aged 18 to 44 with children under 14 years of age constitute 92.2 percent of all employed women with such children.
7. These data do not indicate which days of the week each spouse works. All spouses, however, are full-time workers, so we would expect considerable overlap in days worked.
8. In our sample, there are no couples who both work the day shift according to the BLS definition and have no overlap in employment hours. Almost all (99.2 percent) have 5 or more hours of overlap.
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Barbiturate-Enhanced Detection of Brain Lesions by Carbon-14–Labeled 2-Deoxyglucose Autoradiography

Abstract. *Cerebral glucose metabolism in rats was examined 1 week after the production by ibotenic acid of unilateral striatal lesions. The incorporation of carbon-14–labeled deoxyglucose decreased within the lesion but much less than that of carbon-14–labeled glucose. Barbiturate anesthesia caused a reversal of the asymmetric striatal deoxyglucose labeling, such that the lesioned striatum retained more tracer than the contralateral side. The combined use of barbiturates and radiolabeled deoxyglucose may enhance the identification of recent brain infarction in experimental animals and in man.*

The development of the labeled 2-deoxy-D-glucose (2-DG) technique for measurement of cerebral glucose utilization has made it possible to investigate local metabolic rates under a wide variety of experimental conditions (*1*). The method is based on the measurement of accumulated 2-DG-6-phosphate (2-DGP) in the brain after intravenous injection of 2-DG. The glucose analog 2-DG competes for both the uptake and phosphorylation of glucose in the brain. Unlike glucose 6-phosphate, however, 2-DGP is not readily metabolized and remains trapped intracellularly. A model developed by Sokoloff *et al.* (*2*) makes it possible to determine cerebral glucose metabolic rates, provided that the time courses of plasma glucose and 2-DG concentrations as well as the accumulated tissue 2-DGP are measured.

With the advent of positron emission tomography, it has become possible to apply the 2-DG methodology to the human brain with the use of [^{18}F]2-deoxy-2-fluoro-D-glucose or [^{11}C]2-DG as tracers (*3–5*). Interest has focused on glucose metabolism in pathologic brain tissue, but for such tissue some of the assumptions necessary for quantitation cannot be readily verified (*3, 4*). We have therefore examined the use of [^{14}C]2-DG to determine the rate of glucose metabolism in lesioned rat brain. The ibotenic acid lesion is particularly attractive for this purpose since the toxin causes a marked loss of neurons and induces a glial reaction but does not damage local microvessels (*6*). Thus large areas of abnormal brain tissue retain the capacity for the uptake of tracers from blood. In addition, striatal lesions produced by ibotenic acid (*6*) or the related neurotoxin kainic acid (*7*) have been proposed as animal models for Huntington's disease. Such lesions exhibit reduced 2-DG uptake, which is assumed to reflect decreased glucose utilization (*8, 9*), although interpretation must be guarded in the absence of further information regarding the validity of the 2-DG method in the lesioned brain (*10*).

Unilateral striatal ibotenate lesions were produced in male Sprague-Dawley rats weighing between 180 and 200 g. The animals were anesthetized with diethyl ether and mounted in a stereotaxic frame with reference planes as described by König and Klippel (*11*). Ibotenic acid (20 μg in 1 μl of phosphate-buffered saline, *p*H 7.4) was infused through a 30-gauge cannula over 8 minutes at the following coordinates: 1.0 mm anterior to bregma, 2.6 mm lateral to the midline, and 5.5 mm below the cortical surface. The cannula was left in place for an additional 2 minutes to reduce the reflux of ibotenate up the cannula track. During the first 24 hours after the administration of ibotenate, the animals were generally hypoactive although episodes of seizure-like activity, consisting of chewing and facial clonus, progressing to forelimb clonus, rearing, and truncal torsions, were observed. Although bilateral manifestations were noted, the convulsions appeared to favor the forelimb contralateral to the lesion.

Seven days after the production of the striatal lesions, the animals were prepared for determination of cerebral glucose metabolism or blood flow. Femoral venous catheters were inserted under light ether anesthesia, and the animals were allowed to recover for 4 hours in Plexiglas rodent restrainers. The animals used in the determination of cerebral glucose metabolism received an intravenous bolus injection of [^{14}C]2-DG (100 μCi/kg, 60.3 mCi/mmole) or [^{14}C]glucose (100 μCi/kg) in 0.5 ml of saline followed immediately by a 0.5-ml saline flush of the catheter. The distributions of both [1-^{14}C]glucose (60.1 mCi/mmole) and [6-^{14}C]glucose (52.7 mCi/mmole) were examined. The animals were killed by intravenous injection of KCl and decapitation 45 minutes after [^{14}C]2-DG administration (*2*) or 10 minutes after [^{14}C]glucose (*12*) administration. Cerebral blood flow was studied by the intravenous infusion of [^{14}C]iodoantipyrine (100 μCi/kg, 58 mCi/mmole) for 1 minute, after which the animals were decapitated (*13*).

The brains were removed rapidly, blocked caudal to the level of the inferior colliculus, and frozen onto microtome chucks with crushed Dry Ice. Coronal sections (20 μm) of the forebrain through the level of the lesioned striatum were cut in a cryostat at −18°C, collected on cold glass slides, and rapidly desiccated on a hot plate. Brain sections and calibrated ^{14}C plastic reference standards (*14*) were apposed to Kodak type SB-5 film for 3 to 7 days. We measured the optical densities of the resultant autoradiograms with a microcomputer-assisted spot densitometer (*15*), and we determined the regional brain isotope concentrations by using curves generated from the reference standards. A minimum of eight measurements was obtained from each striatum in at least six sections through the center of the lesion (Fig. 1, A through D). Data on striatal isotope uptake and retention were expressed as the ratio of the lesioned to the contralateral (control) tissue ^{14}C content.

Striatal uptake of 2-DG in awake, lesioned rats was reduced by 20 percent on the lesioned side as compared to that on the contralateral side [lesion/control ^{14}C ratio = 0.80 ± 0.03 (standard error of the mean), N = 6]. The distribution of label within the lesion was heterogeneous; in contrast, there was relative homogeneity in the contralateral striatum and in unlesioned animals (Fig. 1A) (*16*). These results are in agreement with qualitative (*9*) and quantitative (*8, 10*) studies on 2-DG metabolism after the production of striatal kainate lesions.

Also consistent with earlier reports (*2, 17*), we found that rats treated with pentobarbital (40 mg/kg, intravenous, 5 minutes prior to 2-DG injection) showed a generalized decrease in 2-DG uptake. The lesioned striatum, however, appeared resistant to the barbiturate effect (Fig. 1B). Thus barbiturate anesthesia caused a reversal of the pattern seen in its absence such that the lesioned striatum retained more tracer than the control side (lesion/control ^{14}C ratio = 1.31 ± 0.04, N = 2) (*18*). Autoradiograms from animals injected with [^{14}C]glucose demonstrated a much greater reduction in isotope retention within the lesioned striatum (lesion/control ^{14}C ratio = 0.52 ± 0.2, N = 4) than that observed with 2-DG (Fig. 1C). No qualitative or quantitative differences were observed between the autoradiograms from lesioned animals receiving [1-^{14}C]glucose or [6-^{14}C]glucose (*19*).

The pattern of striatal uptake of [^{14}C]iodoantipyrine revealed greater variability between animals than was observed with either of the metabolic tracers. Although the autoradiograms indicated that blood flow to the lesioned striatum tended to decrease (lesion/control ^{14}C ratio = 0.89 ± 0.06, N = 5), it was unchanged or increased (Fig. 1D) in some animals. In any event, the average reduction in blood flow observed within the lesion is less than the decrease in metabolic rate measured with either [^{14}C]2-DG or [^{14}C]glucose. Thus there appears to be a dissociation of the normal coupling between blood flow and metabolism (*1*), resulting in either relative or absolute hyperemia of the lesioned striatum. A similar phenomenon has been described as occurring in the human brain after a stroke. Emission tomographic studies have demonstrated both relative and absolute hyperperfusion in the zone peripheral to ischemic infarctions, where metabolic disturbances are observed concomitantly with relative preservation of the vascular supply (*20*). The nature of the etiologic process underlying these findings is presently unknown, although metabolic acidosis with reactive hyperemia as well as capillary proliferation have been implicated.

The apparent differences in glucose metabolism observed with [^{14}C]2-DG and [^{14}C]glucose could result from the invalidation of assumptions associated with the use of either tracer. (i) The kinetic constants in the 2-DG model may be altered in the lesioned tissue. This has been described on a global basis when blood glucose ranges beyond normal limits (*21, 22*) and when brain glucose concentrations are locally altered (*22, 23*). Were this the case in our study, however, we would expect underestimation of glucose utilization in the lesion by 2-DG, since a reduced metabolic rate in the face of preserved or perhaps increased blood flow should shift the rate constants toward those occurring in hyperglycemia (that is, transport >> phosphorylation) (*24*). (ii) Metabolic trapping of ^{14}C in the brain after the administration of [^{14}C]glucose involves the entry of labeled metabolites into a large pool of glutamate and other amino acids associated with the Krebs cycle (*12*). The relative decrease in ^{14}C found in the lesions after [^{14}C]glucose administration might be attributed to decreased trapping of glucose metabolites and accelerated loss of $^{14}CO_2$ from the brain. (iii) There may be an increase in anaerobic glycolysis within the lesion, which, under steady-state conditions, would lead to increased glucose uptake and phosphorylation and a reciprocal loss of lactate from the lesioned striatum. Thus, 2-DGP might accumulate out of proportion to glucose metabolites, since radioactive lactate derived from [^{14}C]glucose would be cleared from the brain in venous blood. Although [^{14}C]2-DG would measure the rate of glucose phosphorylation, [^{14}C]glucose labeling might better reflect the overall rate of energy production if the net contribution of anaerobic glycolysis to metabolism were minor.

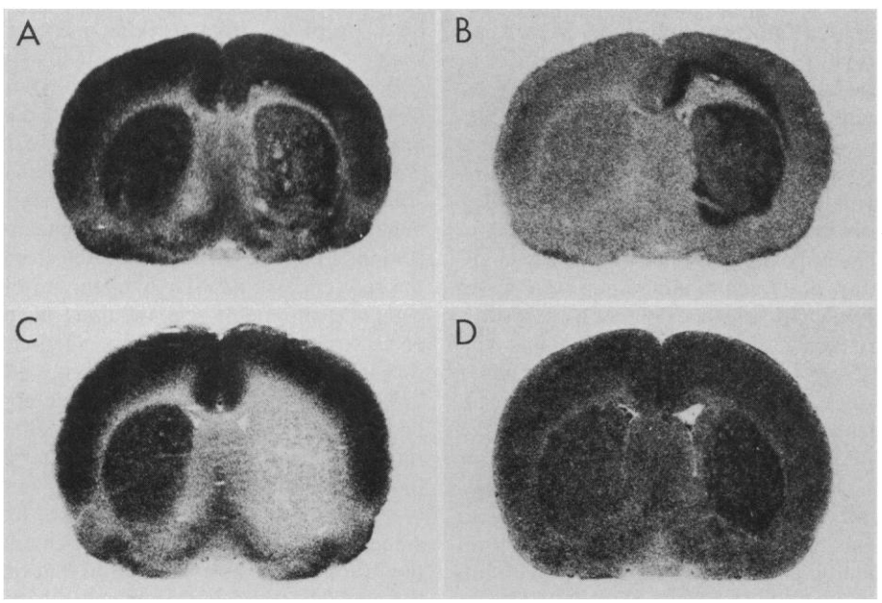

Fig. 1. Autoradiographs of coronal sections of rat brain 1 week after the production by ibotenic acid of a right striatal lesion. (A) Incorporation of [^{14}C]2-DG in an awake animal. Note the decrease in right striatal labeling relative to the control side. (B) Incorporation of [^{14}C]2-DG in a barbiturate-anesthetized animal. Labeling is decreased throughout the brain. The lesion, however, is relatively resistant to the barbiturate effect and appears as the darkest region in the autoradiogram. The lesion in this animal extends into the overlying cortex because of the spread of ibotenate from the injection site. (C) Incorporation of [^{14}C]glucose. Labeling within the lesioned striatum demonstrates a much greater reduction than that observed with [^{14}C]2-DG in (A). (D) Uptake of [^{14}C]iodoantipyrine. The fact that tracer accumulation in this animal is elevated within the lesion suggests an increase in cerebral blood flow. Although cerebral blood flow is usually slightly decreased in the lesion (see text), in this animal the dissociation of cerebral blood flow from metabolism is evident.

Of particular interest is the resistance of 2-DG uptake within the lesioned striatum to suppression by barbiturate anesthesia. Although this labeling could represent an overestimate of glucose utilization, it could alternatively reflect increased metabolic demand within proliferating astrocytes and phagocytes. Since barbiturates reduce the cerebral metabolic rate as well as neuronal activity (*1, 12, 17, 25*), the refractoriness of the lesion suggests an increase in metabolic processes unrelated to neuronal function. This finding is perhaps expected in view of the facts that the neuropil in the ibotenate-lesioned striatum is replaced by a cellular infiltrate, whereas barbiturate-sensitive cerebral glucose metabolism is associated with regions of dense synaptic contact (*2*).

We conclude that lesioned brain regions utilize glucose, but the specifics of this process in relation to the quantitative measurement of regional glucose metabolism may differ significantly from that of the normal brain. The distribution of metabolic tracers may thus reflect both physiological alterations in neuro-

nal activity and pathological changes in the local dynamics of cerebral glucose utilization and intermediary metabolism. As a result, serious underestimates of the extent of neuronal loss can follow from an evaluation of labeled 2-DG uptake alone. Histological examination should serve to demarcate those areas in which metabolic changes cannot be attributed exclusively to alterations in functional state.

The metabolic and hemodynamic alterations observed in this study together with the histological changes (*6*) in ibotenate lesions resemble those seen during the subacute response to a variety of processes that result in focal brain necrosis. We therefore propose that the attenuated barbiturate response is a general characteristic of damaged brain tissue. In clinical studies where direct inspection of tissue is precluded, the combined use of barbiturates and positron-emitting 2-DG analogs should provide a functional contrast enhancement technique for the detection of recently lesioned areas.

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19. The lack of difference between the distributions of [1-^{14}C]glucose and [6-^{14}C]glucose suggests that there is no appreciable increase in glucose metabolism through the hexose monophosphate shunt in the lesioned striatum. Thus, the less expensive [1-^{14}C]glucose appears suitable for evaluation of glucose metabolism in lesioned as well as normal rats. Glucose labeled in the 1 or 6 position was preferred to [2-^{14}C]glucose, used in earlier studies (*12*), because the former compounds give rise to [acetyl-2-^{14}C]acetylcoenzyme A. Label in this position is retained longest during oxidation via the tricarboxylic acid cycle, thus minimizing loss of $^{14}CO_2$ during the experiment.
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Human Brain Tumor–Derived Cell Lines: Growth Rate Reduced by Human Fibroblast Interferon

Abstract. *The biological response modifier human β-interferon had pronounced antigrowth effects on various histologic types of human brain tumor cells but no effects on a nontransformed cell line, MRC-5. The cultures of brain tumor cells showed severe alterations indicative of cell injury and death after exposure to β-interferon for 2 to 6 days. Similar results were obtained with cells freshly explanted from human brain tumors. The results indicate that it may be possible to use fresh, explanted tumor tissue to identify patients who might benefit from therapy with β-interferon.*

It is widely accepted that antiproliferative activity is a property of all three antigenic types of interferon, that is, IFN-α, IFN-β, and IFN-γ (*1*). There is evidence that IFN expresses its antiproliferative activity on target cells both indirectly and directly. Indirect action requires the cooperative action of other elements of the nonspecific defense system, and the clearest example of this is

Table 1. Sensitivity of human brain tumor cell cultures to IFN-β. The cultures received fresh medium with or without IFN-β every 42 to 48 hours as shown. The concentration of IFN-β is expressed as the amount required to reduce the growth rate by 50 percent.

Experiment No.	Cell line	Duration of treatment (days)	Concentration of IFN-β (U/ml)
	Cultures received IFN-β on days 2 and 4		
56-6	A172	6	~ 30
56-6	MRC-5	6	> 3000
	Cultures received IFN-β on days 2 and 4, and medium alone on days 6 and 8		
56-16	A172	10	~ 30
56-16	A382	10	~ 30
56-16	MRC-5	10	> 3000
56-36	A172	10	~ 30
56-36	A382	10	< 30
56-50	MRC-5	10	> 3000
56-69	SK-N-SH	10	< 300 > 30
56-69	U87-MG	10	< 30
	Cultures received IFN-β on days 0, 2, 4, and 6, and medium alone on days 8 and 11		
56-75	SK-N-SH	13	30