i.e. by imprinting. Similarly, preferences between strains vary according to whether females are reared by both mother and father, or by the mother alone. It thus seems important to attempt to find out the importance of early learning in determining mating preferences, although it is known to be important in certain other species[17].

All the mechanisms discussed represent deviations from random mating, and it is difficult to know their evolutionary significance. However, an extreme form of sexual selection is polygyny, which occurs in nearly all anthropoid apes and is likely in primitive hominids[18]. A leader of a group with several wives will contribute a far greater than average share to the genetic composition of the next generation. Thus reproductive success would be closely correlated with genetic superiority, so allowing a more rapid rate of evolution than under random mating.

The density-dependent system where rare genotypes are more successful in mating than common ones, ensures the maintenance of rare genotypes in the population, and so enhances genetic heterogeneity. This is also true for positive assortative mating[19] and may be true for many of the mechanisms discussed. Under certain circumstances, these mating systems may lead to balanced polymorphisms without heterozygote advantage. Another example of this is the preferential mating recently found in the mimetic butterfly, *Papilio glaucus*[20].

For the density-dependent mating system where rare genotypes are favoured, a consequence in a polymorphic situation may be that the component of the genetic load[18,21] due to rare unfit homozygotes is reduced due to their advantage in mating. This may be important in outbreeding species where a great number of polymorphic systems seem likely[22], although it can also be argued that only a proportion of polymorphisms are under selection in a given environment, so that most polymorphisms are relics of previous selection[22]. In a general sense, mating behaviour variations probably enhance genetic heterogeneity without contributing greatly to the genetic load, but this needs further theoretical investigation.

Studies in the field of behaviour genetics have therefore brought to light numerous variations of the breeding system leading to deviations from random mating and so affecting rates of evolution. In any organism exhibiting variable courtship rituals, deviations from random mating may be more common than not for loci involved directly or indirectly with mating behaviour[23].

Résumé. Des expériences portant sur le comportement sexuel chez plusieurs espèces, telles que la drosophile et la souris nous montrent que l'hypothèse qu'elles s'accouplent par hasard, hypothèse dont on fait état assez fréquemment dans la génétique des populations, n'est valable dans cadre des populations naturelles qu'avec de sérieuses restrictions.

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Quantitative Changes in the Phosphorus Fractions of Transplanted Brain Tumors During Complete Ischemic Incubation

A previous investigation has demonstrated that a variety of human and experimentally induced brain tumors, when incubated under conditions of complete ischemia, generated lactate in significant excess to that expected from glucose and glycogen disappearance[1]. Furthermore, P_i (inorganic phosphate) was found to accumulate in excess of that expected from changes in measured phosphorylated metabolites. The fact that the increments of P_i were of about the same magnitude as those of the unexplained lactate seemed a possible clue to the source of this extra lactate. Consequently in the present study measurements have been made of the changes which occur during anaerobic incubation in the levels of various phosphorus containing fractions. In most cases significant decreases in the levels of acid-soluble organic phosphorus and nucleic acid phosphorus were observed, with a concomitant increase in P_i. Lipid phosphorus and residual phosphorus fractions exhibited no significant change during the incubation.

Experimental. 3 different types of experimentally induced malignant mouse brain tumors were studied. Ependymoblastomas and glioblastomas were transplanted in C_3H mice, and medulloblastomas in C-57 mice. The 2 medulloblastomas studied (designated M-1, M-2) were composed of uniform small cells with hyperchromatic nuclei and indistinct cytoplasm. The 2 glioblastomas (G-1, G-2) were extremely cellular tumors with areas of focal necrosis, cellular palisading and vascular proliferation. Ependymoblastomas (E-1, E-2) were cellular tumors containing closely packed epithelial cells. Electron micrographs of the ependymoblastomas demonstrated prominent terminal bars, characteristic of tumors of ependymal origin.

Tumors were selected for study 3 weeks after transplantation, at which time they were about 1 cm in diameter. Half of the tumor was removed and frozen within 2–3 sec of extirpation in Freon-12 (CH_2F_2) chilled to near its freezing point (– 156 °C) by liquid nitrogen. The remaining portion of the tumor was resected and incubated under mineral oil at 37 °C for 4 h, and then frozen. Dissected frozen samples, about 20 mg in weight, were weighed at – 20 °C and homogenized in 70 µl of 3 *M* $HClO_4$ at – 8 °C. 330 µl of a 4 m*M* EDTA solution was added and homogenization continued at 4 °C.

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Aliquots of this homogenate were assayed for total glucose and total phosphorus. Total glucose was measured after acid hydrolysis of a 20 μl aliquot of the homogenate in 1 ml of 1 N HCl (100 °C for 2 h in a sealed tube). It is assumed that this represents free glucose plus glycogen. Glycogen standards were carried through this procedure, and total glucose measured by a fluorometric technique[2]. Total phosphorus was determined in duplicate by ashing 3 μl aliquots of the homogenate (equivalent to about 150 γ wet weight of tumor) in 100 μl of 0.8 N $HClO_4$ in 10 N H_2SO_4. Ashing conditions, extraction, and subsequent color development with 1 ml of 1% ascorbic acid, 0.25% molybdate reagent in 0.1 N sodium acetate were as described by LOWRY et al.[3] for tissue phosphorus fractions.

The insoluble $HClO_4$ precipitate was then removed by centrifugation at 0 °C. The original $HClO_4$ extract was neutralized and analyzed for acid-soluble phosphorus, lactate, glucose, ATP and P_i. The specific spectrophotometric and fluorometric techniques for measuring the latter 4 metabolites have been described and were used without modification[2].

All assays were conducted in quadruplicate with appropriate blanks and standards.

Results. Each of the experimental brain tumors studied generated a significant excess of lactate with respect to total hexose disappearance (glucose plus glycogen) during the anaerobic incubation (Table I). The lactate increments were similar to values found with other experimentally induced brain tumors incubated under similar conditions, but were less variable than those found with a variety of incubated human brain tumors[1]. Levels of ATP fell, but this substrate was still present at readily measurable concentrations (5–6% of zero time values) at the conclusion of the 4 h of complete ischemia. Glucose levels remained at values close to those found at zero time, apparently at the expense of glycogen. The concentration of the latter metabolite decreased significantly during incubation in every case. Initial levels of glucose, glycogen, ATP and P_i were generally alike in all tumors, and the values found were similar to those reported in the previous series of experimentally induced brain tumors[1]. Initial lactate levels were found to be higher in these s.c. brain tumor transplants than in the earlier tumors which were grown in the cranium. However, the increment in lactate during incubation was substantially the same.

Total phosphorus was higher in the 2 ependymoblastomas than in the medulloblastomas and glioblastomas

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Table I. Effect of 4 h of ischemic incubation on glucose, glycogen, lactate, ATP and P_i in 6 experimentally induced mouse brain tumors

Tumor	Glucose		Glycogen[a]		Lactate		ATP		P_i		Δ Total glucose	Δ Lactate		Δ P_i
	t_0	t_f	t_0	t_f	t_0	t_f	t_0	t_f	t_0	t_f		Observed	Calculated	Observed
Ependymoblastoma (E-1)	1.9	1.2	2.9	0.3	19.8	31.7	2.03	0.11	6.6	22.8	3.3	11.9	6.6	16.2
Ependymoblastoma (E-2)	1.8	1.3	2.6	0.2	18.2	29.8	2.52	0.19	6.1	21.9	2.9	11.6	5.8	15.8
Glioblastoma (G-1)	1.2	1.2	1.5	0.4	16.4	27.2	1.10	0.62	6.9	17.5	1.1	10.8	2.2	10.6
Glioblastoma (G-2)	1.1	1.5	3.3	0.6	16.5	23.9	0.83	0.41	9.6	20.2	2.3	7.4	4.6	10.6
Medulloblastoma (M-1)	2.5	1.3	3.4	0.2	13.5	28.6	0.87	0.08	7.6	14.8	4.4	15.1	8.8	7.2
Medulloblastoma (M-2)	1.3	1.3	1.2	0.2	18.5	26.2	0.76	0.54	7.6	21.6	1.0	7.7	2.0	14.0

Conditions of incubation are as described in the text. Samples frozen before and after incubation are designated t_0 and t_f respectively. All values are expressed as mmoles/kg wet weight. The values for Δ lactate calculated are based on Δ total glucose disappearance during incubation (2 moles lactate produced per mole of hexose that disappeared). [a] Total glucose after acid hydrolysis minus preformed glucose.

Table II. Changes in 6 phosphorus containing fractions of experimentally induced tumors during 4 h of ischemic incubation

Tumor		Measured total P	Acid-soluble P	P_i	Acid-soluble organic P	Lipid P	Nucleic acid P	Residual P	Sum of P fractions
E-1	t_0	93.3	37.1	6.6	30.5	16.6	35.3	2.0	91.0
	t_f	91.6	40.2	22.8	17.4	16.8	29.5	2.8	89.3
E-2	t_0	107.0	40.5	6.1	34.4	18.3	39.8	3.9	102.5
	t_f	87.5	37.1	22.9	14.2	16.5	31.4	4.2	89.2
G-1	t_0	67.0	20.5	6.9	13.6	16.1	29.5	2.3	68.4
	t_f	67.0	28.3	17.5	10.8	13.0	23.9	1.6	66.8
G-2	t_0	62.5	22.4	9.6	12.8	12.9	24.7	3.2	63.2
	t_f	58.0	28.0	20.2	7.8	10.3	17.9	2.3	58.5
M-1	t_0	67.5	32.1	7.6	24.5	13.8	21.6	3.6	71.1
	t_f	77.0	36.1	14.8	21.3	16.4	21.9	3.4	77.8
M-2	t_0	60.5	19.1	7.6	11.5	13.3	29.5	2.0	63.6
	t_f	46.3	21.6	18.1	3.5	8.9	20.1	1.7	52.3

Tumors and samples are designated as in Table I. Values are expressed in mmoles/kg wet weight. Values for the acid-soluble organic phosphorus fraction are derived by subtracting P_i levels from those of acid-soluble phosphorus.

(Table II). The sums of the different phosphorus fractions are close to the values for total phosphorus measured independently. Therefore, the substantial differences seen in 3 cases between initial and final sample are attributable to heterogeneity of the tumors rather than to analytical inaccuracy. After 4 h of incubation, a modest but definite increase in the acid-soluble phosphorus content of 5 of the 6 tumors was found (as a percentage of total phosphorus, the increase is to be seen in all 6 tumors). The increases in acid-soluble phosphorus are associated with decreases in the nucleic acid phosphorus fraction. This is most clearly shown when the phosphorus balance sheet is 'normalized' by expressing the changes as a percentage of the total (Table III). Values for lipid phosphorus represented about 20% of the total phosphorus of each tumor, and remained essentially unchanged by the incubation. Residual phosphorus (that associated with the insoluble protein residue after lipid and nucleic acid extraction) amounted to only 2–5% of the total phosphorus content, and did not change significantly during incubation.

Discussion. The values of the various phosphorus fractions found at 'zero time' in these experimental tumors are in substantial agreement with those reported for human brain tumors of the same type by NAYYAR[4], COHEN[5], BRANTE[6], and SELVERSTONE and MOULTON[7]. The latter authors, however, found higher total phosphorus (about 100 m*M*/kg wet weight) in a single human medulloblastoma than in the 2 murine medulloblastomas of this study. Nevertheless, percentage distribution among the phosphorus fractions in the human medulloblastoma agrees with that of the experimental tumors. It is of interest that the relative concentrations of the phosphorus fractions at 'zero time' are in close agreement with values reported for a variety of rodent neoplasms from diverse tissues[8].

It would seem probable that any lactate formed in these tumors in the complete absence of oxygen would derive from a carbohydrate source. In the present experiments only 25% of the lactate production can be accounted for by disappearance of glucose and glycogen. The appearance of extra lactate is accompanied by the formation of much more P_i than can be explained by breakdown of ATP or P-creatine[1]. Therefore, it is possible that the lactate is derived from phosphorylated carbohydrate. The present results suggest that nucleic acid may represent part but probably not all of this phosphorylated carbohydrate source of lactate. For each mole of nucleic acid ribose, a maximum of 1.67 moles of lactate could be generated through the mediation of nucleases, and enzymes of the pentose and Embden-Meyerhof pathways. On this basis, the disappearance of nucleic acid could account for all the extra lactate in only 2 out of the 6 cases (Table III). In view of the decreases observed in acid-soluble organic phosphorus, the balance of the lactate might have come from intermediates present in this soluble fraction. In the previous study[1] it was found that changes in known major carbohydrate metabolites (glucose-6-P, fructose diphosphate, triose phosphate) could not account for the extra lactate. It will therefore be necessary to examine other possibilities, one of which could be the acid-soluble oligonucleotide fraction. In the previous study a substantial decrease in acid-soluble pentose (as measured by the orcinol reaction) was in fact reported.

Table III. Changes in acid-soluble phosphorus, nucleic acid phosphorus and lactate during 4 h of ischemia

Tumor	Δ Acid-soluble organic P	Δ Nucleic acid P	Δ Acid-soluble organic plus nucleic acid P	Δ P_i	Extra lactate
	m*M*/100 m*M* total P				
E-1	− 14.0	− 5.8	− 19.8	+ 18.4	+ 5.5
E-2	− 17.7	− 3.6	− 21.3	+ 19.7	+ 10.4
G-1	− 3.6	− 7.4	− 11.0	+ 16.1	+ 13.6
G-2	− 7.0	− 8.8	− 15.8	+ 19.3	+ 7.9
M-1	− 7.0	− 2.2	− 9.2	+ 8.4	+ 5.0
M-2	− 11.4	− 8.0	− 19.4	+ 22.6	+ 18.9

The tumors are designated as in Table I. The changes are calculated per 100 mmoles of total phosphorus (last column of Table II). 'Extra lactate' is that formed which is not accounted for by disappearance of glucose and glycogen.

Neoplastic tissue has a remarkable ability to survive under conditions of complete ischemia with maintenance of significant levels of ATP[1,9,10]. The incentive for tracing the source of the extra lactate is the hope of explaining how brain tumors can maintain these ATP levels (in contrast to normal brain which loses all ATP during a few minutes of anoxia). In vivo the conversion of the pentose of nucleic acid to lactate might generate enough high energy phosphate to permit tumor cells to withstand conditions of poor blood supply. It may be significant that normal brain does not generate extra lactate when incubated for as long as 4 h anaerobically[1]. Brain is, however, capable ultimately of degrading ribonucleic acid since this substance was found to be diminished in a 24-h brain infarct[1,11,12].

Zusammenfassung. Glukose, Glykogen, Milchsäure, ATP und 5 phosphathaltige Fraktionen wurden an experimentellen, anärobisch-inkubierten Hirntumoren bestimmt. Die Mengen anorganischen Phosphats und Milchsäure waren grösser als die vom Katabolismus niedermolekularer Substanzen erwarteten. Die Möglichkeit besteht, dass die Pentose aus Nukleinsäuren und säurelöslichen Oligonukleotiden als Quelle der überschüssigen Milchsäure entstammt. Dies würde unter anärobischen Bedingungen eine weitere Energiereserve für Tumorzellen darstellen.

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