in his statement that "When matings ... of two males are coincident, then ... numbers of sperm determine paternity" (see, for example, ref. 3). Moreover, this statement made in relation to rabbit, pig and sheep supports our suggestion that the association between breeding system and relative testes size will be found in other mammalian orders.

(4) Finally, while the hypothesis relating breeding system to relative testes size (rather than to some more precise variable) is simple, the good agreement between the data and predictions based on the stated assumption implies that it is not simplistic.

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Hexose transport in hybrids between malignant and normal cells

IT HAS been reported by White *et al.*[1] that a decreased K_m for hexose transport correlates with malignancy in matched pairs of hybrids between malignant and normal cells. Hexose transport was measured by the uptake of 0.1–5 mM 2-deoxy[^{3}H]glucose at 20 °C; in these conditions, uptake was found to be linear for at least 10 min. No doubt the authors are aware that sugars such as 2-deoxyglucose (or D-glucose) enter cells rapidly, soon (generally within <30 s at 25 °C) establish a steady state—at intracellular concentrations which can approach that in the medium—and are, in turn, phosphorylated at slower rates which persist linearly for several minutes[2,3]. Within 5 min of exposing Novikoff[2] or Lettree[4] cells (at 25 or 37 °C) to 2-deoxy[^{3}H]glucose at concentrations of ≤0.2 mM, for example, >85% of the radioactivity associated with the cells is in the form of 2-deoxy-[^{3}H]glucose-6-phosphate, whereas <15% is present as an intracellular pool of free sugar.

In other words, it is likely that the rate-limiting step is phosphorylation, not transport, of the hexose and that what White *et al.* are measuring is the linear accumulation of 2-deoxy[^{3}H]glucose-6-phosphate in cells. While the use of a 'coupled reaction' to measure a preceding one is a commonly used biochemical technique, its validation does depend on confirmation that the second reaction is not rate-limiting. The article by White *et al.*[1], however, contains no mention of the phosphorylation reaction, let alone measurement of intracellular 2-deoxy[^{3}H]glucose and 2-deoxy[^{3}H]glucose-6-phosphate. Moreover, some authors have suggested that it is sugar kinases (not sugar transport *per se*) that are different in transformed and normal cells[5].

Although the results of White *et al.*[1] clearly show that malignant cells take up hexoses at low concentrations better than their non-malignant homologues, the claim of these authors that their "findings leave little doubt that malignancy is closely linked to a decrease in the K_m of the membrane hexose transport system" seems as yet unjustified.

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Table 1 Kinetic parameters of 3-*O*-methyl-D-glucose uptake in malignant and non-malignant cells

Cell type	V_{max}	K_m
$CBAT_6T_6$	325.1±26.8	4.419±0.520
fibroblasts	420.3±90.8	4.868±1.214
PG19	106.6± 5.1	2.114±0.172
	116.6±18.2	2.211±0.460
1Acn2	331.2±40.2	8.495±1.282
1Acn1TG	107.6± 7.3	2.771±0.241

The uptake of 3-*O*-methyl-D-glucose was measured by the technique previously described[1] for 2-deoxyglucose with the following modifications: (1) 3-*O*-methyl-D[1-^{3}H]glucose (0.1–5.0 mM; 10–500 mCi mmol^{-1}) was added to the cells in a volume of 250 μl and its uptake terminated by washing the cells twice with 1 ml of ice-cold phosphate-buffered saline. (2) At 20 °C, uptake of 3-*O*-methylglucose is linear for only 1–2 min, so that correspondingly shorter incubation times were necessary. Kinetic constants were calculated as before from the linear portions of the uptake plots.

WHITE *ET AL.* REPLY—Since our paper[1] was submitted, we have succeeded in establishing conditions in which the uptake of 3-*O*-methyl-D-glucose in our cells is sufficiently linear to permit formal kinetic analysis. This has enabled us to repeat our experiments using a glucose analogue that is not phosphorylated. The V_{max} values for 3-*O*-methyl-D-glucose uptake are comparable with those for 2-deoxyglucose uptake, but the K_m values are two to four times higher, indicating that 3-*O*-methyl-D-glucose is transported less efficiently than 2-deoxyglucose or glucose. Nonetheless, the reduction in K_m that we previously described for 2-deoxyglucose uptake also occurs with 3-*O*-methyl-D-glucose.

Table 1 gives representative values for $CBAT_6T_6$ mouse fibroblasts, PG19 malignant mouse melanoma cells, 1Acn2, a human hybrid cell line in which malignancy is suppressed and 1Acn1TG, a malignant segregant derived from this hybrid. The reduction in K_m that we described previously[1] is thus not primarily determined by the phosphorylation reaction, but by the flux of hexose across the cell membrane. Measurements we have made of the hexokinase activity in cell homogenates indicate that there is no correlation between hexokinase activity and malignancy.

Our conclusion that malignancy is closely linked to a decrease in the K_m of the membrane hexose transport system stands.

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