Dimethyl Sulfoxide: Lens Changes in Dogs during Oral Administration

Abstract. *Oral administration of dimethyl sulfoxide to young dogs reduces relucency of the lens cortex, causing the normal central zone of the lens to act as a strong biconvex lens.*

During routine ophthalmologic examinations of dogs receiving dimethyl sulfoxide (DMSO), unusual changes were observed in the lens. We now describe these changes.

Dimethyl sulfoxide was administered daily by gastric intubation to six male-female pairs of young adult beagles at doses of 0, 2.5, 5, 10, 20, and 40 g/kg 5 days a week; during the 1st week it was given in physiological saline, and thereafter in 50-percent aqueous solution.

During the 2nd week the two dogs receiving 40 g/kg were absolved for 5 days; administration was resumed in the 3rd week at 20 g/kg twice daily, but because the animals would not tolerate this dosage it was reduced to 10 g/kg twice daily.

Both dogs on 20 g/kg daily (the two dogs originally receiving 40 g/kg daily) and one of two dogs on 10 g/kg daily could not tolerate such dosage. Administration of DMSO was continued for 23 weeks (107 doses), at the end of which time the drug was withdrawn from the seven survivors which were then observed for 31 more weeks. The dogs were examined by indirect and direct ophthalmoscopy, under mydriasis induced by 0.5-percent tropicamide (Mydriacyl, Alcon Laboratories, Fort Worth, Texas), before the tests and at least at monthly intervals during and after the dosing.

The first ocular effect of the administration was refractoriness to the normal mydriatic action of 0.5-percent tropicamide (Table 1). Conjunctival instillation of 0.5-percent tropicamide in dogs usually produces wide (10- to 12-mm) dilation of the pupil within 30 minutes. The pupils of these dogs did not dilate maximally except with repeated instillation or with a higher concentration of the mydriatic agent. The refractoriness to dilation appeared to be related to the dose.

Changes in the lens were seen in four dogs during the 9th week of administration; by the 18th week all dogs had lens changes that persisted or became more pronounced with continuing administration (Table 2). By retroillumination the lens appeared to be divided into two zones: a central area simulating the nuclear zone, and a peripheral zone. Both areas were clear, the retina being easily visible though somewhat distorted when viewed by indirect ophthalmoscopy; with direct ophthalmoscopy the distortion was more evident (Fig. 1).

In markedly affected animals the retina could be seen in detail at differing ophthalmoscopic settings, depending on which area of the lens the light beam penetrated. Thus it became necessary to decrease the strength of the ophthalmoscopic lens by −20 diopters to properly examine the retina, when moving from the peripheral to the central area of the lens. The difference was less in dogs receiving lower doses of DMSO. Subsequent slit-beam biomicroscopic examinations of similarly affected dogs showed that the cortical area of the lens was almost optically clear instead of having its normal relucency.

Table 1. Refractoriness to tropicamide mydriasis in dogs after administration of DMSO. Symbols: +, very slight; ++, slight; +++, moderate; ++++, severe. Controls showed no effects. Dog identifications appear in parentheses.

Week	DMSO daily dosage (g/kg)							
	2.5		5		10		40	
	(188)	(119)	(224)	(121)	(125)	(214)	(218)	(129)
	During dosage							
4	++	++	++	++	++	++	0*	0*
9	+	+	++	+++	++++		++++	
13	+	+	++	+++	++++			
18	++	++	++	++++	++++			
21	++	++	0*	++++	+++			
	After 23 weeks of dosage							
26	0	0	0	0	+++			
28 and later	0	0	0	0	0			

* Reported to have vomited drug after dosage.

Table 2. Lens changes in dogs given dimethyl sulfoxide orally. Symbols: ±, trace; +, very slight; ++, slight; +++, moderate; ++++, severe. Controls showed no changes. Dog identifications appear in parentheses.

Week of		DMSO daily dosage (g/kg)					
Test	Withdrawal	2.5		5		10	40
		(188)	(119)	(224)	(121)	(125)	(218)
		During dosage					
4		0	0	0	0	0	0
9		0	0	++	++++	+++	+++
13		0	++	++	++++	++++	—
18		++	++	++++	++++	++++	—
21		++	++	++++	++++	++++	—
		After 23 weeks of dosage					
26	2	++	++	+++*	+++	++++	—
28	4	++	++	++*	++	+++*	—
30	6	++	++	+++*	++	+++*	—
33	9	+	++	+++*	++	+++	—
35	11	+	++	+++*	++	+++	—
38	14	+	++	+++	++	+++	—
41	17	+	++	+++	++	+++	—
44	20	+	++	+++	++	+++	—
48	24	±	±	+++	++	+++	—
52	28	+	+	+++	++	+++	—

* Secondary equatorial ring.

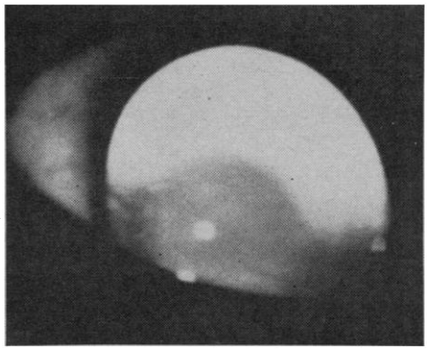

Fig. 1. The lenses of dogs treated with DMSO, seen by retroillumination, contain a central nuclear relucent zone that remains transparent.

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*).

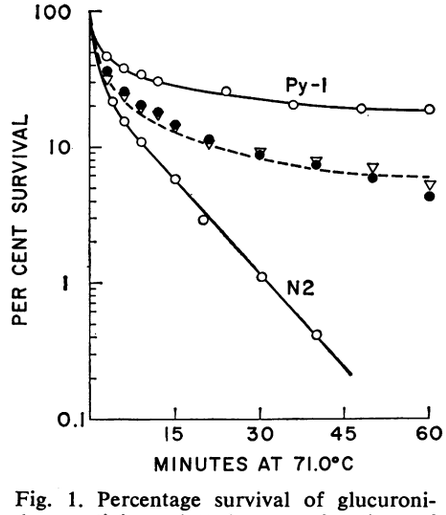

Fig. 1. Percentage survival of glucuronidase activity, plotted as a function of minutes of heating at 71°C. Each solid line represents the inactivation kinetics of enzyme from a parent cell line; dashed line represents the calculated inactivation curve for a mixture of two parts N2 and one part Py-1 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.