Insulin and Brain Metabolism
Absence of Direct Action of Insulin on K^+ and Na^+ Transport in Mouse Brain

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SUMMARY

This is a study of the effect of insulin on the transport of K^+ and Na^+ from the blood into the brains of normal mice. Despite profound reductions in plasma and brain glucose levels, reduction of plasma K^+ concentration and progressive deterioration of neurologic function 30-120 minutes after insulin injection, in 20-22-day-old animals there was no increase in brain K^+ and Na^+ concentrations. In fact, at 120 minutes, when the brain water content increased 0.7 per cent, brain K^+ concentration was significantly reduced, not elevated.

The effect of insulin on brain electrolyte and water content in adult mice was also studied. Although brain water increased 0.5 per cent at 120 minutes, there were no changes in brain Na^+ or K^+ concentrations at any time after insulin injection.

The data from mice do not support a role of insulin in electrolyte transport in brain. DIABETES 25:758-63, September, 1976.

A voluminous literature has established the role of insulin in membrane transport, glycolysis, and the synthesis of glycogen and fat in a number of extracranial organs and tissues. By contrast, there are relatively few studies concerning the role of insulin in brain metabolism, and the findings are highly inconsistent. A brief representative review is relevant to this report.

First, concerning the transport of glucose from the blood into brain; the studies of Rafaelson,[1] Prasannan,[2] Flock et al.,[3] Konitzer et al.,[4] Nelson et al.,[5] and Passonneau et al.[6] suggested that insulin facilitated glucose transport into brain, but those of Park et al.,[7] Buschiazzo et al.,[8] and Betz et al.[9] did not. Studies pertaining to the influence of insulin on brain glycogen synthesis are equally controversial. Prasannan and Subrahmanyam,[10] Nelson et al.,[5] and Passonneau et al.[6] reported significant increases in brain glycogen after injection of insulin (and glucose); but in the laboratories of Held et al.[11] results were variable, and Goldberg and O'Toole[12] found no effect of insulin on glycogenesis in brain. A number of investigators[5,13] have also demonstrated increases in brain glucose-6-phosphate after insulin (and glucose) injection. Stimulated by these conflicting reports, we have recently investigated the effects of alloxan diabetes (four days) on brain carbohydrate metabolism in young mice.[14] In the majority of the animals metabolite changes reflected severe reduction in brain glycolysis and glycogenesis, and administration of insulin (and glucose) had a dramatic effect in correcting these abnormalities. At first glance, the findings strongly support a role of insulin in brain carbohydrate metabolism. However, the untreated diabetic animals were severely dehydrated, and findings in the brain of nondiabetic animals with an equivalent degree of dehydration (produced by water deprivation and mannitol injections for four days) were almost identical with those seen after alloxan. Since the weight of the diabetic animals returned to normal during insulin and glucose administration, at least some of the improvement in metabolite levels could be related to rehydration. Furthermore, in acute insulin deficiency not complicated by dehydration (produced by the injection of anti-insulin serum) glycogen deposition in brain increased 36 per cent at six hours and 63 per cent at 12-24 hours.

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Reports of the effects of insulin on brain electrolyte metabolism are also inconclusive. Ellison et al.[15] studied the changes in K^+ in rat brain during insulin hypoglycemia; based on wet and ash weights, values were unchanged; on a dry-weight basis, potassium increased 4.8 per cent. Sloviter and Yamada[16] could demonstrate no effect of insulin on the efflux of K^+ from the isolated perfused rat brain preparation. Recently, Arieff et al.[17] reported findings that suggest that insulin has a primary effect on the transport of K^+ from the blood into the brain; there was evidence of an effect on brain Na^+ transport as well. The authors propose that the increase in brain electrolyte content increased brain osmolality, which in turn increased the water content of brain, producing cerebral edema. Since the findings could explain the unsolved mechanism of seizures and coma in insulin-induced hypoglycemia, it seemed most important to see whether insulin increased the K+ content of the brain in another species of mammal.

PROCEDURE

Preparation of animals. Six litters of nursing white 20-22-day-old mice were used (total 48 animals). Animals weighed from 10.7 to 18.1 gm.; the average weight of 37 mice was 13.5 ± 0.3 gm. (mean ± S.E.M.). Animals of this age were used because of our clinical interests and previous biochemical experience. Animals were removed from their mothers and other sources of food at the start of all experiments. Water was allowed ad libitum. Experimental mice were injected subcutaneously with 40 U./kg. of crystalline zinc insulin (Iletin, Eli Lilly & Co., Indianapolis, Indiana), in a volume of 10 ml./kg. of normal saline; control littermates received an equal volume of normal saline. At 30 and 120 minutes after insulin injection animals were killed by decapitation. For cerebral metabolite measurements the amputated head was allowed to drop directly into liquid N_2, which was agitated constantly until the specimen fell to the bottom of the Dewar flask. Immediately after decapitation blood was collected from the severed neck vessels in heparinized micro blood-collecting tubes.

The effect of insulin on brain electrolyte and water content in two litters of normal adult mice was also studied. The mice were 62 days old and weighed 31 ± 1 gm. (mean ± S.E.M.) (range, 25-40 gm., n = 21). The dose of insulin, the route of administration, and other details of the experimental procedure were identical to those used in the younger animals.

Preparation of plasma and brain tissue. Blood was centrifuged promptly in a cold room at 4°. Fresh plasma was used for electrolyte determinations. For glucose and lactate assays plasma was deproteinized with 10-20 volumes of 0.5 M perchloric acid (PCA). It was not necessary to neutralize the PCA extracts since the addition of aliquots in the volume required for measurement of these metabolites did not change the pH of the reagent buffer or affect the complete recovery of the standards.

Fresh brain (excluding the cerebellum) was dissected free of meninges and weighed to 0.01 mg. on a microbalance at room temperature.

Frozen brain was dissected from the head (previously stored at −80°) with sharp chisels in a cryostat at −35°. Tissue samples were placed in screw-cap Pyrex culture tubes surrounded by dry ice (in the cryostat) and then dropped into liquid N_2. The brain samples were powdered and weighed in a cold room at −22°. Tissue extracts were prepared according to the procedure of Lowry et al.[18] and were stored at −80° until the time of assay.

Analytic methods. The water content of brain was calculated from the difference in the wet and dry weights (brain was dried to constant weight at 100°). Dried brain was then digested for 24 hours with 0.75 N HNO_3;[19] Na^+ and K^+ concentrations were determined in aliquots of the digest and in plasma with a flame photometer.

All metabolites in plasma and brain were measured in a Farrand fluorometer by specific microenzymatic technics. ATP, P-creatine, glucose, glucose-6-phosphate, fructose diphosphate, pyruvate, lactate, glutamate, and aspartate were measured as described by Lowry and Passonneau.[20] Glycogen was measured by the method of Passonneau and Lauderdale[21] (enzymatic hydrolysis with amylo-alpha-1,4-alpha-1,6-glucosidase followed again by the enzymic measurement of glucose).

RESULTS

Insulin injection in young mice. During the first 30 minutes or so after insulin injection there were no apparent effects of insulin administration in the weanling mice—certainly subjective symptoms and subtle differences in behavior from the controls were not ruled out. Beginning at about 50 minutes the mice began to exhibit various degrees of grossly abnormal behavior—drowsiness, unresponsiveness, intermittent myoclonic jerks, and splaying of the extremities.

Thirty minutes after insulin injection there were

TABLE 1

Effect of insulin on plasma glucose and electrolytes in young mice

Treatment	Glucose	Na^+	K^+
	mM	mEq./L.	
Control	8.19 ± 0.43 (10)*	139 ± 0.5 (17)	7.2 ± 0.3 (17)
Insulin 30 min.	3.33 ± 0.17 (5)†	139 ± 1.0 (9)	5.2 ± 0.2 (9)†
Insulin 2 hr.	1.01 ± 0.21 (6)†	136 ± 1.1 (10)‡	6.0 ± 0.4 (9)‡

In this table and those that follow, all values are given as the mean ± S.E.M.
*Number of animals is given in parentheses.
†p vs. control < 0.001.
‡p vs. control = 0.03.

significant reductions in plasma glucose (59 per cent) and K^+ (28 per cent) concentrations (table 1). Despite this unequivocal evidence of insulin action, there was no change in the water, Na^+, or K^+ content of the brains of these animals (table 2).

Two hours after insulin injection plasma glucose levels were only 12 per cent of those of controls; plasma K^+ concentration had risen slightly but was still significantly less than controls' (17 per cent lower) (table 1). A small but statistically significant drop (2.0 per cent) in plasma Na^+ concentration was also seen. At this time brain water content increased 0.74 per cent on a wet-weight basis and 3.8 per cent on a dry-weight basis; brain K^+ concentration was reduced 2.0 per cent and brain Na^+ concentration was unchanged (table 2).

The cerebral metabolic rate of 20-22-day-old mice has not previously been reported. In 10-day-old mice the cerebral metabolic rate (in terms of high-energy phosphate) is only one half of the adult rate, 13 vs. 25 mmol./kg./min.[18] Therefore, although it seems unlikely, it is possible that the supply of glucose to the brain in these young animals was sufficient to meet the metabolic requirement for age despite the reduced plasma glucose concentration. In view of this possibility, the effect of insulin on brain carbohydrate and energy metabolism in weanling mice was investigated (figure 1). Brain glucose values were down to 15 per cent of controls' at 30 minutes and were only 5 per cent of controls' at 120 minutes. The fall in brain glucose was accompanied by a decrease in glycogen and in all of the glycolytic intermediates from glucose to lactate. With the exception of glucose and glucose-6-phosphate, the levels of these metabolites were significantly lower at two hours than at 30 minutes after insulin injection. Brain aspartate levels increased progressively; glutamate levels were unchanged at 30 minutes but decreased significantly at 120 minutes after insulin injection. Despite the drastic reduction of brain glucose, levels of the high-energy phosphate-containing compounds ATP and P-creatine were unchanged. In fact, at two hours, P-creatine was significantly elevated above the control.

Although the data do not permit a calculation of the cerebral metabolic rate, the metabolite concentration profile in figure 1 is almost identical to that seen in adult mice in response to insulin hypoglycemia.[22-25] The pathophysiology of the paradox of a low energy supply and a high energy reserve in these animals is now fairly well established. Duffy et al.[27] found that in severe hypoxia cerebral metabolism was reduced in the face of high levels of ATP and postulated that a similar mechanism might pertain in insulin hypoglycemia. Ferrendelli and Chang[24] confirmed this hypothesis; in adult insulin-induced hypoglycemic mice the cerebral high-energy phosphate use rate was reduced 40-60 per cent. Later studies from their laboratory[25] showed relatively greater reduction of fructose diphosphate than

TABLE 2

Effect of insulin on brain water and electrolytes in young mice

Treatment	Water		Na^+	K^+
	per cent	gm. H_2O/100 gm. dry	mEq./kg. dry	
Control (17)*	80.26 ± 0.04	406.7 ± 1.1	253 ± 2	493 ± 2
Insulin 30 min. (9)	80.31 ± 0.07	407.9 ± 1.7	254 ± 2	489 ± 3
Insulin 2 hr. (10)	80.85 ± 0.10†	422.3 ± 2.8†	250 ± 2	483 ± 3‡

*Number of animals is given in parentheses.
†p vs. control < 0.001.
‡p vs. control < 0.01.

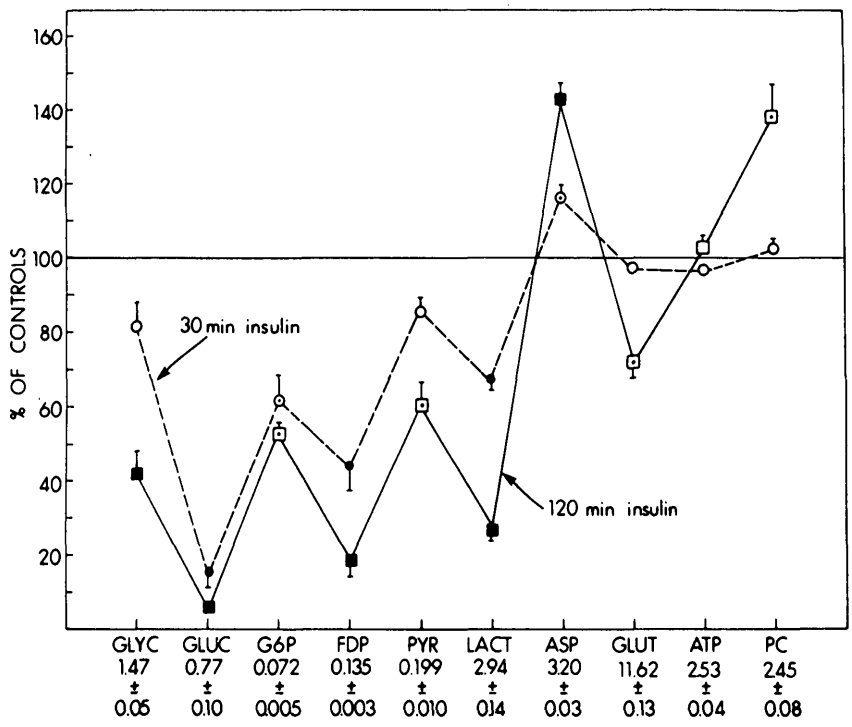

FIGURE 1
Percentage changes in 10 metabolites in mouse brain at 30 and 120 minutes after insulin. The abbreviations used are GLYC, glycogen; GLUC, glucose; G6P, glucose-6-phosphate; FDP, fructose diphosphate; PYR, pyruvate; LACT, lactate; ASP, aspartate; GLUT, glutamate; and PC, P-creatine. The control levels are given below each metabolite in mmol./kg. wet weight (mean ± S.E.M.). The results represent the findings in 11 brains—four controls, four 30 minutes postinsulin, and three 120 minutes postinsulin. The vertical line through each symbol has a length equal to ± S.E.M. Where there is no line, the symbol used has a radius ≥ the S.E.M. Values marked with a filled symbol are significantly changed from control values, $p \leq 0.001$. Open symbols with a central dot represent values significantly changed from control, $p = 0.002$-0.01. Values marked with an open symbol are not significantly different from the control, $p > 0.05$.

glucose-6-phosphate levels, a hallmark of inhibition of glycolysis at the phosphofructokinase step. Similar changes were observed in the present study.

Insulin injection in adult mice. Since the absence of an effect of insulin on brain electrolyte transport in the 20-22-day-old mice (as against adult rabbits) could be related to incomplete development of the brain in the immature mice, the study was repeated using adult animals (see Procedure, preparation of animals).

Whereas young mice showed no effect of insulin 30 minutes after injection, adult animals were visibly affected well before this time. Compared with controls there was obvious blanching of the tails and ears, with decreased vascular markings. Another difference was that all of the adults exhibited abnormal neurologic signs—decreased spontaneous activity and/or ataxia—soon after injection. Beginning at 45 minutes, five of the seven adult mice developed repeated violent tonic-clonic seizure phenomena; one died in status epilepticus 84 minutes after insulin injection.

The effect of insulin injection on plasma glucose and electrolyte concentrations in adult mice (table 3) was similar to that seen in the younger animals (table 1).

In normal adult mouse brain, Na^+ concentration was 5.5 per cent lower ($p < 0.001$) and brain water content 1.5 per cent lower ($p < 0.001$) than in the brains of the weanling animals (table 4). Again, as in the younger animals, there was no change in brain water content 30 minutes after insulin and a smaller increase (0.5 per cent of wet weight and 2.2 per cent of dry weight) at 120 minutes. With one exception, brain Na^+ and K^+ concentrations did not differ from controls' at any time after insulin injection. In the

TABLE 3

Effect of insulin on plasma glucose and electrolytes in adult mice

Treatment	Glucose	Na^+	K^+
	mM	mEq./L.	
Control (5)*	10.13 ± 0.25	140 ± 0.5	7.8 ± 0.4
Insulin 30 min. (5)	2.57 ± 0.38†	140 ± 1.9	5.4 ± 0.2†
Control (5)	10.49 ± 0.48	141 ± 1.3	7.6 ± 0.7
Insulin 2 hr. (5)‡	0.99 ± 0.19†	143 ± 2.8	7.1 ± 0.6

*Number of animals is given in parentheses.
†p vs. control < 0.001.
‡One mouse was not included in these values (see text - Insulin injection in adult mice).

TABLE 4

Effect of insulin on brain water and electrolytes in adult mice

Treatment	Water		Na^+	K^+
	per cent	gm. H_2O/100 gm. dry	mEq./kg. dry	
Control (5)*	78.94 ± 0.05	374.8 ± 1.2	228 ± 3	489 ± 3
Insulin 30 min. (5)	79.16 ± 0.12	379.9 ± 2.6	227 ± 2	489 ± 3
Control (5)	78.54 ± 0.06	365.9 ± 1.3	229 ± 1	485 ± 2
Insulin 2 hr. (5)‡	78.90 ± 0.06†	373.9 ± 1.4†	225 ± 1	485 ± 2

*Number of animals is given in parentheses.
†p vs. control = 0.003.
‡One mouse was not included in these values (see text - Insulin injection in adult mice).

exceptional case, one of the 120-minute-postinsulin group of animals (not included in tables 3 and 4) brain Na^+ concentration (335 mEq./kg. dry) was 55 per cent higher than the control value, and brain K^+ content (456 mEq./kg. dry) 6 per cent lower. Brain water content was 81.2 per cent, 3.4 per cent higher than the control and seven times the increase seen in the other animals 120 minutes after insulin injection. Whether these extraordinary electrolyte changes are related to hypoglycemia or cerebral anoxia cannot be said (see Discussion).

DISCUSSION

In normal young mice classic evidence of insulin action (severe lowering of plasma and brain glucose content, decrease in plasma K^+ concentration, and progressive neurologic dysfunction) was not associated with increased K^+ and Na^+ transport from the blood into the brain at 30 or 120 minutes after injection. Similar negative results were seen in adult mice. This finding is very different from that observed in rabbit.[17] Thirty-five minutes after injection of insulin (50 U./kg. i.v.) brain K^+ concentration increased 7 per cent in fasted, anesthetized, mechanically ventilated rabbits. At 146 minutes after insulin, brain K^+ was still elevated and there was an increase of 7 per cent in Na^+ as well. In the rabbits the increase in brain electrolyte concentration after insulin injection was accompanied by a 6 per cent increase in water content (dry weight) at both time intervals (35 and 146 minutes after insulin). By contrast, in both young and adult mice, the water content of brain was unchanged at 30 minutes after insulin, and the increase seen at 120 minutes in the 20-22-day-old mice was, in fact, accompanied by a significant drop in brain K^+ concentration, not an increase.

After completion of this study, Astrup and Norberg[27] reported on K^+ activity in the cerebral cortex of fasted, anesthetized, mechanically ventilated adult rats during progressive severe insulin-induced hypoglycemia. The dose of insulin was also 40 U./kg. given i.p. No significant changes in brain extracellular K^+ were seen during the shift in the EEG from normal to high-amplitude slow wave activity (normal reserve of brain high-energy phosphate compounds, ATP, ADP, and P-creatine[28]). However, about two minutes after onset of isoelectric EEG (150-224 minutes), (depleted high-energy phosphate reserves[28]) there was a release of cellular K^+ to the extracellular space as shown by an increase of extracellular K^+ from 4 to 50 mmol./L. The increased K^+ release from the cells was not due to anoxia (which produced similar changes). Injection of glucose restored extracellular K^+ to normal within three minutes of injection. Brain tissue K^+ concentration was not measured in this study, but it seems likely that brain intracellular potassium was reduced by an equivalent amount. The data recall the findings in the solitary adult mouse in which brain K^+ concentration fell 30 mEq./L. and brain Na^+ increased 85 mEq./L. 120 minutes after insulin injection. However, since the mice were not mechanically ventilated, anoxia cannot be ruled out as an explanation for these electrolyte changes.

The present study does not offer an explanation for disturbed neurologic function in hypoglycemic mice. Although insulin did not increase brain K^+ content at any time after injection, at 120 minutes brain water concentration increased 3.8 per cent (dry weight) in the young animals and 2.2 per cent in the adults. Since all of the mice exhibited a varied degree of overt neurologic disturbance at this time, it is possible that the increase in cerebral water content contributed to the neurologic abnormality. The mechanism for the increased water content in normal mice 120 minutes after insulin is not apparent. However, it is clearly not due to a direct effect of insulin on K^+ and Na^+ transport in mouse brain. These results emphasize the importance of species and procedural differences and suggest caution in extrapolating the findings to man.

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REFERENCES

[1]Rafaelson, O.J.: Action of insulin on glucose uptake of rat brain slices and isolated rat cerebellum. J. Neurochem. 7:45-51, 1961.

[2]Prasannan, K.G.: Effect of insulin on glucose metabolism in cerebral cortex slices under aerobic and anaerobic conditions. J. Neurochem. *19*:1825-28, 1972.

[3]Flock, E.V., Tyce, G.M., and Owen, C.A., Jr.: Regulatory effects of insulin and liver on brain glucose metabolism. Endocrinology *84*:392-406, 1969.

[4]Konitzer, K., Solle, M., and Voigt, S.: Wirkung von Insulin auf den Hirnstoffwechsel. I. Veränderungen einiger Phosphor-und Stickstoff-metaboliten des Rattenhirns in vivo nach Insulinapplikation. Acta Biol. Med. German. *15*:461-79, 1965.

[5]Nelson, S.R., Schulz, D.W., Passonneau, J.V., and Lowry, O.H.: Control of glycogen levels in brain. J. Neurochem. *15*:1271-79, 1968.

[6]Passonneau, J.V., Brunner, E.A., Molstad, C., and Passonneau, R.: The effects of altered endocrine states and of ether anaesthesia on mouse brain. J. Neurochem. *18*:2317-28, 1971.

[7]Park, C.R., Johnson, L.H., Wright, J.H., and Batsel, H.: Effect of insulin on transport of several hexoses and pentoses into cells of muscle and brain. Am. J. Physiol. *191*:13-18, 1957.

[8]Buschiazzo, P.M., Terrell, E.B., and Regen, D.M.: Sugar transport across the blood-brain barrier. Am. J. Physiol. *219*:1505-13, 1970.

[9]Betz, A.L., Gilboe, D.D., Yudilevich, D.L., and Drewes, L.R.: Kinetics of unidirectional glucose transport into the isolated dog brain. Am. J. Physiol. *225*:586-92, 1973.

[10]Prasannan, K.G., and Subrahmanyam, K.: Effect of insulin on the glycogen synthesis in vivo in the brain and liver of rats under different conditions. Indian J. Med. Res. *53*:1003-09, 1965.

[11]Held, K., Walpurger, G., and Gottstein, U.: Der cerebrale Glycogengehalt unter dem Einfluss von Glucose-und Insulin-Injektionen. Klin. Wochenschr. *46*:15-18, 1968.

[12]Goldberg, N.D., and O'Toole, A.G.: The properties of glycogen synthetase and regulation of glycogen biosynthesis in rat brain. J. Biol. Chem. *244*:3053-61, 1969.

[13]Strang, R.H.C., and Bachelard, H.S.: Effect of insulin on levels and turnover of intermediates of brain carbohydrate metabolism *in vivo*. J. Neurochem. *18*:1799-1807, 1971.

[14]Thurston, J.H., Hauhart, R.E., Jones, E.M., and Ater, J.L.: Effects of alloxan diabetes, anti-insulin serum diabetes, and non-diabetic dehydration on brain carbohydrate and energy metabolism in young mice. J. Biol. Chem. *250*:1751-58, 1975.

[15]Ellison, R.J., Wilson, W.P., and Weiss, E.B.: Changes in cerebral potassium during insulin hypoglycemia. Proc. Soc. Exp. Biol. Med. *98*:128-29, 1958.

[16]Sloviter, H.A., and Yamada, H.: Absence of direct action of insulin on metabolism of the isolated perfused rat brain. J. Neurochem. *18*:1269-74, 1971.

[17]Arieff, A.I., Doerner, T., Zelig, H., and Massry, S.G.: Mechanisms of seizures and coma in hypoglycemia. Evidence for a direct effect of insulin on electrolyte transport in brain. J. Clin. Invest. *54*:654-63, 1974.

[18]Lowry, O.H., Passonneau, J.V., Hasselberger, F.X., and Schulz, D.W.: Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J. Biol. Chem. *239*:18-30, 1964.

[19]Bradbury, M.W.B., and Kleeman, C.R.: Stability of the potassium content of cerebrospinal fluid and brain. Am. J. Physiol. *213*:519-28, 1967.

[20]Lowry, O.H., and Passonneau, J.V.: A Flexible System of Enzymatic Analysis. New York, Academic Press, 1972, p. 175.

[21]Passonneau, J.V., and Lauderdale, V.R.: A comparison of three methods of glycogen measurement in tissues. Anal. Biochem. *60*:405-12, 1974.

[22]King, L.J., Lowry, O.H., Passonneau, J.V., and Venson, V.: Effects of convulsants on energy reserves in the cerebral cortex. J. Neurochem. *14*:599-611, 1967.

[23]Goldberg, N.D., Passonneau, J.V., and Lowry, O.H.: Effects of changes in brain metabolism on the levels of citric acid cycle intermediates. J. Biol. Chem. *241*:3997-4003, 1966.

[24]Ferrendelli, J.A., and Chang, M.M.: Brain metabolism during hypoglycemia. Effect of insulin on regional central nervous system glucose and energy reserves in mice. Arch. Neurol. *28*:173-77, 1973.

[25]Gorell, J.M., Law, M.M., Lowry, O.H., and Ferrendelli, J.A.: Cerebral cortical metabolism during hypoglycemia and clinical recovery. Neurology *25*:360, 1975.

[26]Duffy, T.E., Nelson, S.R., and Lowry, O.H.: Cerebral carbohydrate metabolism during acute hypoxia and recovery. J. Neurochem. *19*:959-77, 1972.

[27]Astrup, J., and Norberg, K.: Potassium activity in cerebral cortex in rats during progressive severe hypoglycemia. Brain Res. *103*:418-23, 1976.

[28]Norberg, K., and Siesjo, B.K.: Oxidative metabolism of the cerebral cortex of the rat in severe insulin-induced hypoglycemia. J. Neurochem. *26*:345-52, 1976.