# Regional Localization of Cerebral Edema Following Fluid and Insulin Therapy in Streptozotocin-Diabetic Rats

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**SUMMARY**

**To evaluate the location and early time course for development of cerebral edema following therapy for diabetes, streptozotocin-diabetic rats were subjected to constant i.v. infusion with saline and regular insulin. At the end of 1, 2, and 5 h of therapy, these rats were killed and tested for density of the cerebral cortex, subcortical white matter, caudate-putamen, thalamus, and medulla. Density data from treated animals were compared with those from control animals. From these data, change in brain tissue volume as water was calculated.**

**Diabetic animals killed after 1 h of fluid and insulin therapy demonstrated a modest increase in brain water content of all areas tested except white matter. Following 2 h of therapy, all regions tested were edematous, with a magnitude of edema that was similar to that seen at 1 h. After 5 h of therapy, there was further increase in water content of the cerebral cortex, but not the other regions examined. Treatment with saline alone did not result in central overhydration.**

**The findings of this study suggest that aggressive therapy with fluids and insulin, but not fluid alone, results in global overhydration of the brains of diabetic animals. Prolonged fluid and insulin treatment with a return of blood glucose to normal values causes further and preferential accumulation of edema fluid in the cerebral cortex. DIABETES 30:762–766, September 1981.**

In 1936, Dillon et al. reported deaths in eight juvenile diabetics who underwent treatment for "uncomplicated" diabetic ketoacidosis.[1] Autopsy material from these cases revealed gross and light microscopic evidence of cerebral edema. Since that time, a number of reports have been published that describe young juvenile diabetics who show initial improvement chemically and clinically after fluid and insulin therapy, only to lapse into coma and die as the result of uncontrolled cerebral edema.[2–6] In spite of a number of provocative theories concerning the fundamental mechanisms associated with the development of cerebral edema resulting from treatment for diabetes, many of the fundamental characteristics of this form of brain swelling remain to be determined.

The present investigation focuses on the early (1–5 h) effects of fluid and insulin therapy on brain water content in streptozotocin-diabetic rats. The brains of these animals were tested for regional changes in brain water content, as measured by change in brain density from control values, to localize fluid accumulation with this treatment.

## MATERIALS AND METHODS

Adult male Sprague-Dawley rats, 300–390 g in weight, were injected intraperitoneally with freshly prepared streptozotocin in a sodium citrate buffer, pH 4.5,[7] at a dose of 75 mg/kg Age- and sex-matched rats were injected with the buffer only to serve as vehicle control animals.

One and two weeks after injection, all animals were tested for whole blood glucose (Dextrostix read with an Ames Reflectance Meter, Ames Company, Elkhart, Indiana) and plasma ketones (Ketostix), using blood expressed from a clipped tail vein. Streptozotocin-injected animals were selected for study on the basis of positive plasma ketones and blood glucose values in excess of 400 mg/dl at both 1 and 2 wk following injection.

**Untreated (time zero) diabetic and vehicle control rats.**

Two weeks (14 ± 1 days) following injection of streptozotocin or vehicle, selected animals were studied as follows: To evaluate central and peripheral characteristics of diabetic animals before fluid and insulin therapy (at time zero), one group of diabetic and one group of vehicle control rats were tested for whole blood glucose (Dextrostix), plasma ketones (Ketostix), and whole blood osmolality (vapor pressure osmometer), using tail vein blood. Each animal was then anesthetized with sodium pentobarbital (25 mg/kg, i.p.) and immediately killed for measurement of brain density.

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To evaluate magnitude of ketosis for diabetic animals used in this study, β-hydroxybutyrate values were measured in separate groups of vehicle and diabetic animals. These animals were tested for blood parameters, using tail vein blood, followed by removal of 1 cc of blood by cardiac puncture. The cardiac blood was centrifuged and the serum measured for β-hydroxybutyrate by an enzymatic-spectrophotometric method.[8]

**Diabetic rats infused with saline and insulin.** Three groups of diabetic rats were tested for whole blood glucose and osmolality and for plasma ketones (Ketostix). They were then anesthetized with sodium pentobarbital (25 mg/kg, i.p.) and subjected to continuous i.v. infusion via an exposed femoral vein, using a 27-gauge Butterfly Infusion Set, 6-cc syringe, and a Sage Infusion Pump. For all groups, the infusion fluid contained 0.45% aqueous sodium chloride (half normal saline) and regular insulin (Iletin, Eli Lilly Co, Indianapolis, Indiana). The rate and time of infusion and concentrations of constituents were adjusted individually to provide: (1) for one group (1-h infusion), 30 U/kg/h regular insulin plus 20 cc/kg/h half normal saline, administered for 1 h; (2) for one group (2-h infusion), dosage as above, administered for 2 h; (3) for one group (5-h infusion), 30 U/kg/h regular insulin plus 20 cc/kg/h half normal saline, administered for 2 h, followed by 15 U/kg/h regular insulin plus 8 cc/kg/h half normal saline for 3 additional hours.

**Control animals for infusion studies:** Control groups for the infused diabetic animals included: (1) 3 groups of vehicle control animals that were anesthetized, without infusion, for 1, 2, or 5 h; (2) 1 group of vehicle control rats anesthetized and infused with half normal saline (fluid only) for 5 h at a dose of 20 cc/kg/h for 2 h, followed by 8 cc/kg/h for 3 additional h; and (3) 1 group of diabetic animals anesthetized and infused with half normal saline (fluid only) for 5 h, using the same saline dose as that described above.

All infused and timed-anesthetized rats were tested at hourly intervals from the time of initiation of infusion (or anesthesia) until the time they were killed for whole blood glucose and osmolality.

All animals were killed by immersion of the head in liquid nitrogen. For each rat, the frozen head was sliced midsagittally, using a band saw. One-half of each head was sectioned coronally into 5-mm slices for measurement of brain density. The other half was used for a separate study.

**Measurement of brain density.** Determination of change in brain density from control values has been shown to be an indirect but very precise method for quantitating change in brain water content.[9] In the present study, an organic density gradient was established in a graduated cylinder by partial mixing of kerosene and bromobenzene.[10] The gradient was calibrated with aqueous sodium chloride standard solutions of known density.

Frozen brain slices were immersed in kerosene and permitted to thaw. Duplicate 2–5-mg samples of parietal cortex, caudate-putamen, dorsal thalamus, subcortical white matter, and medulla were dissected and dropped into a calibrated organic gradient to determine specific gravity.[11]

To determine changes in brain density from normal values in diabetic rats subjected to saline and insulin therapy, data from the treated animals were compared with those of anesthetized, untreated vehicle control rats. To determine the effect of fluid therapy alone on vehicle control and diabetic animals, data from the saline-treated groups were compared with those untreated control and untreated diabetic rats, respectively. To determine the effect of the insulin component of therapy on brain density in diabetic rats, density data from diabetic animals treated with fluid and insulin were compared with those from diabetic rats subjected to fluid therapy only.

To compare data obtained from density measurements with those resulting from other procedures, change in brain density of experimental groups was converted to change in tissue volume as water, using the Nelson equation.[9]

Percent change in tissue volume as water

$$= \frac{(SG - 1)\,con - (SG - 1)\,exp}{(SG - 1)\,exp}$$

where SG = specific gravity, con = control tissue, and

TABLE 1
Body weight, blood values, and regional drain density in unifused rats 2 wk following streptozotocin or vehicle injection

| Factor | Tested for β-hydroxybutyrate | | Tested for brain density | |
|---|---|---|---|---|
| | Vehicle control (N = 6) | Diabetic* (N = 12) | Vehicle control (N = 7) | Diabetic* (N = 7) |
| Total body wt (g) | 392 ±9 | 309† ±11 | 421 ±10 | 307† ±14 |
| Whole blood glucose (mg/dl) | 84 ±3 | 451† ±22 | 85 ±5 | 482† ±26 |
| Whole blood osmolality (mosm/kg) | 296 ±1 | 320† ±2 | 296 ±1 | 316† ±3 |
| Plasma ketones (Ketostix) | neg. | +1 | neg. | +1 to +2 |
| Serum β-hydroxybutyrate (mM) | 0.47 ±0.06 | 4.92† ±0.33 | — | — |

Values reported (except Ketostix) are means ± SEM.
* Selected for positive plasma ketones with Ketostix and for blood glucose values in excess of 400 mg/dl.
† Significantly different from values of matched vehicle controls, $P < 0.01$.

TABLE 2
Regional brain density in anesthetized and/or infused vehicle control and diabetic rats

| Group | N | Duration of anesthesia (h) | Area tested for specific gravity: Parietal cortex | White matter | Caudate-putamen | Dorsal thalamus | Medulla |
|---|---|---|---|---|---|---|---|
| Uninfused vehicle control | 7 | 0 | 1.0504 ± 0.0002 | 1.0502 ± 0.0003 | 1.0505 ± 0.0003 | 1.0511 ± 0.0003 | 1.0492 ± 0.0002 |
| Uninfused diabetic | 7 | 0 | 1.0518* ± 0.0003 | 1.0512 ± 0.0004 | 1.0514 ± 0.0004 | 1.0525* ± 0.0004 | 1.0509* ± 0.0003 |
| Uninfused vehicle control | 7 | 1 | 1.0503 ± 0.0002 | 1.0501 ± 0.0002 | 1.0505 ± 0.0003 | 1.0515 ± 0.0004 | 1.0494 ± 0.0002 |
| Uninfused vehicle control | 7 | 2 | 1.0506 ± 0.0003 | 1.0505 ± 0.0003 | 1.0507 ± 0.0005 | 1.0511 ± 0.0004 | 1.0498 ± 0.0003 |
| Uninfused vehicle control | 7 | 5 | 1.0506 ± 0.0003 | 1.0503 ± 0.0003 | 1.0501 ± 0.0003 | 1.0512 ± 0.0003 | 1.0492 ± 0.0002 |
| Infused diabetic (saline + insulin) | 7 | 1 | 1.0489* ± 0.0004 | 1.0488 ± 0.0004 | 1.0490* ± 0.0003 | 1.0497* ± 0.0003 | 1.0481* ± 0.0004 |
| Infused diabetic (saline + insulin) | 8 | 2 | 1.0488* ± 0.0003 | 1.0492* ± 0.0002 | 1.0488* ± 0.0005 | 1.0497* ± 0.0002 | 1.0480* ± 0.0004 |
| Infused diabetic (saline + insulin) | 8 | 5 | 1.0475*†‡ ± 0.0006 | 1.0483*‡ ± 0.0004. | 1.0486*‡ ± 0.0005 | 1.0500*‡ ± 0.0004 | 1.0477*‡ ± 0.0004 |
| Infused diabetic (saline only) | 7 | 5 | 1.0516 ± 0.0005 | 1.0511 ± 0.0004 | 1.0516 ± 0.0004 | 1.0521 ± 0.0004 | 1.0505 ± 0.0003 |
| Infused vehicle control (saline only) | 6 | 5 | 1.0494 ± 0.0004 | 1.0497 ± 0.0003 | 1.0491 ± 0.0004 | 1.0505 ± 0.0004 | 1.0481 ± 0.0004 |

Values reported are means ± SEM.
* Significantly different from values of matched, uninfused vehicle controls, $P < 0.05$.
† Significantly different from values of diabetic rats treated for 2 hr with saline and insulin, $P < 0.05$.
‡ Significantly different from values of diabetic animals treated with saline only, $P < 0.05$.

exp = experimental tissue. In using this equation, it is assumed that the specific gravity of brain solids remains constant during the experimental period.

The Student's *t* test was used to compare group data.

## RESULTS

**Untreated diabetic rats.** Two weeks following streptozotocin injection, selected diabetic rats had a significant decrease in total body weight and increases in whole blood osmolality and serum β-hydroxybutyrate as compared with values for matched vehicle control animals (Table 1).

Centrally, these animals demonstrated an increase in tissue density (decrease in water content) of the cerebral cortex, thalamus, and medulla (Table 2). Data from a separate study indicated that tissue density changes in the diabetic animals were not due to change in specific gravity of brain

TABLE 3
Percent change in brain tissue volume as water in diabetic rats

| Group | Area tested: Parietal cortex | White matter | Caudate-putamen | Dorsal thalamus | Medulla |
|---|---|---|---|---|---|
| Untreated diabetic* | −2.7% | −2.0% | −1.8% | −2.7% | −3.3% |
| 1-h Treated diabetic* (insulin + saline) | +2.9% | +2.7% | +3.1% | +3.6% | +2.7% |
| 2-h Treated diabetic* (insulin + saline) | +3.7% | +2.6% | +3.9% | +2.8% | +3.8% |
| 5-h Treated diabetic* (insulin + saline) | +6.5% | +4.1% | +3.1% | +2.4% | +3.1% |
| 5-h Treated diabetic† (insulin + saline) | +8.6% | +5.8% | +6.2% | +4.2% | +5.9% |

Values calculated from density data reported in Table 2, using the Nelson equation, with negative values representing a decrease in tissue volume and positive values representing an increase in tissue volume.
* Values based on comparison with data from matched, uninfused vehicle control groups.
† Values based on comparison with data from diabetic animals treated with saline only.

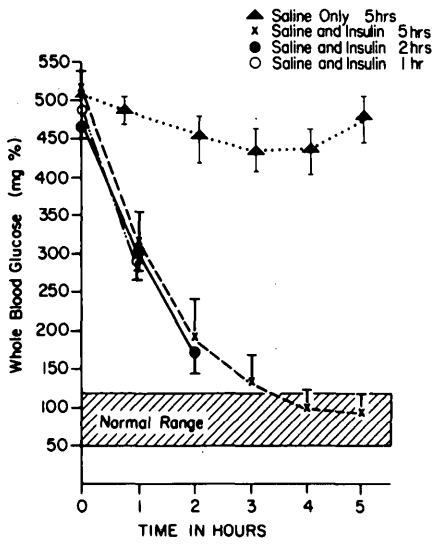



**FIGURE 1. Change in whole blood glucose in diabetic rats subjected to intravenous therapy consisting of saline only or of saline plus insulin.**

solids. Therefore, decrease in tissue volume was calculated and ranged from 1.8% to 3.3% for the 5 regions tested (Table 3).

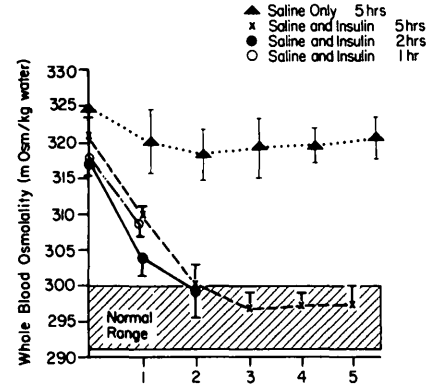
**Vehicle control animals subjected to anesthesia or saline infusion.** Density data collected from the brains of vehicle control groups subjected to 1, 2, and 5 h of pentobarbital anesthesia (Table 2) were similar to those from animals killed immediately after initiation of anesthesia, suggesting that prolonged pentobarbital anesthesia fails to alter brain density in normal animals.

Density data from vehicle control animals subjected to infusion with half normal saline were statistically similar to those of untreated control animals, although there was a trend toward slight increase in water content for all areas tested (Table 2).

**Diabetic animals infused with saline only.** With a 5-h infusion of half normal saline, diabetic rats showed a modest and transient disease in peripheral blood glucose, but not osmolality (Figures 1 and 2).

Centrally, the brain densities for all areas tested in these animals were similar statistically to those of untreated diabetic rats (Table 2).

**FIGURE 2. Change in whole blood osmolality in diabetic rats subjected to intravenous therapy consisting of saline only or of saline plus insulin.**




**Diabetic animals infused with saline and insulin.** Diabetic rats infused with saline and insulin for 1 h demonstrated a reduction in whole blood glucose and osmolality during therapy (Figures 1 and 2), but were hyperglycemic and hyperosmolar peripherally at the time of killing.

Centrally, these animals had a small but significant decrease in brain density (increase in brain water content) over values for untreated control rats in all areas tested except white matter (Table 2). The magnitude of edema, based on change in tissue volume, was rather similar for all areas tested and ranged from 2.7 to 3.6% (Table 3). In comparing density data of the treated rats with those of the untreated diabetic animals (at time zero), there was an increase in tissue volume of 4.7–6.3% for the individual areas tested during the first hour of infusion with saline and insulin.

Diabetic rats infused for 2 h demonstrated a continued decrease in whole blood osmolality and glucose during therapy, with a return of peripheral osmolality to near normal values by the time of killing (Figures 1 and 2).

Centrally, these animals had a significant decrease in brain density from control values for all regions tested (Table 2). Data from the individual areas examined were somewhat similar to those seen after a 1-h infusion with saline and insulin, with an increase in tissue volume over control values that ranged from 2.6 to 3.9% (Table 3).

Diabetic animals infused with saline and insulin for 5 h demonstrated a return to normal values for whole blood glucose and osmolality during the therapy (Figures 1 and 2).

Centrally, these animals had a decrease in density of the cerebral cortex that reflected a 6.5% increase in tissue volume over control values (Tables 2 and 3). Magnitude of edema for this region was significantly greater than that seen after 1- or 2-h infusions with saline and insulin. For the other areas tested, data at 5 h were statistically similar to those seen at 1 and 2 h.

Density data from the diabetic rats treated with saline and insulin differed considerably from those of the diabetic rats treated for a similar period of time with saline only. In comparing these data, the tissue volume of the cerebral cortex was 8.6% greater with therapy that included insulin. For the remaining areas tested, the increase in tissue volume with fluid and insulin therapy ranged from 4.2 to 5.9% over values for animals treated with saline only.

## DISCUSSION

The present study focuses on the early timing, location and magnitude of cerebral edema resulting from fluid and insulin therapy in streptozotocin-diabetic rats.

Concerning timing for development of cerebral edema with this model, an increase in water content over normal control values was detectable as early as 1 h after initiation of therapy. This finding was unexpected, particularly since data collected from untreated diabetic animals suggested that prior to therapy, several areas of the brain were dehydrated. These findings suggest that a very rapid central overyhydration occurs in the diabetic rat during fluid and insulin therapy.

Although the precise mechanism for central overhydration cannot be determined from this study, it appears that the insulin component of the therapy was an essential factor, as infusion with saline only failed to cause brain edema.

Arieff and Kleeman[12,13] have reported data that suggest that insulin enhances the transport of electrolytes into the central nervous system. If that is the case, insulin therapy may cause an addition of osmotically active particles to brain tissue that could cause an uptake of osmotic water. These same investigators,[12,13] as well as others,[14] have also shown that during peripheral dehydration, the central nervous system "adapts" by an accumulation of osmotically active particles. These osmols presumably attract osmotic water and tend to maintain the water content and volume of the brain at near-normal levels in spite of peripheral dehydration. A central excess of osmotically active particles during the dehydration prior to therapy coupled with additional osmols resulting from insulin treatment may represent the basis for a very rapid osmotic edema following fluid and insulin therapy in the diabetic rat.

Concerning location of cerebral edema, 5 areas of the brain were examined in the present study for change in brain water content: cerebral cortex, a superficial cell-rich or gray matter area; caudate-putamen and thalamus, deep gray matter areas with some myelinated fibers; medulla, an area of neuropile or mixture of cells and fibers; and white matter, a cell-poor area consisting largely of fibers and myelin. Data from these areas provided information concerning specific changes in widely-spaced areas with different cytoarchitecture and blood supply.

The location and magnitude of cerebral edema seen with the present model varied with the length of time of infusion. For the caudate-putamen, thalamus, and medulla, an increase in tissue volume over control values occurred that was of small magnitude (less than 5%) and was rather similar for all time periods examined (1, 2, and 5 h). These findings suggested a rather stable and modest overhydration over the periods examined that was somewhat similar for these three areas.

For the cerebral cortex, overhydration of a small magnitude was seen at 1 and 2 h, followed by a further increase in tissue volume, to 6.5% over normal control values, at 5 h following initiation of therapy. The increase in magnitude of cortical edema at 5 h had an interesting variation for the individual animals of this group. For five animals of the group, density data of the cerebral cortex was similar to that seen at 1 and 2 h. For three animals, there was an increase in tissue volume of greater than 9% over control values. These findings suggest a difference in sensitivity to therapy among individual animals of the group, in spite of rather close similarity in modeling and treatment.

The mechanism for further decompensation of the cerebral cortex, and not the other gray matter areas tested at 5 h, is unknown. Sensitivity of the cerebral cortex, however, has been suggested from the findings of a number of investigations[1,5,12,13] and may be related to the structural, metabolic, and/or vascular nonhomogeniety of the central nervous system.

The subcortical white matter had an absence of measurable edema at 1 h, followed by a minimal edema at the later periods examined. These findings suggest that fluid accumulates less quickly in the white matter than in the other, largely gray matter areas tested.

In the three animals of the 5-h group that had substantial edema of the cerebral cortex, however, there was further increase in the tissue volume of the white matter, to greater than 6% over control values. These findings suggest that the water content of the white matter in these animals increased in parallel with overhydration of the overlying cerebral cortex.

It is possible that at least a component of the edema seen with this model involves an interstitial accumulation of fluid that either develops independently within the white matter or runs off from the extracellular space of the adjacent cortex following cortical accumulation of fluid. Interstitial edema of the white matter has been demonstrated by electron microscopy following the development of osmotic brain edema resulting from peripheral injection of distilled water in the rat.[15] Structural examination for the precise location of edema fluid (extracellular or intracellular) resulting from therapy for diabetes has not been accomplished. This type of investigation could lead to important information concerning the basic characteristics of this form of cerebral swelling.

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