A water insoluble fraction was prepared from ox cerebrum by homogenization in water, dialysis against water, washing with water and drying in air at 293°K. The fraction represented 15.5 ± 3% (n = 8) of the weight of the fresh brain; it contained means of 81% (n = 6) of the lipid, found in freshly dried ox brain samples, and 46% (n = 5) (biuret method) or 51% (n = 3) (6) of the protein, respectively. The Na⁺ and K⁺ concentrations were measured by atomic emission flame photometry, firstly, in prepared media, and then after 4 ml of the same media had been mixed with 1 g of the insoluble fraction at 310°K for 20 min; the concentration in the fraction was calculated from that found in the medium. The concentration in the medium (mM) after incubation was compared with that calculated to be in the fraction, (μmol/g). When the insoluble fraction was mixed with Krebs Ringer saline containing bicarbonate and glucose, modified so that the ratios of NaCl and KCl varied—(although together they always added up to 150 mM)—there was a mean of 2.6 times as much Na⁺ or K⁺ in the fraction (μmol/g) as in the media (mM) when the latter contained up to 80 mM Na⁺ or K⁺. When the concentration of the cations in the media exceeded this value, the concentration of Na⁺ in the fraction rose considerably, while the concentration of K⁺ fell to zero. When only Ca²⁺ or only Mg²⁺ was omitted from the Krebs-Ringer bicarbonate glucose saline, the fraction did not take up Na⁺ or K⁺ from the medium at any concentration. On electron microscopy the fraction was granular and amorphous and contained myelin figures. The fraction did not take up any significant volume of oxygen measured manometrically either in the modified Krebs-Ringer solutions or in a mitochondrial substrate.
INTRODUCTION

One may consider that a living tissue consists of insoluble structures which react with the soluble constituents during regulation and metabolism. It was decided to prepare an insoluble fraction of the brain, which must contain all the structural components of the cells, including the membranes, because in vivo these cannot be soluble in the aqueous fluids of the tissue. One would expect chemical components insoluble in water to be present in all subcellular fractions. This paper is a description of the preparation of an insoluble fraction previously suggested, Hillman (5), and a study of its affinity for \( \text{Na}^+ \) and \( \text{K}^+ \) over the physiological range of concentrations. A preliminary report of this has been given (1).

EXPERIMENTAL PROCEDURE

**Preparation of the Insoluble Fraction.** Ox brain was obtained from an abattoir within 10 min of slaughter and placed in plastic bags in a Dewar flask containing ice. It was brought back to the laboratory within 20 min. The meninges were immediately removed and blood was wiped from the surface. The brain was then stored in a deep freezer at 243\(^\circ\)K.

Samples of ox cerebrum consisting of grey and white matter, each of approximately 3 g, were left to thaw at room temperature (293–295\(^\circ\)K), and then weighed. To each sample 20 ml of distilled water was added, and it was homogenized either in a Potter Elvehjem homogenizer for 2–3 min by hand, or in a domestic blender for 15–20 sec; both methods were found to give similar results. The homogenate plus water with a total volume of 64 ml was placed in 8 compartments formed by knotting Visking dialysis tubing 24/32. It was dialysed in a Feinstein (3) dialyser in a glass tank containing 5–6 liters of distilled water; the dialysis was slightly modified in that air bubbles rather than the glass beads used by Feinstein were used for stirring. The dialyser rotated at a rate of approximately 60 rev/min; the total volume of distilled water, against which the homogenate was dialysed, was changed four times in 6 hr, making a dialysis volume of 20–24 liters against 9.6 g of initial brain tissue. The dialysis against water would cause 'osmotic shock'.

The fraction was dried at room temperature (293–295\(^\circ\)K) rather than at 388–393\(^\circ\)K, because it was felt that this would cause less denaturation of proteins, and less breakdown, denaturation and evaporation of proteins and lipids. Heating the fraction above 373\(^\circ\)K causes bubbles of air and steam to leave the fraction which would add to the disruption.

The dialysis bag was emptied into flat glass dishes 20 cm \( \times \) 10 cm and dried at room temperature (293–295\(^\circ\)K) with a domestic blower switched to 'cold' for about 12 hr. During this period it was often necessary to break up the surface with a spatula to prevent a skin forming, which would have caused non-uniform drying. The fraction deposited as a thin film, which was then collected with a spatula and washed with 40 times its volume of distilled water on a Whatman No. 1 filter paper. The residue was again dried as described above, and stored in stoppered tubes in a desiccator containing silica gel at room-temperature. This dried preparation was designated the 'insoluble fraction', and all references will be to the fraction prepared in this way.

Native egg albumin, diluted ten times with water, purified bovine serum albumin (Koch-Light Ltd) diluted 15 times, or 1% solutions of trypsin or pepsin could all pass freely through