Table 1. Effect of Sodium-free Medium on the Metabolism of Slices in the Presence of Glucose or Pyruvate as a Substrate

Animal and tissue	Glucose as a substrate (3·3 m*M*)						Pyruvate as a substrate (6·0 m*M*)	
	Oxygen uptake (μmol./gm./hr.)		Lactate formation (μmol./gm./hr.)		Glucose utilization (μmol./gm./hr.)		Oxygen uptake (μmol./gm./hr.)	
	Control	Sodium-free	Control	Sodium-free	Control	Sodium-free	Control	Sodium-free
Guinea pig brain cortex	55·9 ± 6·0* (10)	58·9 ± 11·7 (6)	16·6 ± 3·2 (10)	48·8 ± 10·4 (6)	17·7 ± 1·4 (10)	36·7 ± 5·4 (6)	54·9 ± 4·2 (8)	93·3 ± 5·2 (5)
Guinea pig liver	34·0 ± 3·4 (3)	32·5 (2)	< 2·0	< 2·0	—†	—	46·1 ± 1·7 (3)	40·0 (2)
Rat diaphragm	54·8 ± 7·8 (4)	28·7 ± 4·3 (4)	9·51 ± 0·88 (4)	8·32 ± 1·21 (4)	5·86 ± 1·44 (4)	3·68 ± 2·31 (4)	63·9 ± 11·4 (3)	35·8 ± 7·2 (4)
Rabbit kidney cortex	80·0 (2)	50·3 ± 1·5 (3)	15·8 (2)	8·18 ± 0·08 (3)	11·7 (2)	6·44 ± 0·34 (3)	94·9 (2)	68·6 ± 2·9 (3)

* Mean ± standard deviation: figures in parentheses, numbers of experiments. † Glucose was rather increased in the medium.

was also increased to 34·3 ± 0·5 (3) μmol./gm./hr. in the sodium-free medium, the control value of which was only 6·2 ± 0·4 (3) μmol./gm./hr. When lactic acid was used as a substrate, the respiration was severely inhibited and the utilization of lactic acid was not detected. On the other hand, when pyruvic acid was added as a substrate, the oxygen uptake was increased greatly. But the respiration of brain slices metabolizing citrate, succinate, fumarate or glutamate was somewhat inhibited. These effects of sodium-free medium on the metabolism are thought to be peculiar to brain tissue, and are not observed with slices of liver, kidney or diaphragm (Table 1).

When potassium ions were added to the medium, replacing sodium, the oxygen uptake, glucose utilization and lactic acid formation were highest on the addition of 60 m.moles potassium; with further decrease of sodium ions, the metabolism was somewhat reduced. It was found that the ratio of the concentration of potassium to sodium is intimately related to the metabolic activity of brain slices.

These phenomena indicate some metabolic characteristics of brain tissue and the functional specificities of nerve cells. A detailed report on the effect of some inorganic ions, including others beside sodium, is being prepared for publication.

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A Non-Destructive Technique for locating Amino-acids

The electrophoresis of free amino-acids on paper strips by the technique of Durrum[1] results in close discrete bands which are difficult to locate by running parallel pilot substances.

The fluorescence produced by heating the paper at 100° C. for 15 min.[2] proved to be too insensitive for satisfactory location. Heating after immersion in acetone increases the sensitivity, but we have now found that treating amino- or imino-acids with *o*-coumaric acid dissolved in acetone produces a compound which gives a bright yellow fluorescence. The amino-acids can easily be regenerated quantitatively.

Following electrophoresis in 2 *N* acetic acid, the paper strips are dried overnight at room temperature, and then immersed in acetone containing 0·1 per cent *o*-coumaric acid. The lengths of paper are air dried for 2–3 min., warmed in an oven at 100° C. for 1–2 min., and then briefly exposed to ultra-violet light from a Mazda *MBW/U* bulb. The yellow fluorescent amino-acid complex can then be accurately delineated against a background blue fluorescence which rapidly fades due to the heat produced by the ultra-violet lamp. Finger pressure also produces yellow fluorescent areas.

The separate bands of amino-acids can be extracted and then chromatographed by the descending butanol/acetic acid/water (40–10–50) system. The *o*-coumaric acid contamination runs with the solvent front and has no effect upon either the speed of running or upon the separation of the eighteen common amino-acids investigated. After chromatography, the paper sheets are dried overnight at room temperature, and the individual amino-acids located by the fluorescence technique. Under these conditions the sensitivity is such that 2 μgm. of amino-acid/sq. cm. of paper can be detected without difficulty; the lower limit appears to be about 1·2–1·4 μgm./sq. cm. The area of paper containing the amino-acid is cut out and the amino-acid content determined by the pyridine–phenol–ninhydrin method of Troll and Cannan[3].

The excised paper contains less than 30 μgm. of *o*-coumaric acid per sq. cm., and experiments have shown that the colorimetric determination is unaffected by the presence of 300 μgm. of *o*-coumaric acid. Recovery experiments employing the method of location described have given quantitative yields of chromatographed amino-acids, but extending the time of heating at 100° C. to 5 min. will produce losses of up to 10 per cent. For this reason overexposure to the hot ultra-violet lamp should be avoided.

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