ferent probabilities within an age class of being caught.

However, these biases should not alter the general shape of the predicted relations. The hypothesis has three parts. (i) If the proportion of first breeders acting as females (y variable) is plotted against the ratio (in numbers) of older to first-breeders (x variable), we expect a negative relation for small x values and no relation for large x values. (ii) If the proportion of older breeders acting as males is plotted against the ratio of first to older breeders, we expect the same shape of relation as given in (i). (iii) We expect to find some big males and some small females only if both of the relations given above have some y values at zero. These three predictions are a qualitative description of Eqs. 5 and 6.

For California, the proportion of first breeders acting as females is negatively correlated with the ratio of older breeders to first breeders (Fig. 1). Least-squares regression gives the fit $y = .60 - .20x$ ($r = .67$, $N = 12$, $P < .05$). However, recent years (1973 through 1975) show a relation different from the rest of the data. If only data before 1972 are used, the regression is considerably improved ($y = .68 - .3x$, $r = .87$, $N = 9$, $P < .05$).

Since the usual assumptions of regression and correlation are probably violated in these data, we also calculated a Spearman rank correlation coefficient (r_s). With all the data, $r_s = .51$, which just reaches the .05 significance level ($N = 12$). Again, recalculating r_s without the years 1973 through 1975 greatly improves the fit ($r_s = .85$, $N = 9$, $P < .01$). The theory also predicts that no older breeding shrimp should be males; none were found.

The two Oregon populations differ from the California one in that both show years in which some older individuals reproduced as males. The data support our hypothesis (Figs. 2 and 3) (Table 1). All but one regression (for points in the positive region) are significant, and, for these, the rank correlation is also significant.

Several other systems may be used to further test the model developed here. The situation discussed by Trivers and Willard (*1*) is one such case. If P is the fraction of the mothers in poor condition, W_1 the relative fitness of a son of a mother in good condition, and W_2 the relative fitness of a daughter of a mother in good condition, the same sex-ratio rules apply. The same rules may also apply to some solitary wasps and bees (*2, 12*) and to various orchids (*13*). Some orchids are sexually labile, with individuals found in the bright sunlight mostly reproducing as females and those in the shade as males. Other organisms with ESD may be treated similarly.

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20 September 1977; revised 20 December 1977

5-Thio-D-Glucose Selectively Potentiates Hyperthermic Killing of Hypoxic Tumor Cells

Abstract. *To investigate the mechanisms by which heat affects cancer cells, we used 5-thio-D-glucose, an inhibitor of glycolysis in HeLa S-3 cells, under aerobic and hypoxic conditions at temperatures ranging from 37° to 43°C. Drug alone or heat alone killed a minimum number of cells under aerobic or hypoxic conditions. Exposure to drug and hyperthermia selectively increased the number of cells killed under hypoxic conditions at temperatures as low as 40.5°C but had little effect on cells incubated under aerobic conditions. These results suggest that the glycolytic pathway is a primary site of hyperthermic damage leading to cell death.*

It has been claimed repeatedly for more than a century that hyperthermia (temperatures above 40°C) can have a selective lethal effect on cancer cells. However, only sporadic attempts have been made to use these observations clinically, partly because of a poor understanding of the mechanism of enhanced thermal sensitivity of tumor cells, and partly because of the difficulty of selectively heating a chosen tumor volume at depth. Recently, more conclusive evidence of the action of heat on cancer cells both in vitro and in vivo has generated a renewed interest in this subject (*1*).

In cell culture studies (*1*), numerous cellular factors involved in the thermal response of tumor and normal cells have been identified. Thermal sensitivity is strongly dependent on such factors as cellular growth states, cell cycle phases, nutritional status, ambient oxygen concentration, and intra- and extracellular pH (*2*). Of importance to clinical cancer therapy is the finding that hypoxic cells are sensitive to heat. Indeed, several laboratories reported hypoxic cells were far more sensitive to heat than cells cultured under aerobic conditions, though caution must be exercised in interpreting these data because the experimental induction of hypoxia is invariably associated with changes in the nutritional status of cultured cells, for example, the glucose concentration and extra- and intracellular acidity. When tumor cells are cultured at 42° to 43°C, their respiratory activity, including anaerobic glycolysis, is significantly reduced (*3*). In contrast, in normal cells cultured at the same temperature the respiratory activity is not as severely affected. The importance of anaerobic glycolysis in the pathogenesis of the cancer cell was discussed by Warburg (*4*). His original observation was that when both normal and malignant tissue slices were incubated in a medium containing

0036-8075/78/0414-0206$00.50/0

glucose, in the absence of oxygen, there was a rapid and continuous production of lactic acid, indicating an increased rate of anaerobic glycolysis. However, when these tissue slices were incubated in the presence of oxygen, glycolysis virtually ceased in normal tissues whereas in tumor tissue glycolysis continued actively. Warburg therefore regarded the continued anaerobic glycolysis as being an integral part of the biochemical life-sustaining processes of malignant cancer cell tissues, and as being particularly important for survival of such cells under long periods of hypoxia.

We reasoned that if hypoxic cells derive their life-sustaining energy by way of anaerobic glycolysis (as opposed to oxidative phosphorylation for cells under aerobic conditions), then selective metabolic inhibitors of glycolysis should modify the thermal sensitivity of hypoxic cancer cells. A finding such as this would have not only important clinical implications but also could lead to the elucidation of the apparent selective heat effect on cancer cells. Here we report that 5-thio-D-glucose (5TG), a near analog of D-glucose, interferes with the cellular metabolism of D-glucose, though its exact mechanism of action remains obscure (*5*).

We used HeLa S-3 cells grown in Eagle's minimum essential medium supplemented with 10 percent fetal calf serum. The cell culture procedures, including maintenance, trypsinization, and the test for contamination of the cultures with mycoplasma, were as described (*6*). No antifungal agent was used in this study. We determined the colony-forming ability of plated single cells so that we could construct dose-survival curves for cells grown under both aerobic and hypoxic conditions. Flasks containing exponentially growing HeLa monolayers were flushed with water-saturated gas (95 percent N_2 and 5 percent CO_2) at a flow rate of 150 cm^3/min for 10 minutes. This method of induction of hypoxia yields an oxygen enhancement ratio (oxygen factor) of about 2.6 to 2.9 when cultures are irradiated with 250-kV x-rays. To obtain aerobic conditions we used a similar procedure except that the flasks were flushed with water-saturated air for 10 minutes. Cells were heated by immersing the flasks in a water bath heated by a Haake model 52 temperature circulator and verified by a National Bureau of Standards' thermometer.

The results are summarized in Fig. 1. Incubation of cells cultured under either hypoxic or aerobic (control) conditions at 41°C without 5TG resulted in the minimum number of cells being killed. When hypoxic or control cells were cultured at 37°C, the addition of 5TG had no effect. When 5TG was added to hypoxic cells at 41°C the number of cells killed increased with time of exposure to the drug. The addition of 5TG to control cells had no effect. Figure 2 shows that 5TG became toxic at temperatures from 40.5°C. Thus,

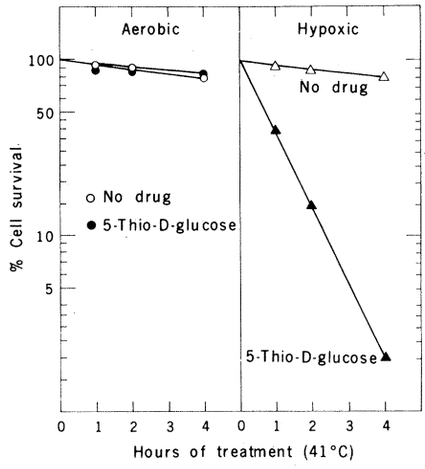

Fig. 1. The HeLa S-3 cells were cultured at 41°C with or without 5-thio-D-glucose (1 mg/ml) under aerobic or hypoxic conditions. Cell survival, in terms of colony-forming ability, was estimated as a function of time of temperature treatment at 41°C. There was no evidence of cytotoxicity with 5-thio-D-glucose at 37°C under aerobic or hypoxic conditions. The plating efficiency of cells under aerobic and hypoxic conditions was 60 percent.

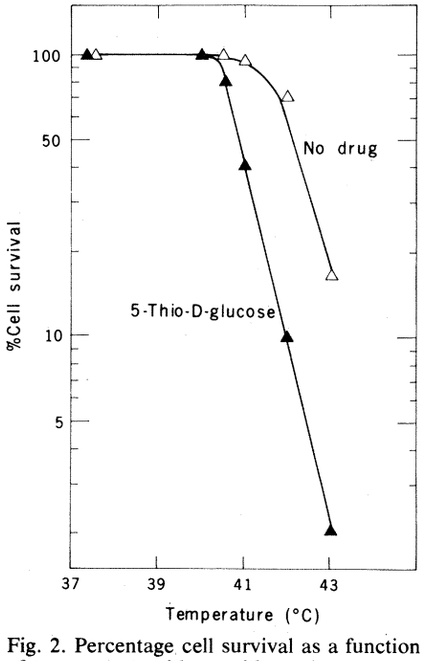

Fig. 2. Percentage cell survival as a function of temperature with or without the presence of 5-thio-D-glucose (1 mg/ml, 1 hour of treatment) under hypoxic conditions. The hypoxic state was achieved as described in the text.

if the glycolytic process is altered under anaerobic conditions, even a moderate degree of hyperthermia is effective in killing cells. Throughout these studies the *p*H of the medium was kept at 7.3 to 7.4 under both control and hypoxic conditions.

The specific biochemical mechanism of action of 5TG is not known. This substance has recently attracted attention as a potential male contraceptive (*5*). It effectively inhibits spermatogenesis in mice at doses of 50 mg/kg, well under the mean lethal dose of 14 g/kg. It is interesting that spermatogenesis is one of the most heat-sensitive normal processes and that spermatozoa heavily depend on anaerobic glycolysis for energy (*7*). According to Chen and Whistler (*8*), the phosphates of 5TG are competitive inhibitors of phosphorylated D-glucose and thus interfere with the cellular metabolism of D-glucose. It may be that 5TG is similarly metabolized as D-glucose 6-phosphate and that one of the intermediary glycolytic products is specifically toxic to hypoxic cells. In any event, it is clear that interference of glucose metabolism under hypoxic conditions when combined with even mild hyperthermia can significantly decrease cell survival. This synergistic effect suggests that the glycolytic may be a primary site of hyperthermic damage.

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6 September 1977; revised 28 December 1977