sugars inhibit the hydrolytic activities of yeast invertase and yeast lactase, but not their synthetic activities, may point to the existence of two separate enzymes or of two different glycosyl-enzyme intermediates, one concerned with the hydrolysis of the substrates into their monosaccharide constituents, the other with the synthesis of oligosaccharides.

S. SRINIVASAN
J. H. QUASTEL
McGill–Montreal General Hospital Research Institute, Montreal, Quebec

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Increased Radioresistance through Heterosis

Strain differences in radiosensitivity of mice have been recognized for some time (*1–4*) even when the variables of age (*3, 5*), sex (*2, 6*), and dose rate (*7*), have been eliminated or controlled. These strain differences have not been explained, except that Brecker and Cronkite (*8*) tried to correlate the degree of injury to bone marrow with species differences.

Inbreeding has long been recognized as a condition leading to reduction in vigor of the species; the opposite practice—namely, outcrossing between fertile strains—has often resulted in first-generation hybrids which are, in certain respects, more vigorous than either of the parental stocks. This is known in genetics as "heterosis." When two divergent strains are crossed, only the first generation resulting therefrom tends to show the maximum beneficial effects in terms of size and vitality. When two diverse groups of heritable factors are brought together, homozygosity is reduced, and the more frequently deleterious recessive characters will be temporarily masked. The individual thus tends to exhibit vitality exceeding the vitality of either of the parent stocks.

Realizing that there are species and strain differences in radiosensitivity (or its reciprocal, radioresistance) and that even closely related and readily interbred mice do not necessarily have the same $LD_{50/30}$ value, we devised an experiment (*9*) to determine whether there might be any heterotic effect in hybrids between the CF_1 and C_{57} black/6 strains of mice. These two strains were inbred randomly for many generations prior to the production of the animals used in this experiment. At the same time a cross was made between C_{57} males and CF_1 females to produce the hybrid strain. The cross was made in this direction because it was found that C_{57} males were somewhat more aggressive than the CF_1 males, and mating resulted more frequently. It is not believed that the opposite cross would have produced F_1 genotypes which were any different.

A total of 485 mice were used in this experiment, divided into six categories of strain and sex, with a minimum of 64 mice in the smallest group and a maximum of 107 in the largest.

The radiation facilities used consisted of a Westinghouse Quadrocondex constant potential therapy x-ray machine at 210 kv (peak) and 15 ma, with 0.28-mm Cu and 0.50-mm Al filters, and at a distance of 40 cm from the target to the center of the body of the mouse. Seven mice were x-irradiated simultaneously in a plastic box in which they were free to move within the uniform field of exposure. The dose rate was 152.7 r/min.

It had previously been found that the $LD_{50/30}$ for the C_{57} black mice was a bit higher than that for the CF_1 white mice, and it has been known for some time that the males are more radiosensitive than are the females of the CF_1 strain (*2*). In order that the physical factors of x-irradiation might be the same for all mice of the same sex, it was decided to x-ray the males to 525 r and the females to 600 r when they were 2 months of age. It was hoped that this would give no 0 percent or 100 percent values in mortality and that all of the data would fall between these extremes. The data are all presented in Table 1.

Species differences in radiosensitivity, expressed in different $LD_{50/30}$ values, have never been explained. There is certainly no correlation with the phylogenetic or evolutionary relationships. Why, for instance, should the guinea pig be so sensitive, the hamster more resistant than man, and the chicken about twice as resistant as man? Nevertheless, the radiation-lethality data are highly reproducible and reliable and are used in radiobiological research for the testing of the effects of the so-called protective drugs and of numerous environmental variables.

Many of the factors which condition or alter radiosensitivity are known. Several of these are age, sex, metabolic or physical activity, or both, amount of oxygen in the environment, presence of infection, previous exposure to insult, and presence of certain so-called protective drugs. When all of these variables are equalized for the animals to be tested, we still find that the C_{57} black mice are more resistant to x-irradiation death than are the CF_1 white, even though they are so closely related that they can be freely interbred. The F_1 generation of a cross between these two strains is identical with the black parent in coat color, and yet the mice manifest survival values even better than that of either parent strain. This implies that the sum total of the effect of a large group of dominant characters coming from both parent strains to the heterozygous hybrids must give to these mice an improved radiotolerance. It would be impossible at this stage to point out those dominant characters which provide the increased radioresistance, or even the possible synergism involved. However, the data do substantiate the presumption that recessive mutants tend to be deleterious.

When one examines these data statistically (*10*) the increase in the female mice is seen to exceed the 5 percent level of probability, indicating that the hybrid female is very probably more radioresistant than either of its pure parent stocks. On the other hand, the relatively small increase in the male hybrids is not statistically significant (see Table 1). This might appear to weaken the thesis

Table 1. Heterosis (hybrid vigor) and radiosensitivity. F_1 mice were tested at 2 months of age. The numbers in parentheses represent the number of animals of the category which were irradiated; C_{57} mice are black, CF_1 mice are white, and the hybrids are black (dominant); χ^2 was determined between the hybrid and the C_{57} pure stocks which have the greater resistance.

Mice and radiation dose	Percentage survival				
	Pure	Pure	Hybrid	χ^2	P
F_1 generation	$C_{57} \times C_{57}$	$CF_1 \times CF_1$	$C_{57} \times CF_1$		
Females (+600 r)	20.8 (77)	8.6 (72)	36.5 (96)	5.055	< 0.05
Males (+525 r)	70.3 (64)	33.3 (69)	80.4 (107)	2.262	> 0.10

of hybrid vigor, or heterosis, until it is pointed out that the ovarian hormones are notably more potent than are the hormones of the testes with respect to radioprotection. It is even known that the male hormone is deleterious, with respect to radiation exposure (*2*), and combining the male hormone factors from two strains would tend, in all probability, to counteract any otherwise heterotic effect. In contrast with this, since the ovarian (female) hormones are beneficial (with respect to radiation resistance) one would logically expect that, added to other heterotic factors, the resulting hybrid females would be statistically more radioresistant than females of either parent stock. This is in fact our finding.

One conclusion is obvious—namely, that the combination of the haploid C_{57} black genome with the CF_1 white genome shows recessivity of the higher sensitivity of the latter, so that the hybrids exhibit radiosurvival in percentage values approximately three times that of the pure whites. Since, also, the male hybrids showed better tolerance than did the pure blacks (even though the finding is not statistically significant), one must admit to the probable influence of heterosis.

A recent paper by Uphoff (*11*) shows that F_1 marrow from a hybrid cross had more radioprotective value than did the marrow from either of the parental strains. The heterotic effect, therefore, probably pervades the entire hybrid.

The hybrid offspring obtained when two strains of mice are crossed (C_{57} and CF_1) have higher survival values than do either of the pure strains when exposed to whole-body $LD_{50/30}$ day x-rays. The increase is significant for the female offspring but not for the males. The results are explained in the case of the males on the basis of the combined and deleterious male tendencies being inadequate to counterbalance completely the beneficial effects of heterosis; the combined and beneficial female tendencies from the two strains, plus heterosis, resulted in statistically significant survival values for the F_1 females.

ROBERTS RUGH
JOAN WOLFF
Radiological Research Laboratory, Columbia University, New York

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Induction of Neural Tissue in Ventral Explants from Frog Gastrulae by Carbon Dioxide Shock

If the speculation that the differentiation of cells depends in part upon cellular protein synthesis has any validity, then the possibility exists that the culture of ventral mesoderm and ectoderm of the early *Rana pipiens* gastrula (stage 10) under conditions which might accelerate protein synthesis conceivably might also effect the type of cell differentiation. In the vast majority of control experiments, the ventral parts of the frog gastrula showed differentiations of mesenchyme, epidermal and blood cells, and also oral suckers, when cultured at 17°C in small stender dishes containing about 10 ml of a buffered saline solution (Niu-Twitty solution) (*1*) for periods of 6 to 14 days. Neural, muscular, nephric, or chordal tissues appear in less than 2 percent of the cultures. At the end of the culture period the explants were fixed in Bouin's fixative, sectioned at 10 μ, and stained with hematoxylin eosin.

In the various experimental series of cultures, the ventral explants were cultured at higher temperatures, in various nutrient media and were also subjected to brief shocks with alkaline and acid media. For the control and experimental series a total of 230 cultures, which included 1900 explanted tissues, were made. The aim of these experiments was to increase the level of metabolism, or of available nutrient, and thus possibly to accelerate the synthesis of cytoplasmic proteins and determine the effect upon differentiation (*2*).

Possibly the most direct means of raising the rate of metabolism of the ventral explants is to culture them at higher temperatures. Although Gilchrist (*3*), Huxley (*4*), and Margen and Schechtman (*5*) applied local high-temperature stimuli to ventral regions of whole eggs without altering morphogenesis, this method has the disadvantage that it may not overcome any dominance factors that might emanate from the differentiating dorsal structures. This would not be a problem in culturing the isolated ventral tissues at the higher temperatures. When gastrula ventral mesoderm-ectoderm was cultured at temperatures of 28, 30, and 33°C for 3 to 4 days and then at 17°C for 6 more days, differentiation was merely equivalent to that of the control cultures. Thus it appears that higher temperatures alone, although they certainly raise the level of respiration, cannot alter the prospective fate of these tissues. However, it is known from experiments in vitro (*6*) that higher temperatures alone cannot increase the extent of solubilization of yolk platelets.

Another possible means of attempting to accelerate protein synthesis in the explants is to supply the cells with nutrient in the culture medium and thus attempt to bypass the yolk as the nutrient source. To this end, bits of ventral mesoderm-ectoderm from early gastrulae were cultured in various concentrations of a nutrient medium containing all the essential amino acids, most of the water-soluble vitamins, glucose, and each of the nucleotides; in saline extracts from hatched larvae, adult frog hearts, brains, and muscle; and also in livetin (the water-soluble yolk protein) dissolved in a *p*H 5.7 acetate buffer-saline medium. None of these series of explants showed greater specialization than the controls cultured in Niu-Twitty solution. The combination of added nutrient and culture at higher temperatures also failed to induce the formation of dorsal tissues in a significant number of cases.

Acid and alkaline shocks have been utilized in eliciting the formation of neural tissue in isolated ventral tissues of urodele gastrulae (*7*), but such experiments have not yet been successfully carried out with the frog embryo. Holtfreter (*8*) has demonstrated that a *p*H below 4.0 and above 9.6 partially solubilizes yolk platelets, and I have put forth the idea that solubilization of protein, including ribonucleoprotein, from the yolk platelets and the initiation of embryonic differentiation may be causally related (*6*). In order to ascertain whether inductions could be obtained in the anurans with low and high *p*H, ventral explants were exposed to 0.02 percent NH_4OH in tap water (*p*H 11.0) for 3 to 6 minutes, washed several times in sterile saline, and cultured for 6 to 12 days. The acid shocks were carried out by bubbling CO_2 into the nonbuffered "A" part of the culture medium until the *p*H was 3.7, and explants were left in this acidified medium for periods ranging from 10 minutes to 1 hour and then cultured in the normal saline medium for 12 days. Most of the CO_2 shocks were for 10 or 20 minutes since longer shocks sometimes killed the explants.