10. Eadie, G. S., Brown, I. W., Jr., *ibid.*, 1953, v8, 1110.

11. Dornhorst, A. C., *ibid.*, 1951, v6, 1284.

12. Eadie, G. S., Brown, I. W., Jr., *J. Clin. Invest.*, 1955, v34, 629.

Received March 3, 1958. P.S.E.B.M., 1958, v98.

Changes in Cerebral Potassium During Insulin Hypoglycemia.* (23962)

R. J. ELLISON, W. P. WILSON, AND E. B. WEISS
(Introduced by J. W. Everett)
Department of Psychiatry, Duke University School of Medicine, and Vet. Admin. Hospital, Durham, N. C.

Little is known about metabolism of cerebral potassium during insulin hypoglycemia. Most recently La Fran *et al.*(1) have postulated that electroencephalographic changes and alterations of consciousness observed in both acute and prolonged insulin coma are in part due to shifts in electrolytes and water within the brain. They have postulated a decrease in intracellular potassium as a result of the deposition of brain glycogen. This results in a distortion of the intra- and extracellular relationship of potassium and sodium giving rise to extracellular edema. Several investigations are not in accord with this hypothesis, since it has been shown that there is an increase in intracellular potassium in liver, muscle, and erythrocytes during hypoglycemia(2-6). Further, Himwich has demonstrated a drop in cerebral glycogen content during hypoglycemia(7). Finally, *in vitro* and *in vivo* studies of the effect of insulin on cerebral metabolism have not demonstrated increased glucose utilization(8-10). These studies would tend to refute the arguments offered by La Fran *et al.*(1) to support their hypothesis, and would indicate that the changes observed electroencephalographically and clinically are primary or secondary results of a lowered level of metabolic substrate. This study was undertaken in an effort to clarify if possible, the changes that might be observed in the total brain potassium following a marked lowering of the blood sugar.

Method. 12 adult white male rats of the Osborne-Mendel strain were given 50 units of 80 units/cc crystalline regular insulin intravenously in a tail vein. The rats were then sacrificed by decapitation at 1½ to 5½ hours after injection, and in varying depths of hypoglycemic reaction ranging from stupor to coma. The whole brain including cerebellum was then analyzed for potassium by modification of the method of Elliott and Jasper(11). Following determination of wet and dry weights, samples were ashed in an electric furnace at 650°C for 4 to 5 hours and remained overnight at 350° to 400°C(11). The ashed weight was determined. The samples were then dissolved in 10 cc of concentrated hydrochloric acid, diluted to 100 cc with distilled water. Potassium was determined using an internal standard flame photometer (12). 12 control rats were lightly anesthetized with ether, decapitated, and the above procedure was carried out.

Results. In comparing brains obtained from experimental and control groups, there appeared to be no observable edema in either group; on close inspection, however, it was noted that in the insulin-treated group the brains appeared to be more friable, hyperemic and glistening. Also, the meninges were more adherent to the surface of the cortex.

There was no statistically significant increase in water content of brains of hypoglycemic rats. The values obtained were: controls 78.16%, hypoglycemic 78.38% ($p = > .50$).

Comparison of the amount of potassium on the basis of wet brain showed no statistically significant difference. The values were: control 92.89 meq. potassium/kg, hypoglycemic

* This work was supported by grant from Nat. Inst. of Mental Health.

TABLE I. Cerebral Potassium Levels during Hypoglycemia.

	Mean	S.D.	t	p
meq K/kg				
Wet wt control	92.89	6.11	1.07	.30
Insulin	95.45	3.08		
Dry wt control	425.8	25.0	2.19	<.05
Insulin	446.2	9.0		
Ash wt control	6158.0	719.0	2.04	>.05
Insulin	6726.0	344.0		
% water				
Control	78.16	.55	.37	>.50
Insulin	78.38	1.67		

95.45 meq. potassium/kg ($p = .30$).

In contrast to the above negative findings there was a statistically significant increase in dry grain potassium. These findings were: controls 425.8 meq. potassium/kg, hypoglycemic 446.2 meq. potassium/kg ($p =< 0.05$). The ash weight values showed a neat statistically significant increase ($p =>0.05$). These values were: controls 6158.0 meq. potassium/kg, hypoglycemic 6726.0 meq. potassium/kg.

Discussion. The data show a statistically significant increase in dry brain potassium. Although values for potassium in the ashed samples were not significant, it is possible that the combination of a near significant value for ashed brain plus a small increase in water content combined to give a significant dry weight value. The distribution of potassium in the brain is not known, but it may be assumed that the increase in cerebral potassium is on the basis of increase in intracellular potassium, since the intracellular values have been estimated to be approximately 99 times those of the extracellular fluid(13). It is obvious that there would have to be a large increase in the extracellular potassium to account for these changes.

Previous investigators have demonstrated, *in vitro,* promotion of glycogenesis by potassium. This glycogenesis *and* metabolism of glucose is facilitated by insulin. The knowledge that 0.36 meq. of potassium is bound with each gram of glycogen and is released by glycogenolysis would explain, at least in part, the transport of potassium into an intracellular position in the brain(14). However, to date, no evidence, either *in vitro* or *in vivo* has been accumulated to demonstrate a facilitation of cerebral glucose metabolism by insulin(12-13).

Finally, it must be pointed out that the results of this study raise the question of the influence of insulin on cerebral glucose utilization. The significant increase in potassium seen here is similar to that produced in other tissues when glucose metabolism is stimulated by insulin. In view of the fact that most studies of insulin coma have been done in the hypoglycemic state it would seem necessary to study the effects of insulin on glucose metabolism of the brain where an adequate supply of substrate was available.

Conclusions. 1. A significant increase in cerebral potassium levels of dry brain was demonstrated in insulin-treated rats. 2. Hypoglycemia had no significant effect on water content of brains. 3. The importance of this finding as related to the present concept of cerebral glucose metabolism is discussed.

1. La Fran, R., Billet, M., Billet, B., *Montpellier Med.,* 1954, v46, 292.

2. Fenn, W. O., *J. Biol. Chem.,* 1939, v128, 297.

3. Overman, R. R., *Physiol. Rev.,* 1951, v31, 285.

4. Danonski, T. S., *J. Biol. Chem.,* 1941, v139, 693.

5. Flock, E., Bollman, J. L., Mann, F. C., Kendall, E. C., *ibid.,* 1938, v125, 57.

6. Himwich, H. E., *A. Research Nerv. and Ment. Dis., Proc.,* 1954, v32, 345.

7. ———, *Brain Metabolism and Cerebral Disorders,* 167, Baltimore, Md., Williams and Wilkins Co., 1951.

8. Kety, S. S., Lubens, F. D. W., Woodford, R. B., Freyhan, F. A., Schmidt, C. F., *Fed. Proc.,* 1948, v7, 64.

9. Elliott, K. A. C., Libet, B., *J. Biol. Chem.,* 1942, v143, 227.

10. Elliott, K. A. C., Scott, D. B., Libet, B., *ibid.,* 1942, v146, 251.

11. Elliott, K. A. C., Jasper, H. H., *Am. J. Physiol.,* 1949, v157, 122.

12. White, J. V., *Anal. Chem.,* 1952, v24, 394.

13. Katzman, R., Leidermann, P. H., *Am. J. Physiol.,* 1953, v175, 263.

14. Walker, B. S., Boyd, W. C., Asimov, I., *Biochemistry and Human Metabolism.* p504, Baltimore, Md., Williams and Wilkins Co., 1954.

Received March 10, 1958. P.S.E.B.M., 1958, v98.