the immunoprecipitates are analyzed on sodium dodecyl sulfate-polyacrylamide gels, two bands are observed (Fig. 2, lanes 2 and 3) that, on electrophoresis, migrate with the P-1 and P-2 polypeptides immunoprecipitated from ^{125}I-labeled purified HBsAg (Fig. 2, lane 1). These bands are two of the seven polypeptide components reported for HBsAg (*18*) and correspond to the p23 and p28 components identified from purified 22-nm particles and the PLC/PRF/5 cell line (*15*). The additional bands observed in lanes 2 and 3 are nonspecific and are observed in the absence of antiserum to HBsAg. The one exception to this is the 68,000-dalton band that is thought to be copurified albumin (*19*). The P-1 and P-2 subunits are probably the only major viral components of HBsAg (*15, 20*). No HBsAg components were found in the cell culture medium from Hep G2 (Fig. 2, lane 4).

One of the most interesting aspects of these tumor-derived cell lines is that they have the biosynthetic capabilities of normal liver parenchymal cells. Indeed, the Hep 3B and PLC/PRF/5 cell lines are capable of tumor formation in athymic mice. We currently have no evidence for the presence of the hepatitis B viral genome in the Hep G2 cells, even though the line is phenotypically similar to the Hep 3B cell line. The retention of the differentiated liver cell functions in this replicating cell line does not therefore relate to the presence of the viral genome. Quantitative comparison of the production of plasma proteins and HBsAg by Hep 3B cultures indicates that the expression of the viral coded product is regulated by the host cell (*10*). The availability of these human parenchymal cells in continuous culture, synthesizing a large number of readily identifiable secreted products, should provide a major tool for investigation of the control of their biosynthesis.

BARBARA B. KNOWLES
CHIN C. HOWE
DAVID P. ADEN

Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

References and Notes

1. T. Peters, Jr., *Am. J. Physiol.* **224**, 1363 (1973).
2. K. N. Jeejeebhoy, J. Ho, G. R. Greenberg, M. J. Phillips, A. Bruce-Robertson, U. Sodtke, *Biochem. J.* **146**, 141 (1975).
3. L. J. Crane and D. L. Miller, *J. Cell Biol.* **72**, 11 (1977).
4. G. Grieninger and S. Granick, *J. Exp. Med.* **147**, 1806 (1978).
5. M. E. Kaighn and A. M. Prince, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2396 (1971).
6. H. L. Leffert and S. Sell, *J. Cell Biol.* **61**, 823 (1974).
7. Y. Tsukada, M. Mikun, H. Hirai, *Int. J. Cancer* **13**, 196 (1974).
8. I. Doi, *Gann* **67**, 1 (1976).
9. H. P. Bernhard, G. J. Darlington, F. H. Ruddle, *Dev. Biol.* **35**, 83 (1973).
10. D. P. Aden, A. Fogel, I. Damjanov, S. Plotkin, B. B. Knowles, *Nature (London)* **282**, 615 (1979).
11. G. M. Macnab, J. J. Alexander, G. Lecatsas, E. M. Bey, J. M. Urbanowicz, *Br. J. Cancer* **34**, 509 (1976).
12. W. Szmuness, *Prog. Med. Virol.* **24**, 40 (1978).
13. G. R. Martin and M. J. Evans, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1441 (1975).
14. O. Ouchterlony, *Acta Pathol. Microbiol. Scand.* **26**, 507 (1949).
15. J. Skelly, J. A. Copeland, C. R. Howard, A. J. Zuckerman, *Nature (London)* **282**, 617 (1979).
16. P. O'Farrell, *J. Biol. Chem.* **250**, 4007 (1975).
17. L. Anderson and N. G. Anderson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5421 (1977).
18. W.-K. J. Shin and J. L. Gerin, *J. Virol.* **21**, 347 (1977).
19. Y. Sanchez, I. Ionescu-Matiu, F. B. Hollinger, J. L. Melnick, G. R. Dreesman, *J. Gen. Virol.*, in press.
20. D. L. Peterson, I. M. Roberts, G. N. Vyas, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 1530 (1977).
21. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
22. Purified HBsAg was provided by J. Gerin, Division of Molecular Virology and Immunology, Georgetown University School of Medicine, Rockville, Md.
23. W. M. Hunter and F. C. Greenwood, *Nature (London)* **194**, 495 (1962).
24. Antiserum to guinea pig HBsAg/ad was obtained from the Research Resources Branch of the NIH-NIAID, request V801-502-058.
25. S. E. Cullen and B. D. Schwartz, *J. Immunol.* **117**, 136 (1976).
26. D. P. Aden, R. Mausner, B. B. Knowles, *Nature (London)* **271**, 375 (1978).
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Cerebral Regional Oxygen Consumption and Supply in Anesthetized Cat

Abstract. *The study involved quantitative measurement of arterial and venous oxygen saturation, oxygen extraction, blood flow, and oxygen consumption in specific areas of the brain. No regional differences in oxygen consumption were found in anesthetized cat brain, and the amount of oxygen available to all regions studied was more than 2.5 times the consumption throughout the brain.*

Roy and Sherrington (*1*) were the first to propose that brain blood flow was regulated to meet the requirements of metabolism. This hypothesis was never tested on a quantitative basis in different brain regions because of limitations of existing technology. Our investigation represents, to our knowledge, the first quantitative determination of the normal regional relationship between oxygen supply and consumption in the brain.

Some areas of the brain are known to be susceptible to oxygen lack, even for a short time. It would be interesting to determine which areas are more susceptible than others. Estimates that may be used as indicators of regional metabolic rates are available for regional oxygenation (*2*), reduced nicotinamide adenine dinucleotide, and cytochrome aa_3 oxidation state (*3*) in various parts of the brain. These methods provide only an estimate of oxygen supply with respect to consumption rate rather than a measure of oxygen supply and consumption.

We report a new microspectrophotometric method for determining oxygen saturation in small blood vessels of quick-frozen brain. Combining this measure with regional blood flow measured by the radioactive microsphere method, we have determined oxygen consumption in defined regions of the brain by application of the Fick principle. No other methods are available for determining oxygen consumption in such small areas and deep structures of the brain.

Unlike most other organs, the brain is a conglomeration of a large number of diverse functional areas. At any moment some of these units may be active and others quiescent. The energy need and, therefore, the metabolic rate of a given region are related, in part, to the instantaneous amount of activity. Some in vivo measurements of regional metabolic rate in the brain may be used as an index of average neuronal activity in that region. The recognized usefulness of monitoring the metabolic rate in the brain in relation to mental activity has led to the development of several procedures; however, attempts to measure oxygen consumption, glucose utilization, or oxygenation are limited to localized brain regions, are indirect, or are not quantitative measures of regional brain oxygen consumption (*2-5*).

Our method is a technique for determining oxygen consumption in small areas of superficial and deep structures of the brain. The arterial-venous oxygen extraction can be determined by a microspectrophotometric method by examining small vessels regionally in quick-frozen tissue (*6, 7*). This method is highly accurate and—since we looked at blood within blood vessels—independent of the organ studied. We measured blood flow through the use of radioactive mi-

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crospheres. This method has been validated in the cat brain (*8*). The Fick principle allows the regional consumption of oxygen to be computed as the product of flow and extraction. Our technique in gracilis muscle had an accuracy of ± 8.7 percent when compared with standard techniques.

Twelve adult mongrel cats of either sex, ranging in weight from 2 to 4 kg were deprived of food overnight and tranquilized with ketamine (33 mg per kilogram of body weight, injected intramuscularly). Normal body temperature was maintained during the experiment with a heating pad. An endotracheal tube and a femoral artery catheter were inserted. The cats were then anesthetized with α-chloralose (75 mg/kg, injected intra-arterially), and artificial respiration was instituted. A left thoracotomy was performed at the fifth intercostal space; a partial pericardiotomy exposed the heart, and a left atrial catheter was inserted. A femoral artery catheter was coupled to a pressure transducer (Statham) for monitoring heart rate and systemic arterial pressure. This femoral artery catheter was used to obtain reference blood flow samples and for subsequent withdrawal of anaerobic arterial blood samples, which, along with an anaerobic blood sample from the superior saggital sinus, were analyzed for P_{CO_2}, P_{O_2}, *p*H, hemoglobin, and hematocrit. A 0.2-ml bolus of approximately 1 million ^{141}Ce-labeled microspheres (15 ± 3 μm) was injected into the left atrium and flushed with 0.5 ml of saline. A 3-minute timed reference blood sample was withdrawn from the femoral artery with a monostaltic pump (Buchler). Weighed brain tissue and blood samples were counted to a standard error of less than 2 percent on a gamma scintillation counter (Hewlett-Packard). From these data, blood flow in milliliters per minute per 100 g was calculated (*8*).

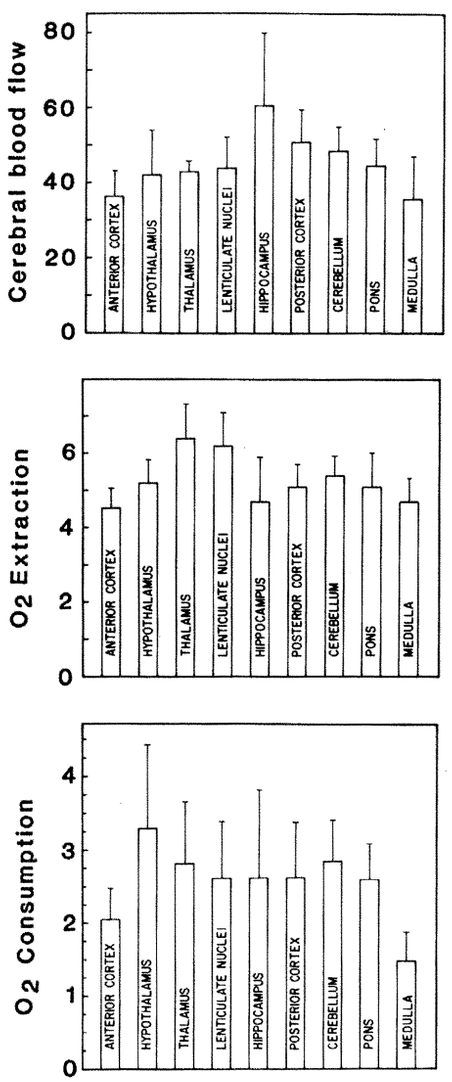

Fig. 1. Regional cerebral blood flow (in milliliters per minute per 100 g), arterial-venous extraction (in milliliters of O_2 per 100 ml of blood), and oxygen consumption (in milliliters of O_2 per minute per 100 g) in nine examined brain regions. No significant regional differences were found.

The cats' heads were quickly guillotined in two places and frozen in liquid nitrogen. The frozen brain was cut into wafers on a band saw. Nine different regions were isolated and examined: anterior cortex, hypothalamus, thalamus, lenticulate nuclei, hippocampus, posterior cortex, cerebellum, pons, and medulla. The mean oxygen saturation value of five veins and one artery (20 to 100 μm in diameter) per region in 12 different cats was determined. A total of 476 veins were examined in 30-μm frozen transverse sections at three wavelengths—560 nm, 568 nm, and 503 nm (*6*, *7*). Data were evaluated by factorial analyses of variance followed by Duncan's multiple range procedure ($\alpha = .05$).

Blood pressure (mean ± standard error) (systolic, 143 ± 9 mm-Hg; diastolic, 102 ± 7 mm-Hg), heart rate (185 ± 11 beat/min), and blood gas values were within physiologically normal ranges. Blood flow in all the regions examined averaged 46.3 ± 3.1 ml/min per 100 g. There were no significant differences in mean blood flow among the areas examined (Fig. 1).

Venous oxygen saturation was variable (from 0 percent to 96 percent) (Fig. 2). No significant differences existed among the regions examined. The overall arterial-venous oxygen differences averaged 33.86 ± 1.27 percent. Oxygen extraction was calculated as the arterial-venous oxygen difference times the hemoglobin concentration times 1.36, where 1.36 represents the number of milliliters of oxygen per gram of hemoglobin (Fig. 1). No significant differences were noted.

Oxygen consumption was determined as the product of flow and oxygen extraction. Oxygen consumption averaged 2.6 ± 0.2 ml of O_2 per minute per 100 g. There were no statistical differences in mean oxygen consumption among the various regions of the brain (Fig. 1). Analysis of variance revealed a significant between-animal variation in all factors.

The ratio of oxygen supply to consumption was computed (*7*). The highest ratio was 3.99 ± 0.45 in the medulla; the lowest, 2.76 ± 0.45, was found in the thalamus. There were no statistical differences in this ratio among the various brain regions.

The venous oxygen saturation in the cat brain reveals considerable heterogeneity (Fig. 1). The heterogeneity in the venous oxygen saturation in the brain quite closely parallels the results reported by Leniger-Follert *et al.* (*9*) for local tissue oxygen tension. They reported that for animals under control conditions and breathing room air, local P_{O_2} values varied from 0 torr to almost arterial values of 90 torr. Our data indicate that oxygen consumption must also be heterogeneous within any given region. We have presented the mean value. This con-

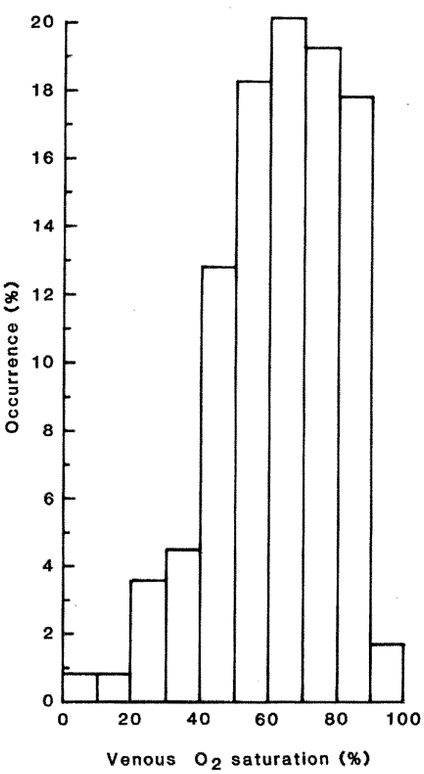

Fig. 2. Venous oxygen saturation measurements. No regional differences were found in the 476 veins examined.

tention of heterogeneity is supported by the work of Sokoloff *et al.* (*5*), who have reported heterogeneous rates of glucose utilization within specific brain structures, often related to the pattern of cytoarchitecture. This heterogeneity of venous oxygen saturation, tissue oxygen tension, and glucose utilization does not appear to lead to large regional (for example, pons versus medulla) differences in oxygen consumption. Our results indicate that regional oxygen consumption in anesthetized cat brain is relatively uniform despite this heterogeneity.

Our blood flow values are similar to those reported by others for the anesthetized cat (*8*) although slightly lower than that reported for conscious animals (*10*). Chloralose as well as barbiturate anesthesia tend to reduce both cerebral blood flow and regional differences in that flow (*11, 12*). These results may be due to the tendency of anesthesia to reduce perfusion of most brain regions to a basal value, one similar to that found in the pons and medulla. The relative homogeneity of regional blood flow in the brain may be due to a reduction in the rate of cerebral metabolism (and possibly in neuronal activity) to a relatively uniform level throughout the brain. Small differences in microflow revealing a heterogeneity of capillary blood flow in the brain—which may be related to differences in capillary length, flow, or both (*13*)—may also be rendered more uniform by anesthesia.

Our regional oxygen consumption values for the brain are similar to those global values reported by others (*5, 12*). Our study reveals a lack of regional differences in brain oxygen consumption. Studies of significantly smaller brain structures have shown heterogeneity of glucose utilization (*5*). This heterogeneity appears to be averaged out in structures as large as the thalamus or lenticulate nuclei. Anesthesia may have depressed regional brain oxygen consumption and metabolism so that metabolic rate throughout the gray matter became more uniform at a lower level. The moment-by-moment differences in microflow (*9*), which may be dampened by anesthesia, when combined with a depression of differences in activity among the various types of neuronal and glial components of a region, may result in a relatively uniform oxygen consumption throughout the anesthetized brain. Although there were no regional differences in cerebral oxygen extraction, flow, and consumption, there were significant differences between animals that might be due to differences in age, sex, weight, strain, and depth of anesthesia.

Our study demonstrated that the supply of oxygen to the various brain regions studied was at least 2.5 times the metabolic needs throughout the brain. Oxygen extraction was relatively low in any given region. This is consistent with hypotheses that the brain is able to maintain adequate blood flow under most circumstances to meet its relatively constant metabolic needs (*14*). The relation of oxygen supply to demand in the brain is higher and more uniform than in the heart. In the heart, the subendocardial region of the left ventricle has a lower oxygen supply-to-demand ratio than the rest of the organ, and the ratio is much lower throughout the heart than the brain (*7*). Whereas under α-chloralose anesthesia all brain regions have an adequate and uniform oxygen supply-to-demand relation, it is not possible to predict what would happen under stressful conditions, such as hypoxia. Different brain regions may have very different blood flow and oxygen extraction reserves.

ELLEN BUCHWEITZ
ARABINDA K. SINHA
HARVEY R. WEISS
Department of Physiology and Biophysics, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway 08854

References and Notes

1. C. S. Roy and C. S. Sherrington, *J. Physiol. (London)* **11**, 85 (1890).
2. H. R. Weiss, P. J. Levy, H. D. Kleinert, V. S. Murthy, *Circ. Shock* **5**, 115 (1978).
3. B. Chance, C. Barlow, Y. Nakase, H. Takeda, A. Mayevsky, R. Fischetti, N. Graham, J. Sorge, *Am. J. Physiol.* **235**, H809 (1978).
4. U. Gleichmann, D. H. Ingvar, N. A. Lassen, D. W. Lübbers, B. K. Siesjo, G. Thews, *Acta Physiol. Scand.* **55**, 82 (1962); M. E. Raiche, K. B. Larson, M. E. Phelphs, R. L. Grubb, Jr., *Am. J. Physiol.* **193**, 228 (1975).
5. L Sokoloff, M. Reivich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada, M. Sinohara, *J. Neurochem.* **28**, 897 (1977).
6. A. K. Sinha, J. A. Neubauer, J. A. Lipp, H. R. Weiss, *Microvasc. Res.* **10**, 312 (1975); *ibid.* **14**, 133 (1977); H. R. Weiss and A. K. Sinha, *Circ. Res.* **42**, 119 (1978).
7. H. R. Weiss, J. A. Neubauer, J. A. Lipp, A. K. Sinha, *Circ. Res.* **42**, 394 (1978).
8. M. L. Marcus, D. D. Heistad, J. C. Ehrhardt, F. M. Abboud, *J. Appl. Physiol.* **50**, 501 (1976).
9. E. Leniger-Follert, D. Lübbers, W. Wrabetz, *Pflugers Arch.* **359**, 81 (1975).
10. M. Reivich, J. Jehle, L. Sokoloff, S. S. Kety, *J. Appl. Physiol.* **27**, 296 (1969).
11. H. Goldman and L. A. Sapirstein, *Am. J. Physiol.* **224**, 122 (1973).
12. W. M. Landau, W. H. Freygang, Jr., L. P. Rowland, L. Sokoloff, S. S. Kety, *Trans. Am. Neurol. Assoc.* **80**, 125 (1955); P. Sandor, M. Nayary, M. Reivich, A. Kovach, *Stroke* **8**, 432 (1977).
13. M. Hertz and A. B. Paulson, *Acta Physiol. Scand. Suppl. 64*, **56**, 488 (1977).
14. W. Kushinsky and M. Wahl, *Physiol. Rev.* **58**, 656 (1978).
15. This work was supported by grants NS-13118 and HL-21172 from the U.S. Public Health Service, a grant in aid from the American Heart Association (New Jersey affiliate), and by a grant from the Foundation of the College of Medicine and Dentistry of New Jersey.

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Multiple Mating-Type Specificities in the Flax Rust *Melampsora lini*

Abstract. *The results of selfing and intercrossing two strains of the flax rust fungus* Melampsora lini *indicate that this species does not possess a simple (+) and (−) mating system. Instead, the data are consistent with the assumption that the genetic control of mating type in* Melampsora lini *is similar to that in the mushroom* Schizophyllum commune, *in which mating type is determined by two factors, each of which is controlled by two linked loci.*

In heterothallic rust fungi (Uredinales), the haploid, monokaryotic infections (pycnia) develop dikaryotic aeciospores only after they are fertilized with "nectar" (a liquid exudate containing haploid pycniospores) from another pycnium of a different mating type. In 1927, Craigie (*1*) proposed that the pycnia of sunflower rust, *Puccinia helianthi*, and wheat stem rust, *P. graminis*, were of two mating types, which he designated (+) and (−). Since then, a (+) and (−) mating system, assumed to be controlled by two alleles at a single locus, has been accepted as common to all heterothallic rusts (*2*). Under this system, 50 percent of crosses between two pycnia should result in aecia formation, irrespective of whether the pycnia being crossed are from the same or different strains.

During a recent study of the inheritance of pathogenicity in the flax rust organism *Melampsora lini*, I found that crosses between pycnia of one strain (CH_5) and those of an unrelated strain (I) resulted in aecia formation 47 out of 48 times. Since this result is clearly inconsistent with a (+) and (−) mating system, I undertook a study to investigate more fully the genetic control of mating type in *M. lini*.

Strains CH_5 and I were used in this study. Strain CH_5 is a hybrid strain derived from intercrossing strains C and H. Strain C was obtained by self-fertilizing New Zealand race 5 (*3*). Strain H is phenotypically the same as race 228 of North American origin (*4*), but was shown to be genotypically different (*5*) from the race 228 used by Flor (*6*). Strain

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