the intermediate and medial part of the hyperstriatum ventrale—completely prevent the acquisition of a learned preference prior to imprinting, but result in only a reduced preference (impaired retention) afterward [B. J. McCabe, J. Cipolla-Neto, G. Horn, P. P. G. Bateson, *Neurosci. Lett. Suppl.* **3**, S381 (1979); *Exp. Brain Res.* **48**, 13 (1982)].

10. Our results stand in contrast to a result frequently obtained in similar studies—namely, that adult lesions disrupt a variety of behaviors more than do early (perinatal) ones [G. E. Schneider, *Neuropsychologia* **17**, 557 (1979)].
11. The volume of RA was 0.368 mm (n = 5; range, 0.28 to 0.51) and 0.436 mm (n = 5; range, 0.41 to 0.46) in birds with lesions of the MAN and control lesions, respectively (U = 5, $P > 0.10$). Of course, MAN lesions may exert subtle effects on RA or HVc neurons (such as on dendritic morphology) that gross volume measurements do not detect.
12. M. E. Gurney and M. Konishi, *Science* **208**, 1380 (1980).
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Decreased Oxidation of Labeled Glucose by Dissociated Brain Cells in the Presence of Fetal Bovine Serum

Abstract. *The effect of serum on the rate of substrate oxidation by dissociated brain cells in vitro was examined. At a serum protein concentration of approximately 0.55 milligram per milliliter, oxidation of [6-^{14}C]glucose to $^{14}CO_2$ was decreased more than 50 percent. Oxidation of [3-^{14}C]-3-hydroxybutyrate and [U-^{14}C]glutamine was decreased much less. Serum from cows, rats, horses, and humans produced similar effects, as did serum from young and old animals and from both sexes. The effect on [6-^{14}C]glucose oxidation was proportional to serum protein concentration, and significant inhibitory activity was obtained with dialyzed serum. Heating (80°C for 10 minutes) significantly reduced the inhibitory activity. These results suggest the presence of a factor in serum that can preferentially decrease glucose oxidation. Such a factor would have profound implications for metabolic regulation in vivo and for studies of cells in vitro in which serum is included in the growth medium.*

It is an accepted practice to add serum (5 to 20 percent) to most tissue culture systems, including media used for neuronal and glial cell cultures (*1, 2*). This practice is based on the idea that serum is required to support the proliferation and survival of cells in culture (*3, 4*). However, the value of including serum in cell culture media has been questioned (*5, 6*), mainly because of the variability of the components in serum. This has led to the identification of an increasing number of "growth factors" required for the maintenance of neuronal cells in culture (*7–10*).

One rationale for using defined media is that every cell type may need a specific microenvironment for survival and growth (*6, 11*). Kaufman and Barrett (*12*) recently identified a serum fraction that supported long-term survival of dissociated rat neurons more reliably than unfractionated serum and suggested that some serum fractions may be toxic to nerve cells in culture. The implication is that a variety of factors in serum may affect several different aspects of the cell. Most investigators have described the effects of serum or isolated factors in terms of cell viability, proliferation, or survival; relatively few have studied the effects of serum on substrate oxidation (*13*). We (*14–16*) and others (*17–19*) have investigated the metabolic characteristics of brain cells in culture. Most of the studies concerned the nature of nutrients required by these cells and possible differences in the capacities of various cell types to use different substrates. Since serum produces a variety of effects on the proliferation and survival of cells in culture, we attempted to examine the effect of serum on the rate of substrate oxidation. We report that the addition of serum causes a much greater decrease in the oxidation of labeled glucose by dissociated brain cells than in the oxidation of other substrates.

Dissociated brain cells were prepared from adult albino Wistar rats (200 to 250 g) (*14–16*). After rapid removal of the brain, the tissue was immersed in 0.9 percent NaCl, cut into small pieces, incubated with 0.2 percent trypsin, washed three times, and further dissociated by gentle titration. The suspension was centrifuged at 600 rev/min for 5 minutes and the supernatant was removed. Cells were resuspended in 0.9 percent NaCl and filtered through Nitex. Trypan blue exclusion and lactate dehydrogenase leakage revealed that cell viability exceeded 90 percent.

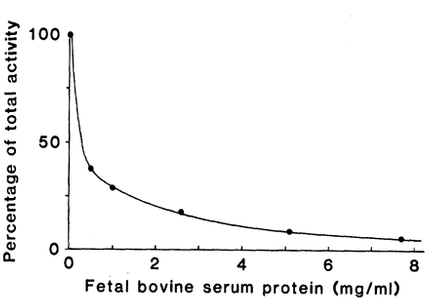

Fig. 1. The effect of increasing fetal bovine serum protein concentrations on the rate of [6-^{14}C]glucose oxidation by dissociated brain cells. Oxidation rates are expressed as percentages of the control value (2.12 nmole/hour per milligram of protein). Each point represents the average of triplicate determinations with variations less than 10 percent. The specific activity of [6-^{14}C]glucose was 296,000 dpm/μmole.

Measurement of ^{14}C-labeled substrate oxidation by dissociated brain cells was performed as previously described (*14*). In these experiments $^{14}CO_2$ produced was trapped on Hyamine hydroxide–saturated filter paper in hanging center wells and counted in a liquid scintillation spectrometer. Previous studies revealed that the rate of $^{14}CO_2$ production is linear for up to 2 hours and is proportional to protein concentration. Cell protein concentrations were maintained between 0.75 and 1.5 mg per sample. The specific activity of [6-^{14}C]glucose was 0.1 μCi/μmole; similar specific activities were used for other labeled substrates.

Addition of 10 percent rat serum greatly reduced the rate of $^{14}CO_2$ production from [6-^{14}C]glucose (Table 1). Similar results were obtained with horse, human, and bovine serum. The inhibitory activity was not affected by freezing for up to 3 weeks and appeared to be stable at 4°C for more than 72 hours. The activity was present in a commercial preparation of dialyzed fetal calf serum and in rat serum samples that had been dialyzed for 18 hours, although the amount of inhibitory activity was considerably reduced in these preparations.

Figure 1 shows the inhibitory effect of fetal bovine serum on [6-^{14}C]glucose oxidation to $^{14}CO_2$ by dissociated brain cells as a function of serum protein concentration. At a relatively low serum protein concentration (0.5 mg/ml), the rate of oxidation was decreased more than 50 percent. With increasing concentrations of serum, the rate decreased exponentially. A considerable amount of the activity was lost when fetal bovine serum was heated (80°C for 10 minutes).

Figure 2 shows the effects of rat serum on the oxidation of several different substrates. Serum had its most pronounced effects on [6-^{14}C]glucose oxidation, decreasing the rate > 75 percent at a serum protein concentration of 2.6 mg/ml. In contrast, an equivalent amount of serum caused only a 35 percent decrease in [U-^{14}C]glutamine oxidation and a < 20 percent decrease in [3-^{14}C]-3-hydroxybutyrate oxidation. Increasing the serum concentration fourfold resulted in a relatively small increment in the inhibition of the latter two substrates. However, the rate of [6-^{14}C]glucose oxidation was decreased to < 10 percent of the control value.

These results show that oxidation of [6-^{14}C]glucose is greatly reduced in the presence of serum. The inhibitory effect of serum on oxidation of [1-^{14}C]glucose and [6-^{14}C]glucose was observed by Larrabee (*13*) in a study of lumbar dorsal root ganglia, and similar effects have been observed with fibroblasts in culture (*20*). A generalized role for this factor is suggested by its presence in most species.

Since considerable inhibitory activity remains after extensive dialysis, it seems unlikely that the observed decrease in oxidation could be the result of substrate dilution by metabolites in serum. This conclusion is also supported by the significant inhibition that results from the addition of only 0.01 ml of serum, representing a dilution of more than 70-fold. At this dilution the contribution to the substrate pool by the added serum would be negligible. Although the inhibitory activity was stable when frozen, it was susceptible to thermal denaturation, suggesting a requirement for conformational integrity.

An interesting aspect is the preferential inhibition of the oxidation of glucose relative to that of 3-hydroxybutyrate or glutamine. It is well established that, under certain conditions, ketone bodies can serve as fuel for the brain, especially in young animals (*21*), and studies of substrate oxidation (*22, 23*) indicate that glucose and 3-hydroxybutyrate are oxidized in similar metabolic compartments. However, our results suggest a separation of these metabolic activities. On the other hand, glutamine oxidation does not appear to occur in the same metabolic compartment as glucose (*15, 16*), even though the rate of glutamine oxidation by dissociated brain cells is two to five times higher than the rate of glucose oxidation (*23*) and most of this oxidation occurs in the tricarboxylic acid cycle (*19, 23*).

Our results seem best explained by the presence of an inhibitor or factor that causes intracellular dilution of glycolytic metabolites before the formation of acetyl coenzyme A. However, since these experiments were carried out with a mixed population of dissociated cells, the possible contribution of metabolic compartmentation cannot be excluded.

The presence in serum of a factor inhibitory to glucose oxidation has major implications for studies of cells in culture. Serum supplementation is used in most tissue culture systems, usually in a concentration equivalent to 10 percent by volume, and at that concentration glucose oxidation would be decreased > 90 percent. The immediate conclusion is that the amount of glucose oxidized by cells in culture is much less than the amount of glutamine oxidized under the same conditions. Recent studies show that glutamine can serve as a major energy source for mammalian cells in culture (*23–25*). The presence of an inhibitor of glucose oxidation would increase this dependence on glutamine oxidation. The use of unfractionated serum in tissue culture may promote the growth of cells that do not utilize glucose.

Table 1. Decrease in [6-^{14}C]glucose oxidation in the presence of serum. The serum in each test system was equivalent to 10 percent (by volume), which is similar to the amounts added to cells in culture. Values are means (and standard deviations) for four different preparations. The control rate for $^{14}CO_2$ production was 3.94 nmole/hour per milligram (standard deviation, 0.38).

Serum	Protein concentration (mg/ml)	Percentage of control value
Human	7.86	18 (1.4)
Horse	7.54	22 (2.4)
Rat	6.89	17 (0.9)
Rat (frozen)	8.38	19 (2.2)
Fetal bovine	4.4	14 (1.0)
Fetal bovine (dialyzed)	4.1	40 (7.8)

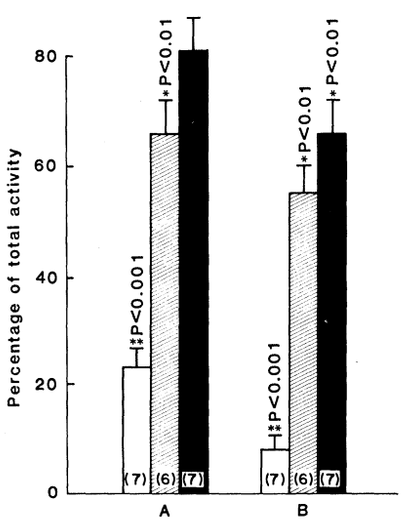

Fig. 2. The effect of serum on the oxidation of [6-^{14}C]glucose (open bars), [U-^{14}C]glutamine (crosshatched bars), and [3-^{14}C]-3-hydroxybutyrate (closed bars) by dissociated brain cells. Values are percentages (± standard errors) of the control values (rates obtained in the absence of serum). The average rates for controls were 4.24, 3.69, and 8.95 nmole/hour per milligram for glucose, 3-hydroxybutyrate, and glutamine, respectively. The numbers of individual animals are shown in parentheses. Concentrations of serum protein added were (A) 2.56 and (B) 10.2 mg/ml. Specific activities of the substrates were 69,000, 195,000, and 32,700 dpm/μmole for [6-^{14}C]glucose, [3-^{14}C]-3-hydroxybutyrate, and [U-^{14}C]glutamine, respectively. Statistical significance was evaluated with the Scheffé test for multiple comparisons.

Simmons *et al.* (*26*) recently showed that defatted albumin inhibited oxygen uptake and energy utilization. They proposed a regulatory mechanism for energy utilization involving the ratio of myo-inositol and albumin. In another study, Kaufman and Barrett (*12*) reported the presence of one or more factors in serum that are toxic to neuronal cells. These results emphasize the need to evaluate further the presence of a factor that affects substrate oxidation, to identify its molecular nature, and to determine its physiological role. The isolated inhibitory factor may provide a tool for delineating metabolic compartments in the brain.

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References and Notes

1. H. M. Temin, R. W. Pierson, N. C. Dulak, in *Growth, Nutrition, and Metabolism of Cells in Culture*, G. H. Rothblat and J. Cristofalo, Eds. (Academic Press, New York, 1972), vol. 1, pp. 49–81.
2. G. D. Fischbach and P. G. Nelson, in *Handbook of Physiology*, section 1, *The Nervous System*, vol. 1, *Cellular Biology of Neurons*, E. R. Kandell, Ed. (Williams & Wilkins, Baltimore, 1977), p. 119.
3. M. Sensenbrenner, J. Booker, P. Mandel, *Z. Zellforsch.* **117**, 559 (1971).
4. E. Richelson, *Int. Rev. Biochem.* **26**, 81 (1979).
5. J. E. Bottenstein and G. H. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 514 (1979).
6. G. Brunner, K. Lang, R. A. Wolfe, D. B. McClure, G. H. Sato, *Dev. Brain Res.* **2**, 563 (1982).
7. J. Bottenstein *et al.*, *Methods Enzymol.* **58**, 94 (1978).
8. D. Barnes and G. H. Sato, *Anal. Biochem.* **102**, 255 (1980).
9. A. Rizzino, H. Rizzino, G. H. Sato, *Nutr. Rev.* **37**, 369 (1979).
10. S. Varon, *Exp. Neurol.* **54**, 1 (1977).
11. J. E. Bottenstein, S. D. Skaper, S. S. Varon, G. H. Sato, *Exp. Cell Res.* **125**, 183 (1980).
12. L. M. Kaufman and J. N. Barrett, *Science* **220**, 1394 (1983).
13. M. G. Larrabee, *Anal. Biochem.* **79**, 357 (1977).
14. J. T. Tildon, S. Merrill, L. M. Roeder, *Biochem. J.* **216**, 21 (1983).
15. L. M. Roeder, J. T. Tildon, D. Holman, *ibid.*, in press.
16. L. M. Roeder, J. T. Tildon, J. H. Stevenson, *ibid.*, in press.
17. M. S. Patel, J. J. Russell, J. J. H. Gershman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7214 (1981).
18. G. Dawson, *J. Biol. Chem.* **254**, 155 (1979).
19. A. C. H. Yu, A. Schousboe, L. Hertz, *J. Neurochem.* **39**, 954 (1982).
20. J. T. Tildon, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 210 (1973).
21. A. M. Robinson and D. H. Williamson, *Physiol. Rev.* **60**, 143 (1980).
22. T. Itoh and J. Quastel, *Biochem. J.* **116**, 641 (1970).
23. J. T. Tildon, in *Metabolic Relationship Between Glutamine, Glutamate, and GABA in the CNS*, L. Hertz, E. Kvamme, E. McGeer, A. Schousboe, Eds. (Liss, New York, 1983), pp. 415–430.
24. H. R. Zielke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4110 (1976).
25. ———, *J. Cell Physiol.* **95**, 41 (1978).
26. D. A. Simmons, A. I. Winegrad, D. B. Martin, *Science* **217**, 848 (1982).
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