THE ACID-SOLUBLE PHOSPHORUS COMPOUNDS OF RAT MUSCLE

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This report describes the application of a paper chromatographic procedure[1] to the separation and identification of acid-soluble phosphorus compounds in muscle from normal rats and rats which had been injected with metabolic inhibitors. Parallel experiments with homogenates and soluble enzyme extracts were carried out and assisted in the identification of the compounds in the intact muscle. Some results with ox-muscle extracts have been described previously[2].

EXPERIMENTAL

Adult rats (200 to 300 g weight) were injected with "myanesin" (25–125 mg/100 g body weight) until the muscles were completely relaxed. The animals were killed by a blow on the head and, in the cold room, the muscle from one hind-limb was removed immediately and frozen in liquid nitrogen. The frozen muscle was broken into small pieces and homogenised in ice-cold perchloric acid. The remainder of the procedure was as described previously[1]. In the experiments with inhibitors, sodium iodoacetate (50 mg/100 g body weight) or sodium fluoride (420 mg/100 g body weight) were injected intraperitoneally after relaxation with "myanesin" and the rats killed 15–20 min later. In the isotopic experiment, 150 μC of ^{32}P as orthophosphate in sterile saline was injected aseptically into the right thigh, the animal then relaxed with "myanesin" and killed 30 min later. Autoradiographs were obtained by exposure of the chromatograms to double coated "Kodak" No-Screen X-ray film for 11 days.

Homogenates were prepared by blending muscle with 2 vol. of ice-water. A soluble muscle enzyme system was prepared from an extract of an acetone powder of enzymes made as described by UMBREIT, BURRIS AND STAUFFER[3] and the cofactors recommended by NEIFAKH AND MEL'NIKOVA[4] were added.

RESULTS

Normal rat muscle

The pattern of radioactive spots obtained when barium fractions A, B and C, were run on two-dimensional chromatograms in GW3 twice/isobutyric acid[1] is shown in Fig. 1**. G-1-P was reported by THRELFALL[5] in rat muscle but was not detected on the

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** Abbreviations are those used in ref. 1.

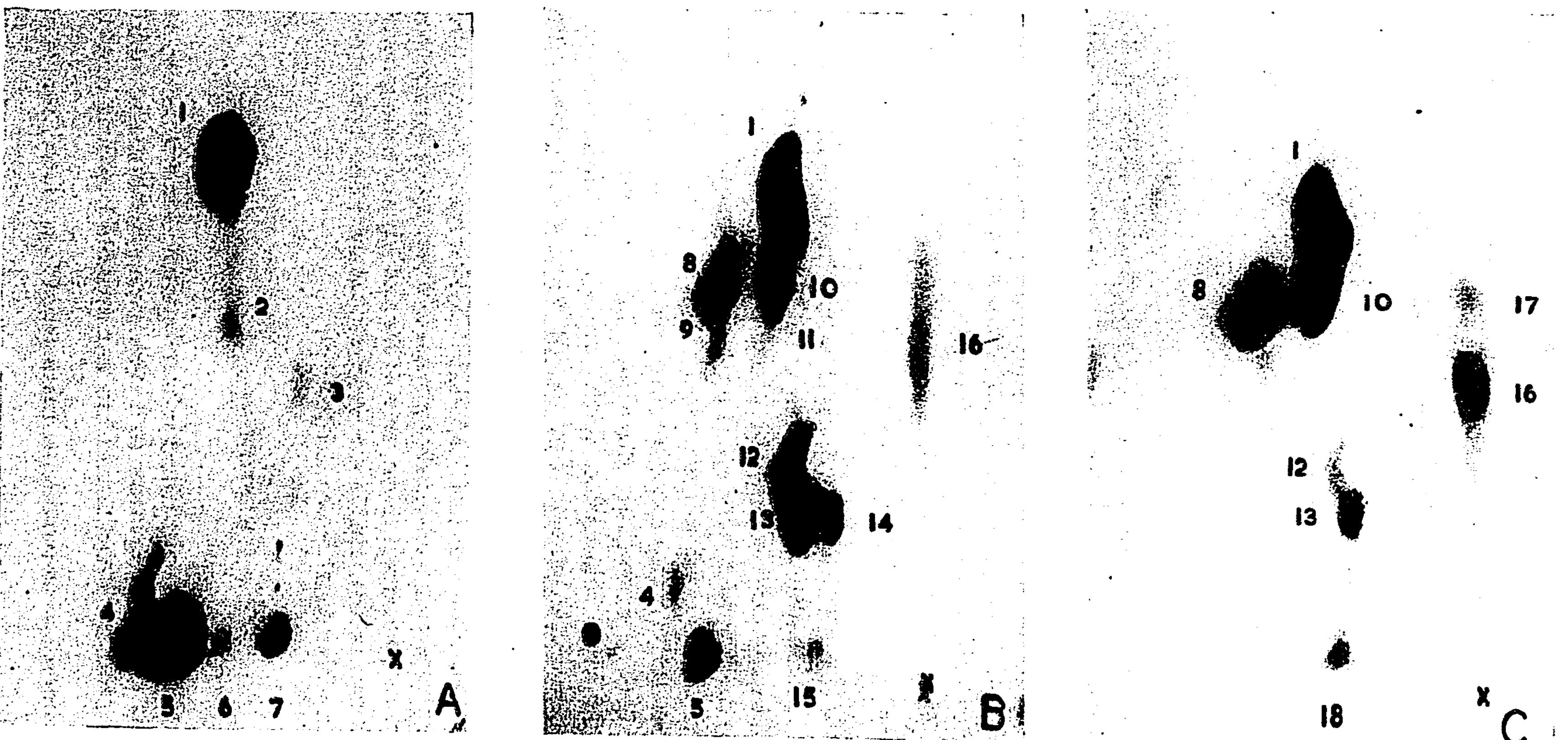

Fig. 1. Autoradiographs of two-dimensional chromatograms in GW3 twice/isobutyric acid of barium fractions A, B and C of the acid-soluble phosphates of normal rat muscle. (?) indicates that the identification is tentative. (1) PO_4, (2) "W", (3) 2,3-PPGA (?), (4) ADP, (5) ATP, (6) GTP, (7) UDPG (?), (8) Cr-P, (9) Gl-2-P (?), (10) PO_4 from Cr-P plus Gl-1-P, (11) DHAP, (12) F-6-P, (13) G-6-P, (14) F-1:6-PP, (15) 3 unidentified compounds, (16) "X", (17) "Y", (18) "Z". In addition non-radioactive spots of DPN and AMPS were present on the chromatogram of fraction C.

chromatograms illustrated since it was very close to G-6-P. However, the small amounts present were detectable on one-dimensional chromatograms in GW3 twice. The spot of the unknown "W" was cut out, eluted with water, and the eluate tested for phosphoglyceric acid by the method of RAPOPORT[6] and for glycollic acid with 2,7-dihydroxynaphthalene[7]. In the latter test, a purple (positive) colour was obtained; Gl-1-P, Gl-2-P and G-6-P gave yellow or orange colours, F-6-P an orange-red and DHAP a green colour, 3-PGA and 2-PGA both gave a purple colour, presumably due to decarboxylation to phosphoglycollic acid. RAPOPORT's test was negative and "W" did not co-chromatograph with added 3-PGA or 2-PGA.

During chromatography in GW3 twice, part of the creatine phosphate remained intact and a portion of this was broken down when the paper was dried to remove the solvent. During the subsequent run in isobutyric acid the orthophosphate liberated ran to the position of spot 10. This spot was a compound one consisting of a stable, non-reducing, phosphate partly obscured by the orthophosphate liberated as described. Treatment of the papers with the periodic acid reagent for *cis*-diols of GORDON, THORNBURG AND WERUM[8] gave a white spot on a blue background. This stable, non-reducing phosphate was also present in homogenates glycolysing glucose-1-phosphate and isolation and elementary analysis confirmed it to be glycerol-1-phosphate.

The two unknowns "X" and "Y" (Fig. 1) do not correspond in position to any of the substances run during the course of this work[1] and have quite unusual R values. A sample of isotopically-labelled blood phospholipids was run in the same solvent system but none of the constituents appeared in a similar region of the chromatogram.

In addition to the radioactive spots, there were two other spots on chromatograms of fraction C corresponding to unlabelled DPN and AMPS. DPN is known to be synthesised in the cell nucleus[9,10] and its labelling would be expected to be slow, and the terminal phosphate of AMP is generally recognised not to turn-over during most metabolic reactions.

Iodoacetate- and fluoride-poisoned muscles

Similar compounds to those in normal muscle were found, but, in addition, IMP and DHAP had accumulated *in vivo*. In the iodoacetate treated animals creatine phosphate was absent, but present in the muscle of animals poisoned with fluoride. In the latter, no 3-PGA could be detected but two new spots having a bright blue fluorescence under ultra-violet light had appeared. Similar compounds could be produced by the action of 0.1 N NaOH on DPN, and the spots were believed to be due to DPN breakdown products.

Homogenates and soluble enzyme systems glycolysing glucose-1-phosphate

The same compounds as those present in intact muscle were found to accumulate anaerobically. However, in homogenates under aerobic conditions a new acid-labile phosphate (R_{PO_4} in GW3 twice = 0.80; R_{AMP} in isobutyric acid = 0.40) appeared. This substance had similar chromatographic properties to glycolaldehyde phosphate prepared by oxidising glycerol-1-phosphate with periodate[11,12].

DISCUSSION

In normal resting rat muscle the compounds reported by THRELFALL[5] and by THRELFALL AND STONER[13] were found, with the exception of 3-PGA, which did not accumulate

in the living muscle under any of the experimental conditions used although it readily accumulated in glycolysing homogenates and enzyme extracts. The unknown "W" appeared to be the compound identified by these authors as 3-PGA, but it is suggested that in fact it has some of the properties of phosphoglycollic acid which is known to form a sparingly soluble barium salt[11] and gives a purple colour with 2,7-dihydroxynaphthalene[14]. AMPS and traces of DHAP were found and radioisotope experiments showed the presence of at least 3 unidentified phosphates.

Investigation of muscle from normal and poisoned animals, of muscle homogenates, and of enzyme extracts glycolysing glucose-1-phosphate, failed to reveal any evidence for the accumulation of ribose-5-phosphate or other intermediates of the pentose cycle or other known alternatives to the Embden-Meyerhof pathway. Some slight evidence was obtained that glycollic acid derivatives may be present, which is of interest since appreciable quantities of the free acid occur in muscle[15,16] and phosphoglycollic acid has been reported in erythrocytes[14].

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SUMMARY

The acid-soluble phosphorus compounds in muscle from normal rats and from rats poisoned with iodoacetate and fluoride were investigated using a previously described paper chromatographic technique and compared with the compounds accumulating in homogenates and enzyme extracts glycolysing glucose-1-phosphate. Autoradiographs of the compounds in normal rat muscle are shown.

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