15559

Swelling of Brain Slices and the Permeability of Brain Cells to Glucose.

K. A. C. ELLIOTT.

From the Department of Neurology and Neurosurgery, McGill University, and the Montreal Neurological Institute, Montreal.

Sperry and Brand[1] found that the percentage dry weight of rat liver slices immersed in physiological saline solutions decreased rapidly for 15 minutes and then more slowly. They concluded that the effect was due to absorption of water by the slices. The observations of Sperry and Brand have been confirmed, though somewhat smaller effects than theirs were obtained. But by weighing the slices before and after immersion, which the previous authors did not do, it was found that the decrease in percentage dry weight of liver slices is largely due to leaching of solid matter out of the slice and that the actual swelling or increase in weight amounts to only about 10%.

Rat brain cortex slices in isotonic saline solutions swell more than this and some factors affecting such swelling are here described.

Methods. The slices were prepared in a humid chamber similar to that described by Sperry and Brand.[1] It was illuminated by a lamp behind a trough of water. The bottom of the chamber was covered with several layers of blotting paper which were thoroughly moistened with water, and air was passed through an aquarium aerator stone immersed in a beaker of water in the chamber. (A glass shield against the beaker prevented spray from falling on the tissue). By this method the humidity was kept at approximately 100%, judging by equal readings of wet and dry bulb thermometers, and the high and uneven temperatures, and the dripping of condensed vapor, which occur when the humidity is produced by hot water, were avoided.

Uniform slices of brain cortex were cut by means of a Stadie and Riggs[2] microtome designed to cut slices about 0.45 mm thick. Four surface slices, each weighing 45 to 75 mg, were cut from each brain. Thoroughly satisfactory slices were obtained without moistening the blade or microtome provided the surfaces of these were wiped clean after cutting each slice. The slices were spread on a watch glass in the chamber until used and then weighed outside on a torsion balance.*

Individual weighed slices were immersed in small beakers containing the fluids to be tested and these were aerated continuously with oxygen or, in the case of bicarbonate-containing fluids, with oxygen-5% carbon dioxide mixture. All media contained at least 0.2% of glucose. After immersion the slices were drained on a glazed perforated disc, spread onto hardened filter paper (Whatman No. 50) gently wiped with torn edges of soft filter paper, removed from the paper, and weighed. This procedure involved an appreciable loss of weight, which was estimated on control slices which were immersed in saline solutions for a few seconds. Experimental slices were immersed for 20 minutes; longer immersion gave relatively little further changes. The fluids were in general kept at room temperature since tests at 38° gave similar results.

Results. Significant results are summarized in Table I and Fig. 1. Concentrations of solutes are expressed in terms of isotonicity with an 0.308 osmolar solution such as normal saline. All figures for percentage swell-

1 Sperry, W. M., and Brand, F. C., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 147.

2 Stadie, W. C., and Riggs, B. C., *J. Biol. Chem.*, 1944, **154**, 687.

* This procedure whereby uniform slices are prepared without moistening has been used here (see also Fuhrman and Field,[3]), and is recommended for preparing slices for metabolism studies since it allows calculation of results on the basis of true, directly determined tissue weight.

3 Fuhrman, F. A., and Field, J., 2nd, *J. Pharm. and Exp. Therap.*, 1943, **77**, 229 and 392; *Am. J. Physiol.*, 1943, **139**, 193; *J. Cell. Comp. Physiol.*, 1943, **21**, 307; *J. Neurophysiol.*, 1944, **7**, 117.

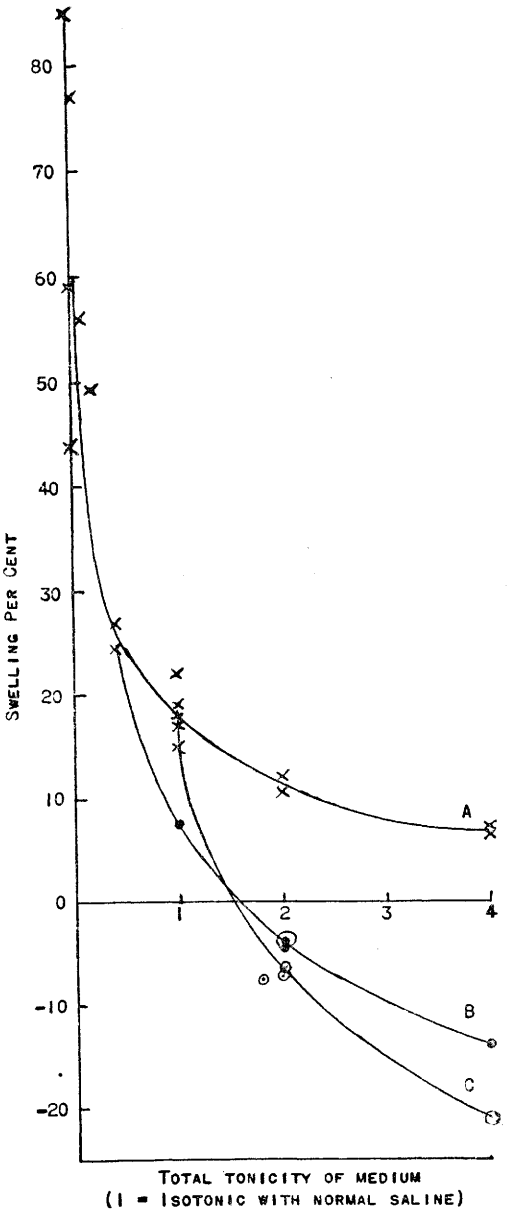

Fig. 1.

Effects of glucose and sodium chloride on the swelling of brain cortex slices.

Curve A—Tonicity made up with NaCl alone.

Curve B—0.4 isotonic NaCl with glucose added to give total tonicity indicated.

Curve C—1.0 isotonic NaCl with glucose added to give total tonicity indicated.

ing or shrinkage, that is increase or decrease in weight, are corrected for the average loss of weight of control slices. This loss varied between 5 and 12%, and in 9 experiments averaged 8%. (Determinations of dry weight on some slices, directly and after brief immersion, indicated that the percentage dry weight of the substance lost on brief immersion and draining was roughly the same as that of fresh tissue. After 20 minutes immersion little or no more dry substance was lost).

In water or pure sugar solutions the swelling of slices was very pronounced and the jelly-like consistency of the tissue prevented accurate drainage and determination of the extent of swelling. With increasing salt concentration the swelling decreased, but in isotonic sodium chloride solution the swelling still averaged about 18%, and even in 4 times isotonic solution some swelling occurred (Fig. 1, Curve A). The extent of swelling was about the same in isotonic sodium chloride, Ringer, Ringer-phosphate or Ringer-bicarbonate solution or in human spinal fluid. It was not affected by additions of manganese or cobalt (2×10^{-4}M). In isotonic potassium chloride the swelling was a little greater, and in isotonic calcium or magnesium chloride it was lower.

In horse or rat serum, the swelling was appreciably lower than in saline. Addition of gelatin,† as colloid material, to Ringer solution reduced the swelling appreciably only if large amounts were used.

The behavior or nonelectrolytes was of special interest. In isotonic (0.308 M) glucose, fructose or sucrose solutions, the tissue swelled as though it were in water, and the swelling was very marked even in 4 times isotonic solutions. However, in the presence of some sodium chloride, the effect of the sugars in decreasing swelling was even greater than that of the salt (Fig. 1, Curves B and C). The potentiating effect of salt is apparent when a glucose solution contains only 0.1 isotonic sodium chloride, and is almost maximal with twice this amount (Table I). The effect seems to be common to all electrolytes since equi-osmolar concentrations of sodium chloride, bromide, sulfate and phosphate, and potassium chloride gave similar results.

It would appear that the tissue cells are

† Knox P-20 Gelatine Solution, kindly supplied by Dr. D. Tourtelotte, Knox Gelatine Products, Inc.

TABLE I.
Percentage Swelling (Increase in Weight) of Brain Slices in Various Media.*

Medium†	% swelling‡	Medium†	% swelling‡
Water†	44, 59, 101, 77	Isotonic glucose	65
Isotonic NaCl	15, 19, 17, 22, 18	2 × Isotonic glucose	41, 25
Ringer sol.	16, 17, 14, 18, 17, 15.5, 17.5, 18	2 × " fructose	58
		2 × " sucrose	50
Spinal fluid (human)	15.5, 17	4 × " glucose	33, 36
Ringer + 1% gelatin	15.5, 15	1.9 " " + 0.1 Isotonic NaCl	11.5
" + 3% "	9.5, 6	1.8 " " + 0.2 " "	—2
Serum (horse)	7, 7.5, 7	1.6 " " + 0.4 " "	—4, —4.5
" (rat)	7	1.3 " " + 0.7 " "	—6.5
Isotonic KCl	24	1.6 " " + 0.4 " Na_2SO_4	—4.5
" $CaCl_2$	5	1.6 " " + 0.4 " NaBr	—3
" $MgCl_2$	4.5	1.6 " " + 0.4 " KCl	—4.5
		1.6 " " + 0.4 " Na phos. buffer	—8.5
		1.6 " fructose + 0.4 " NaCl	—6.5
		1.6 " sucrose + 0.4 " NaCl	—11.5

* This table does not include some of the results illustrated in Fig. 1.
† All media contained at least 0.2% glucose.
‡ All figures have been corrected by the addition of 8, which is the average percentage weight lost on brief immersion and draining. Negative figures indicate shrinkage (loss of weight).

partially impermeable to electrolytes so that solutions of these can oppose swelling by osmotic action, though they cannot, even in high concentration, produce shrinkage. In the absence of electrolyte, the tissue behaves as though it is almost completely permeable to the sugars, while in the presence of even small amounts of electrolyte it becomes almost completely impermeable to sugars.‡ Danielli[5] had discussed physical chemical factors concerned in effects of this type observed with red cells and *Arbacia* eggs. As is indicated in Fig. 1, slices can be maintained without appreciable swelling or shrinkage in a somewhat hypertonic solution consisting of Ringer or normal saline solution containing 0.15 M glucose.

The greater part of the swelling of slices in saline solutions isotonic with serum or stronger, seems to be due to intracellular colloidal osmotic pressure which can be balanced by serum proteins. If this is so it would indicate that the normal intercellular environment of brain cells is not simple spinal fluid but a protein-containing lymph. Even in serum some swelling occurs which is probably a result of damage due to cutting of cell processes or to effects on cell membranes of temporary anoxia or deprivation of circulation during the preparation of slices.

Small additions of materials known to affect permeability *in vivo* or nervous activity, namely adrenal cortical extract (10% of Connaught Laboratory Preparation), acetylcholine chloride and/or eserine (0.1%), metrazol (0.2%) or sodium pentobarbital (0.2%), did not significantly affect the swelling in isotonic sodium chloride solution or in a solution, containing 0.1 isotonic salt and 0.9 isotonic glucose, in which the permeability relations are delicately balanced.

Summary. 1. Brain slices swell considerably in salt solutions isotonic with serum and appreciably even in 4 times isotonic solutions. In serum the swelling is less marked.

2. Brain tissue behaves as if freely permeable to glucose, fructose and sucrose in the absence of electrolyte but, in the presence of small amounts of salt, these sugars behave as impermeable solutes.

‡ In previous work[4] it was concluded, from studies on the respiration of brain tissue homogenized in various media, that brain tissue cells are impermeable to glucose and sucrose. This conclusion was probably correct for the conditions of the experiments concerned in which all media contained some electrolyte, 0.017 M phosphate, and a certain amount of other salts contributed by the tissue in the strong suspensions used.

[4] Elliott, K. A. C., and Libet, B., *J. Biol. Chem.*, 1942, **143**, 227.

[5] Danielli, J. F., in Davson, H., and Danielli, J. F., *The Permeability of Natural Membranes*, New York, 1943, p. 324.