

to the possible biological activity of this chromogen the content of which was estimated to be less than that of vitamin B₁₂ of blood⁶⁻⁹ are in progress.

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Thin-layer Chromatography of Sugar Phosphates from Muscle

SEPARATION of sugar phosphates occurring in plant material was achieved by Bandurski and Axelrod¹ using paper chromatography, while Bove² and Randerath^{3,4} separated a mixture of known nucleotides by thin-layer chromatography. Since the usual methods for separating and identifying the sugar phosphates in muscle are long and tedious, separation and identification by thin-layer chromatography were investigated.

Standard solutions of sugar phosphates were made up to contain 80 per cent ethyl alcohol after freeing them from their metallic salts by shaking with 'Dowex 50', filtering and adjusting to pH 7.0 with NH₄OH. The standards were stable for at least a week at 4° C. Extraction with ethanol was found to be more satisfactory than with water, since in the latter case removal of the water-soluble proteins posed special problems. Extraction of the sugar phosphates from 5 g of muscle tissue was accomplished by homogenization with 50 ml. of ice-cold ethyl alcohol in a Waring blender for 1 min followed by centrifugation at 0° C for 15 min. The supernatant containing the sugar phosphates was stored at -15° C until chromatographed.

A number of absorbents were tried and DEAE cellulose was found to be the most satisfactory. 15 g of DEAE cellulose were shaken with 100 ml. water for 45 sec before spreading. After drying in air overnight, the plates were tailored to a semicircular fan shape, which allowed for radial development⁵. A mixture of known standards and the extract from muscle were both applied in 1.5-cm bands at the base of the fan. The most satisfactory solvent system was found to be isopropyl alcohol, formic acid and water in the ratio of 30 : 20 : 10. The minimum application required for detection was 0.2 mg sugar phosphate per 1.5 cm band. Approximately 1.5 h was required to develop each plate. The nucleotides were visualized under ultra-violet light before spraying. The phosphates were detected using the spray reagent of Hanes and Isherwood⁶. After drying for 1 min at 85° C, the plates were developed under ultra-violet light for 5-10 min. It was often considered necessary to re-spray after developing. A grey-blue line of ortho-phosphate just below the solvent front indicated a slight hydrolysis of the standards.

Adenosine triphosphate, fructose diphosphate, adenosine diphosphate, glucose-6-phosphate, glucose-1-phosphate and adenosine monophosphate could be separated by this

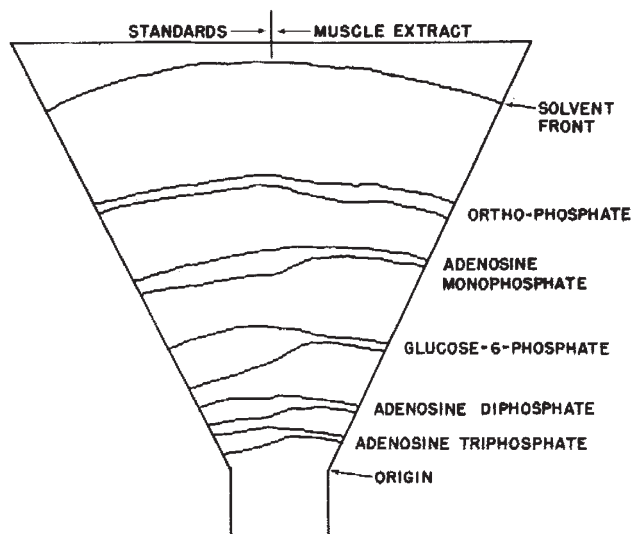


Fig. 1. Thin-layer chromatogram showing separation of sugar phosphates in pork muscle

procedure. Ribose-5-phosphate migrated to the same position as adenosine monophosphate and they could not be distinguished if both were present, while glyceraldehyde-3-phosphate occupied the same position as ortho-phosphate. Fig. 1 shows the separation achieved for the sugar phosphates in pork muscle taken 0.5 h after slaughter and compared with known standards. Other chromatographs of muscle tissue have shown similar separations and indicate that thin-layer chromatography can be utilized to separate the sugar phosphates in muscle.

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PHYSIOLOGY

Hypertension resulting from Renal Arterial Injection of Microspheres

It has been known for some time that alteration of renal function may lead to hypertension. If both kidneys are removed, renoprival hypertension ensues¹. If one renal artery is partially constricted and the opposite kidney removed, blood pressure will increase to hypertensive levels², although renal function is sufficient to maintain essentially normal fluid and electrolyte balance. The latter method is in widespread use as the means of producing hypertension. This communication deals with another method by which hypertension of renal origin may be produced, that is, renal arterial injection of microspheres.

It was previously demonstrated that injection of plastic microspheres into one renal artery altered renal function in such a way that the injected kidney elaborates a urine which is similar to that of a pyclo nephritic kidney³. The