unit time, per unit mass of gradient-containing tissue.

Reduction of the surface area available for water permeation while fixing the cell width across which the concentration difference exists will reduce the total energy expenditure per unit time but *will not affect* the energy expenditure per unit mass of gradient-maintaining tissue, per unit time. Furthermore, such a reduction of "available" area demands that pores of cross-sectional area equal to that of the "available" surface extend across the fixed cell width like microcapillary tubes.

Incidentally, such a structural arrangement differs markedly from the Franck-Mayer scheme, where the gradient existed in an entire cell [*Arch. Biochem.* **14**, 297 (1947)]. Since the gradient would have to be maintained within the pore boundaries, a region of cell fluid would have to be insulated against diffusion between itself and the ambient intracellular fluid. Moreover, there must be a cellular device for funneling a significant fraction of energy from the surrounding cell fluid into the pore, since the energy expenditure per mass of gradient-maintaining region is much greater than that of the rest of the cell. Even if the tubular surface area were reduced to zero, the problem of maintaining a fixed gradient within a localized region (pore) of cell fluid would be the same as that reported previously [*Science* **121**, 302 (1955)]. If the pore extends through the cell membrane only, the osmotic gradient would be increased a 1000-fold, thereby offsetting the reduction of energy expenditure resulting from a decreased surface area.

The effect of reducing "available" surface area during the active transport of a finite volume of water is one of reducing the total energy expenditure while *increasing* the intensity of energy expenditure of the gradient containing tissue. Thus, selection of the appropriate surface area will yield reasonable rates of energy expenditure, but the aforementioned restrictions on pore geometry, funneling of energy, and intensity of respiration still apply. The velocity of flow of transported water increases as the permeation area is decreased, and the energy expenditure over and above that required for gradient maintenance during "idling" can be a small value. This means, as stated previously [*Science* **121**, 302 (1955)], that one cannot exclude rigorously any type of osmotic-gradient hypothesis for the active transport of water. It is pertinent to note that this analysis assumes no frictional resistance to the flow of transported water, no matter how high the velocity, and 100-percent thermodynamic efficiency of the gradient-maintaining mechanism. The minimum value for intensity of cell respiration during water transport is always greater than the value of 21,000 kcal/kg hr, reported previously for the condition of no water transport.

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Ultrafractionated Ultraviolet Irradiation of Yeast Cells

The biological effect of a certain dose of ultrafractionated ionizing radiation was recently studied by Witte (*1, 2*), Witte and Sigmund (*3*), and by Hofmann *et al.* (*4, 5*). Using x- and beta-rays, they found definite minima in the effect on *Drosophila* pupae and eggs and *Escherichia coli* for certain selected frequencies, depending on the ratio of single exposure time (up to 10 μsec) to pause time, which varied from 1/2 to 1/9. This paper presents studies on the effects of ultrafractionated ultraviolet radiation on yeast cells.

The experimental arrangement consisted of a Hanovia quartz lamp (143 v, 1.3 amp direct current, no filter, 7-cm distance) with a motor-driven, aluminum strobic disk (8 circular apertures, each 7/8 in. in diameter, ratio of exposure time to pause time of 1/2). A powerstat provided regulation of motor speeds up to 2100 rev/min; disk speeds were measured with a strobotac. The test specimen was a strain (*6*) of Saccharomyces cerevisiae (*7*). Stock cultures of yeast were grown for 1 to 2 weeks on slants having the following composition: 1 percent dextrose, 0.5 percent yeast extract, 0.5 percent peptone, and 2 percent agar. After incubation at room temperature for 36 to 48 hours, the fresh slants were stored at 5°C. Cells washed from a stored slant into Sorenson's phosphate buffer at *p*H 5.4 served as inoculum for the liquid culture to be used in the experiments. By suitable dilution, the inoculum was brought to a standard optical density of 0.60 to 0.67 (compared with 0.00 for distilled water) as read on a Coleman spectrophotometer at a wavelength of 6000 A. Three 20-ml liquid cultures (media composition: 5 percent dextose, 0.5 percent yeast extract, and 0.5 percent peptone) were inoculated with 0.5 ml of cell suspension and then incubated without shaking at 30°C for 16 hours. After incubation, the pooled cultures were centrifuged at 200g for 5 minutes. The supernatant was discarded, and the residue was resuspended in an equivalent volume of Sorenson's phosphate buffer at *pH* 5.4. After the suspension had been centrifuged and resuspended twice more, the optical density of the suspension was brought to a standard of 0.60 to 0.67.

One-milliliter aliquots of the cell suspension contained in glass planchets (suspension depth of 0.4 mm) were used for radiations. The suspension was magnetically stirred to provide a more homogeneous dose. Times of 1 minute for

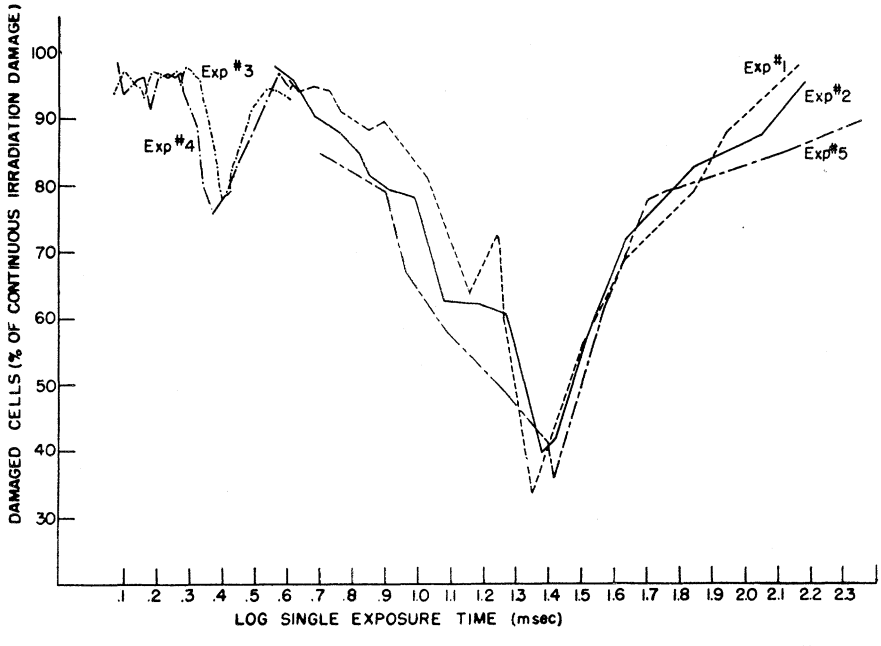

Fig. 1. Effects of ultrafractionated ultraviolet irradiation on yeast cells.