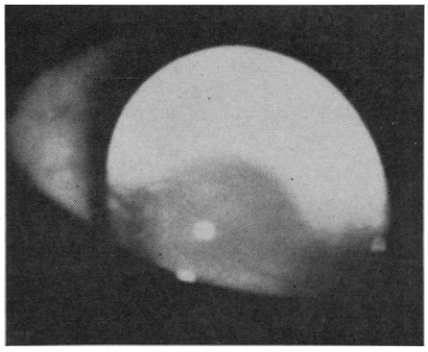

Fig. 1. The lenses of dogs treated with DMSO, seen by retroillumination, contain a central nuclear relucent zone that remains transparent.

The division between the optically clear and normally relucent areas was sharp. While such lens changes persisted after DMSO was withdrawn, they became slightly less pronounced, the lens "nucleus" becoming relatively smaller.

In two dogs a third zone was observed in the lenses (giving a target appearance) in the 2nd and 4th weeks of withdrawal from administration; the new areas persisted for 11 and 6 weeks, respectively, before disappearing ophthalmoscopically. Routine histologic examination of the lens failed to reveal any anatomic changes.

It appears that DMSO affects the new cortical lens fibers as they are formed, causing them to appear optically clear instead of relucent. The refractive index, as judged by the biomicroscopic appearance, must be approximately that of the aqueous. This would have the effect of neutralizing the refractive power of the cortical shell, thereby making the relucent area an effective independent lens because of differences in refractive index. The radius of curvature of the nucleus, less than that of the anterior lens surface, would make the nucleus a stronger biconvex lens, necessitating increasingly negative ophthalmoscopic lens interposition for neutralization. The lens change caused by DMSO administration is unique as far as we know.

*Note added in proof*: There have been no lens changes in rhesus monkeys receiving DMSO at 5g $kg^{-1}$ $day^{-1}$ for 100 days.

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## Glucuronidase Gene Expression in Somatic Hybrids

Abstract. *Evidence at the enzymic level confirms the dual origin of the genetic material of somatic hybrids. Furthermore, the pattern of glucuronidase gene expression in somatic hybrids qualitatively resembles that in livers of animals heterozygous for this gene.*

The original demonstration by Barski *et al.* (*1*) of somatic hybridization was based on karyological evidence. In mixed cultures of two mouse-cell lines possessing different karyotypes, the presence was shown of cells containing the chromosome complements of both "parental" cell lines. Further application of this method soon extended these observations to a number of other mouse-cell lines (*2*). Spencer *et al.* (*3*) have presented immunologic evidence that the genetic information of both somatic parents is expressed in their hybrid derivatives. I now report examination of gene expression at the biochemical level in the same system by comparison of a somatic hybrid cell population with each of its two parental cell lines, using the enzyme β-glucuronidase as a marker. I compare the results with those from genetic studies of the mouse, with the same marker.

The hybrid cells that I tested were fusion products of tissue-culture cell lines originally derived from C3H and Swiss strains of mice. The β-glucuronidase activity in tissue homogenates of most strains of C3H mice is subject to rapid heat inactivation at 71°C, at which temperature the same activity from wild-type mice, such as Swiss, is relatively stable (*4*); genetically, a single Mendelian factor is responsible for this difference (*5*). One may ask whether the properties of the enzyme in each of the parental cell lines reflect its strain of origin, and, if so, whether the characteristics of the enzyme activity present in hybrid cells reflect the properties of both parent enzymes.

The two parental cell lines that I used were clones NCTC 2555 and Py-198-1, which I shall call N2 and Py-1, respectively. The former was derived from subcutaneous connective tissue of a C3H/He inbred mouse (*6*); Py-1, from polyoma virus–"converted" embryonic cells of a noninbred Swiss mouse (*7*). The hybrid cells, designated M109, originated in a mixed culture of N2 and Py-1 cells (*8*). For several years no parental cell mitoses have been observed in this population of hybrid cells (*9*).

All cells were grown to stationary phase in Dulbecco's modified Eagle's medium plus 10 percent calf serum, collected with 0.05 percent trypsin in balanced saline, and washed twice with Earle's balanced saline. Partially purified β-glucuronidase was prepared from frozen cell pellets of the hybrid and both parental cell lines (*10*). The pellets were thawed and homogenized with appropriate volumes of 0.1*M* acetate buffer (*p*H 4.6) in a Potter-Elvehjem apparatus. Homogenates were incubated at 56°C for 4 hours to release β-glucuronidase into solution, and then centrifuged at 105,000*g* for 30 minutes. The high-speed supernatants from the different cell types were then simultaneously dialyzed in the same container against two 1000-ml changes of 0.1*M* acetate buffer (*p*H 4.6). In order to determine the decay kinetics of enzymes from the different cell types, portions of each extract were heated at 71°C for periods of up to 60 minutes; the surviving activity of each portion was assayed by the *p*-nitrophenyl glucuronide procedure of Nimmo-Smith (*11*).

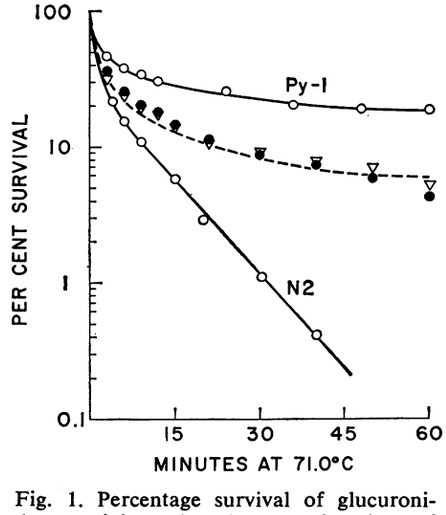


Fig. 1. Percentage survival of glucuronidase activity, plotted as a function of minutes of heating at 71°C. Each solid line represents the inactivation kinetics of enzyme from a parent cell line; dashed line represents the calculated inactivation curve for a mixture of two parts N2 and one part Py-1 enzyme activity. The denaturation kinetics of a real mixture of two parts N2 and one part Py-1 enzyme activity is indicated by the open triangles. Inactivation of the somatic hybrid cell (M109) enzyme is represented by solid circles.

Figure 1, a semilogarithmic plot of the inactivation kinetics of β-glucuronidase activity, shows that the enzyme of N2 is more heat labile than that of Py-1. When N2 and Py-1 decay curves were compared with those of β-glucuronidase in C3H and Swiss mouse-liver homogenates, respectively, the inactivation kinetics proved to be similar, indicating that the properties of enzyme in each of the parental cell lines reflect its mouse strain of origin. Figure 1 shows, moreover, that the activity-decay curve of the somatic hybrid M109 closely approximates that of a mixture of the two parental types in the proportion of two parts N2 to one part Py-1 (*12*).

Presence of both parental activities has also been detected in liver homogenates from $F_1$ mice heterozygous for the mutant and wild-type β-glucuronidase alleles (*13*). Thus the expression of alleles in the heteroploid somatic hybrid cells appears to resemble qualitatively that in liver tissue of analogous murine heterozygotes.

The fact that the inactivation kinetics of the Py-1 and N2 activities were not linear (Fig. 1) suggests the presence of more than one component in each. A similar phenomenon was observed with enzyme activities from wild-type and mutant mouse-liver homogenates (*13*). However, the nature of the multicomponent kinetics is not understood, since only one enzyme band is observed after electrophoresis of wild-type enzyme under various conditions (*14*), and the Michaelis constants of wild-type enzyme activity surviving after 0 and 12 minutes at 71°C did not differ significantly. On the other hand, the inactivation kinetics were not artifacts of the heating procedure, since enzyme that had been heated for 30 minutes, cooled, and then reheated did not exhibit the initial rapid inactivation.

Although the decay rates of each parental enzyme activity are not linear, the similarity of the M109 inactivation kinetics with those of an N2–Py-1 mixture indicates that both parental activities are coexistent in the hybrid-cell population. This finding is confirmation at the enzymic level that genetic material derived from both parental cell types is expressed in somatic hybrids obtained by cell fusion.

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10. Frozen pellets of each cell type were donated by Boris Ephrussi.
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12. In my experiments the decay kinetics of M109 activity varied between those of 1 : 1 and 2 : 1 mixtures of the parental activities. As yet one cannot interpret this fact genetically.
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14. Disc electrophoresis, with the standard 7-percent gel at *p*H 9.5, and starch-gel electrophoresis, with several buffer systems ranging widely in *p*H, have been performed on wild-type liver glucuronidase; always only one activity band was detectable.
15. Aided by PHS grants CA-04090 and CA-5016. The work resulted from suggestions and encouragement by Boris Ephrussi and Kenneth Paigen. The author is a predoctoral fellow of the State University of New York.

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## Sleep Deprivation in the Rat

Abstract. *Sleep deprivation, induced by injections of dextroamphetamine or by forced treadmill activity, resulted in a temporary increase in daily sleep time. However, increasing the period of sleep deprivation above 24 hours to 72 or 120 hours did not result in increased recovery sleep above that present in the 24-hour group.*

A recent book has stressed the motivational characteristics of sleep (*1*), but there is a paucity of data demonstrating that subjects respond to sleep deprivation as if sleep were a drive. Webb (*2*) found that rats deprived of sleep had shorter sleep latencies (time to fall asleep) than control animals. Kleitman (*3*) has reported an increase in sleep time in humans on the first night after prolonged deprivation of sleep. A number of studies have reported changes in the stage characteristics of sleep after deprivation (*4*); however, in these studies, sleep was recorded on only one or, at the most, two recovery nights.

In my experiments, sleep cycles were monitored for prolonged periods before and after varying periods of sleep deprivation. Two methods of producing sleep deprivation were used, injection of dextroamphetamine and a slowly revolving treadmill. Svorad and Novikova (*5*) have induced up to 7 days of sleep deprivation in rats with periodic injections of dextroamphetamine. Sleep deprivation induced by forcing rats to walk a slowly revolving water-immersed treadmill has also been reported (*2, 6*).

Twelve 110- to 120-day-old male Long-Evans rats were subjected to dextroamphetamine-induced sleep deprivation in the first experiment. In addition to recording sleep cycles, food and water intake and body weight were also measured, because of the known anorexic action of dextroamphetamine. An ultrasonic movement-sensing device capable of differentiating the sleeping and waking states in rats has been developed (*7*) and correlated with electroencephalographic (EEG) records (*8*). The correlation of the results of the two methods for differentiating sleep-waking periods is .97, and the movement-sensing system is amenable to recording prolonged sleep cycles. Although this method does not require surgery or the presence of an experimenter during recording, neither does it permit the discrimination of sleep stages; thus, total sleep time during a prolonged period is the dependent variable in these studies.

Ultrasonic sound (nonaudible) is emitted into a test area containing the rat. Movement by the animal produces disturbances in the received portion of the wave, which activates a pulse. The record is read out on an Esterline-Angus operation recorder, model AW. Scoring rules, calibration procedures, and specifications for cages and test-area enclosures have been described (*8*). Briefly, each minute is scored for activity level on a 20-point scale based on the number of pen deflections during that minute. A minute with a score of 0 to 3 denotes sleep and 4 to 20, awake. The calibrator consists of a pendulum placed in the test area. The pendulum is started by moving it a specified distance from the vertical; calibration setting is the time from starting motion until the movement-sensing device stops responding to decreasing pendular swings.

There were four rats at each of the three deprivation levels (24, 72, and 120 hours). The sleep-waking cycle was recorded for 4 days before and 8 days after the period of sleep deprivation, in addition to recording during the sleep-deprivation period itself. The