MEASUREMENT OF EXPERIMENTALLY INDUCED BRAIN SWELLING AND SHRINKAGE

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In studies of the mechanisms of cerebral edema and swelling a simple method for estimating the extent of changes in brain volume in experimental animals is needed. In the present paper it is shown that determinations of dry weight provide a simple method for estimating variations in brain tissue volume, and some observations on swelling and shrinkage are described.

MEASUREMENT OF CHANGE IN BRAIN VOLUME

White et al. (1, 2) have measured changes in brain volume in cats by careful determinations of the volume of the brain and of the cranial cavity. The normal difference between these two volumes is about 10 per cent and variation from this figure represents swelling or shrinkage of the brain. This method is difficult, and could hardly be applied after craniotomy. Assuming that changes in brain volume are due to changes in water content, and that the percentage of dry matter in the brains of normal animals is constant, then a difference from normal in the percentage dry weight of the brain of an experimental animal must be a measure of a change in volume. The swelling or shrinkage may be simply calculated without any need to determine the actual volume of the brain.

If $W$ and $D$ are respectively the fresh and dry weight of the brain of a normal animal and $P$ is the percentage dry weight, then $W = D \times \frac{100}{P}$. If, as a result of treatment, the weight and percentage dry weight change to $W_1$ and $P_1$, then $W_1 = D \times \frac{100}{P_1}$. Whence $W_1 = P \frac{P_1}{W} W$. The swelling or percentage change in weight (or volume, since the tissue density is about unity) is given by swelling percentage $= \frac{W_1 - W}{W} \times 100 = \frac{P - P_1}{P_1} \times 100$. It should be noted that presentation of results

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2 If the swelling or shrinkage is caused by the absorption or loss of fluid which itself contains some solid matter, then a larger change in volume would correspond to a given change in percentage dry weight. If $p$ is the percentage dry weight in the fluid absorbed or lost, it can be shown that

$W_1 = \frac{P - p}{p_1 - p} W$ and percent swelling $= \frac{P - P_1}{P_1 - p} \times 100$. 

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in terms of percentage moisture, as is commonly done, rather than dry weight, tends to give a misleading impression. If the normal moisture content is 80 per cent, a change to 81 per cent indicates a 5 per cent increase in weight since \( W_1 = \frac{20}{19} W = 105W. \)

**DETERMINATION OF DRY WEIGHT**

Rabbits were anesthetized with Nembutal and decapitated. The roof of the skull was removed and the cerebrum was removed from the skull by cutting through the mid-brain along the bony edges of the incisura of the tentorium. This method was adhered to carefully so that the parts of the brain treated, particularly with respect to the relative proportions of grey and white matter, were always the same. The cerebrum was halved midsagitally and the determination was carried out on one half, or on both halves separately as duplicates. All free fluid was carefully wiped out of the ventricles with filter paper. The whole hemisphere was then pushed into a tared weighing bottle with a helmet-type cover and containing a short sealed glass tube with a mace-like head (figure 1). This process was done rapidly, or in a humid chamber, since loss of moisture by evaporation could be appreciable. After determining the fresh weight of the hemisphere, 2 ml. of acetone were run into the bottle and the tissue was carefully reduced to a suspension by mashing with the 'mace'. The acetone was then evaporated away by directing a current of filtered air into the bottle. When the tissue could be spread as a paste around the sides of the bottle, it was placed in an oven at about 108° for 24 hours or more, cooled in a desiccator and weighed. Some care was necessary to insure that foaming of residual acetone did not cause loss of material when the temperature was first raised. After 24 hours, further loss in weight was negligible. Without the acetone treatment, complete drying took much longer. There was never any increase in weight, as has been reported for some fatty tissues on prolonged heating, following this method of acetone treatment.

The agreement between values obtained for the percentage dry weight of left and right hemispheres from the same animal was good. In 25 such pairs of determinations the widest difference was 0.3; usually the difference was much less. However, rather wide variations between individual animals were found (table 1), which could not be correlated with depth or period of anesthesia, weight, or method of killing (bleeding, decapitation, or constriction of the neck).
The average value for normal animals was 21.16 per cent with a standard deviation of the distribution equal to 0.5. In view of the range of variability of the normal animals, values for brains of individual experimental animals between 20.2 and 22.1 per cent cannot be regarded as significantly different from normal. That is to say, a change of less than 4 per cent in brain volume, calculated on the basis of the average normal percentage dry weight, cannot be reliably ascribed to the treatment of the animal.

While this work was in progress a paper by Windle et al. (3) appeared in which a similar method for determining brain dry weight was described. This method appears to be extremely accurate but somewhat more cumbersome than the present method. The tables of Windle et al. show that the dry weight content of normal guinea pig brains varied between 20.5 and 21.7 per cent, a range of variability approaching that found by us for rabbits. Windle et al. found a statistically significant increase of 0.5 per cent in the mean moisture content of brains from concussed animals and an increase of 0.7 per cent with animals which had been waterlogged by stomach tube. It was concluded that edema following concussion is significant but slight. Calculation shows that an 0.5 per cent increase indicates about 2.5 per cent swelling which is appreciable if the available space is only 10 per cent. In earlier work Pilcher (4, 5) attempted to detect changes of brain water in traumatized dog brains, by determinations of dry weight in various parts of the brain. His tables also show great variability; values were reported for the dry weight content of cerebral grey matter between 20.1 and 21.7 per cent in 5 normal animals and between 18.7 and 22.5 per cent in unexposed sides of unilaterally exposed brains. Average figures indicated only slight, though definite, increases in moisture content following trauma with the skull intact and none with the skull exposed.

The variability of the percentage dry weight of normal brain seriously limits the precision of determinations of swelling or shrinkage by the dry weight method. Donaldson (6, 7) in extensive studies on rats, has shown that the moisture content of the brain is affected in a regular manner by the age and size of the animal and by the size of its brain. Even with animals from the same age group there is rather wide variability (s.d. 0.2 to 0.5) but this variability is considerably less among litter mates (s.d. 0.13).

**EXPERIMENTALLY INDUCED CHANGES IN BRAIN VOLUME**

Weed et al. (8-12) showed that considerable changes in brain bulk and spinal fluid pressure could be produced in cats by intravenous injections of hypo- and hypertonic fluids. Similar procedures have been used here. Portions of the skulls of rabbits, anesthetized with Nembutal, were removed and the dura reflected to expose the brain. Either 0.1 per cent glucose solution (hypotonic fluid) or 25 per cent glucose (hypertonic fluid) was infused into the femoral vein, at a rate of about 2 ml. per minute, usually for 60 to 75 minutes. Sometimes the fluid was infused into the

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8 It should be noted that the value, 21.16 per cent, for the average dry weight content has no absolute significance since it represents only a particular mixture of grey and white matter from a variety of brain regions. It is of value only for comparison with similar brain samples from different animals.
internal carotid artery but the results were about the same. The percentage dry weight of the brains of animals thus treated and the change in brain volume calculated therefrom are shown in table 2.

Hypotonic infusion caused definite swelling of the brain tissue. Volume changes were found between 6 and 15.5 per cent, calculated on the basis of average normal dry weight. Hypertonic infusion caused very marked shrinkage, up to 31 per cent in the case of one animal infused for 3 hours. The shrinkage of the brain relative to the cranium was very obvious in all cases of hypertonic infusion and noticeable within 20 minutes of starting the infusion. Chemical determinations on the brain of one of these animals showed that excess of glucose or lactate in the brain could not account for an appreciable fraction of the increased percentage dry weight.

### Table 1. Variation in dry weight content of normal rabbit brains

<table>
<thead>
<tr>
<th>% dry weight</th>
<th>20.1</th>
<th>20.3-20.5</th>
<th>20.6-20.8</th>
<th>20.9-21.1</th>
<th>21.2-21.4</th>
<th>21.5-21.7</th>
<th>21.8-22.1</th>
<th>22.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 2. Effects of intravenous infusion of hypotonic and hypertonic fluids on percentage dry weight of brain

<table>
<thead>
<tr>
<th>HYPOTONIC INFUSION</th>
<th>HYPERTONIC INFUSION</th>
</tr>
</thead>
<tbody>
<tr>
<td>% dry weight</td>
<td>% swelling</td>
</tr>
<tr>
<td>Brain exposed</td>
<td>Skull intact</td>
</tr>
</tbody>
</table>

1 Calculated on the basis of 21.16% dry weight for normal brain. Values less than ±4 are probably within the normal range.

* Infused 3 hours, till death of animal.

The marked changes in brain volume described above all occurred with the brain exposed. When infusions, hypo- or hypertonic, were carried out with the skull intact the effects on the brain volume were less marked (table 2). Evidently mechanical-hydrostatic effects in the closed system counteract osmotic effects. Reid (13) observed less marked histologic effects with cats following water infusion with the skulls intact. Weed and McKibben (12), however, found histological changes following hypo- and hypertonic injections only when the skull was intact.

It may be mentioned that the amount of 25 per cent glucose solution administered during 60 to 75 minutes would correspond to 2.5 to 4.0 liters to a 70-kg. man. Yet not one of the animals so treated, with the brain exposed or the skull intact, showed any obvious signs of distress. Diuresis was prolific, but there was no hemo-
concentration; usually there was slight hemodilution as judged by hematocrit and hemoglobin determinations before and after infusion. Only the animal treated for 3 hours died. With hypotonic infusion there was no diuresis and several of the animals died.

Histological observations were made by Dr. Karl Stern. After hypotonic infusion there was swelling of many cortical nerve cells and enlargement of intercellular and perivascular spaces. The nerve cell change was most characteristic in silver stain (Bielchowsky). There was an unstained halo around the slightly enlarged nucleus and the argentophile substance was 'squeezed' in fragments to the periphery of the cell. Reid (13) found less effect on nerve cells, but constant marked swelling of oligodendroglia and no significant change in other cellular elements. The picture in areas of edema surrounding a brain tumor in man was quite different from that seen in his animals after experimental edema. The brains which had been dehydrated and shrunken by hypertonic infusion in our experiments showed, in silver stain, a peculiar nerve cell picture not unlike the one encountered in the early stages of 'senile' changes in man. The intracellular fiber strands were markedly argentophile and showed clumping and coarseness. The intercellular and perivascular spaces showed a normal picture. Details of these observations on shrunken brains are discussed in another publication (14).

These experiments and histological observations indicate that the dry-weight method does detect and roughly measure cerebral edema and dehydration. Edema from causes other than infusion of hypotonic fluid can apparently also be detected. In a series of 5 experiments no infusion was administered but, after unilaterally exposing the brain for periods up to two hours, muscle and scalp were sutured over the skull defect and the animals kept alive for two days. With 3 of these animals, dry-weight determinations indicated swelling of 3, 5.5 and 6.5 per cent. With two animals, in which the exposure was very brief, no swelling was measurable. In no case was there measurable swelling of the unexposed hemisphere.

In 9 experiments no fluid was infused into the venous system but the brain was exposed for about two hours during which it was either left dry or its surface was irrigated continuously with normal saline, Ringer's, hyper- or hypotonic glucose solution or plain water. The animal was then decapitated and the brain dry weight determined. Results showed no correlation with the type of irrigation fluid used and all were within the normal range. But the average of the series, 21.6 (s.d. 0.6) corresponding to a 2 per cent shrinkage, was significantly different from the normal average and suggests a slight tendency to dehydration of the brain during exposure.

Brains removed from animals which had been left with skull defects for two days, and from some of the animals infused with hypotonic fluid, showed elevated areas moulded to the outline of the skull defect. These are presumably regions of local edema probably developing as a result of interference with circulation by the pressure of the herniating brain against the skull defect. The excess of moisture in these small zones would be too small to affect measurements on the whole brain. Attempts to measure local edema by dry weight determinations on small local areas of the rabbit brain were defeated by too great variability in tissue samples from normal brains.
Other methods for chemical evaluation of cerebral edema have been tested in a preliminary way. These depended upon the possibility that the edematous process involved a change in the amount of brain water which is free to dissolve various substances present in the blood. For instance the chloride space of the brain is about 40 per cent of the total tissue volume instead of 80 per cent which would be expected if all the tissue water were free to dissolve chloride. If the extra fluid entering the tissue in edema contained the same concentration of chloride as the plasma, the chloride content of the brain should increase to a relatively greater extent than does the water content. It can be shown that an increase of 5 per cent in the fluid content of the brain should under these circumstances change the chloride space to 43 per cent, making a 7.5 per cent increase in chloride space. The thiocyanate space is only about 15 per cent and a 5 per cent increase in brain volume made up entirely of water, free to dissolve thiocyanate, would increase the thiocyanate space to 19.7 per cent, which is a relative increase of 31 per cent. With inulin, which normally enters the brain fluid only slightly, the relative increase in edema might be very high. All these possibilities have been tested by determinations of the substances in question in the plasma and in the brain, correcting the amount in the brain for the portion accounted for by blood remaining in the brain. Sodium thiocyanate was administered intravenously or potassium thiocyanate by stomach tube at least 2 hours or 8 hours, respectively, before killing the animal; inulin was given intravenously about 35 minutes before sacrificing the animal. The chloride space of normal brains was found to be reasonably constant, values of 37.1 to 39.6 being obtained, the thiocyanate space seemed to vary widely and no inulin at all appeared to enter the normal brain. All these methods involved accurate determinations of the materials in the blood and in the brain and of the blood content of the brain. The thiocyanate and inulin methods involved interference with the animal, while the chloride method offered little increase in sensitivity. These methods were therefore not pursued when the simple dry-weight method was found to be reasonably satisfactory. But this type of experiment might give valuable information concerning the mechanism of the development of edema.

BRAIN SWELLING WITHOUT TRUE EDEMA

A rapidly developing swelling of the brain, with tendency to herniation through the skull opening, is an occasional, but disconcerting, experience of anyone who has done extensive surgery of the brain in man or in experimental animals. Pilcher (4) observed marked bulging of the brain in one out of 6 dogs following exposure of the cortex without trauma, and in 4 out of 5 when trauma to the head preceded exposure. He was unable to show, however, that this was accompanied by a significant increase in water content of the brain in these animals. He concluded (5), "It seems probable that other factors, such as cerebrospinal fluid volume and intracranial blood volume are of greater importance than cerebral edema in producing the increased intracranial pressure which follows trauma to the head."

Prados et al. (15) in studies on the effects of exposure on cat brains, reported swelling usually observed about two hours after exposure. "The degree of swelling varied a great deal from one experiment to the other, and it depended on some factor the nature of which we are not yet able to determine." In our experiments on rabbits, marked herniation of the brain occurred in 4 out of 18 cases after simple exposure of the cortex for an hour or more without obvious trauma. We were unable to determine the conditions which provoked this swelling, since it did not seem related to the type of irrigating fluid being used, nor did it depend upon whether the cortical surface was kept moist or allowed to dry. The herniation subsided upon severing the neck of the animal. Brain dry-weight determinations in two such cases where herniation had occurred indicated that no swelling of the tissue due to increased fluid content had occurred.
These observations serve to emphasize again the importance of a type of swelling which can occur independent of actual change in brain tissue volume. It presumably results from blood vessel dilatation or dilatation of ventricles and cisterns. The mechanism is one of 'inflation' rather than edema. It is commonly seen in very acute form when the animal struggles and cerebral vessels become engorged. It can be readily imitated by increasing the blood volume by rapid intravenous injections or by increasing the spinal fluid volume by intracisternal injections. It may result from changes in blood volume or pressure or from increased spinal fluid volume produced by increased rate of secretion, decreased rate of absorption, or displacement of fluid from the spinal canal into the ventricles. Such events may occur as a reaction to chemical products of trauma or as a result of nervous reflex reaction to certain cerebral stimuli. Obrador and Pi-Suner (16) have described sudden inflation of exposed dog brain on the production of lesions near the fourth ventricle. The mechanism should be susceptible to partial analysis by determinations of blood in the cranium following sudden constriction of the neck by the method of White et al. (2). Results of some preliminary trials did not indicate that excess blood could account for swelling and herniation observed.

The observations of inflation of the brain have all been made upon exposed brains. When the skull is intact, factors which tend to produce inflation may still operate and it seems probable that some cases of raised intracranial pressure may be due partly to these factors and not entirely to true tissue edema or space-occupying lesion.

In the experiments described earlier, on infusion of hypotonic fluid into rabbits with exposed brains and on rabbits which had been kept for two days with a large skull defect, marked herniation of the brain occurred. This herniation usually subsided considerably on decapitation even though dry-weight determinations showed that there was an appreciable true increase in brain volume. In these animals, therefore, both true edema and inflation were induced.

SUMMARY

The approximate extent of swelling or shrinkage of brains of experimental animals can be readily calculated from the dry weight of the brain without knowledge of the actual brain volume. A simple method for determining the dry weight is described.

The moisture content of normal rabbit brain varies considerably. Changes in moisture content well beyond the range of normal variability were produced by intravenous infusions of hypo- or hypertonic solutions. Such changes were more marked when the brain was exposed than when the skull was intact. The difference between true edema, or swelling due to excess water in the brain, and an apparent increase in volume due to hydrostatic effects is discussed.

REFERENCES