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Direct Evidence for the Hexose Monophosphate Pathway of Glucose Metabolism in Skin

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Barron, Meyer & Miller (1948) first suggested the possibility of the existence of the hexose monophosphate pathway in rat skin. This hypothesis was supported by Hershey & Mendle (1954), who demonstrated the presence of 6-phosphogluconate dehydrogenase in human skin. Glucose 6-phosphate dehydrogenase has also been found in skin by Hershey, Lewis, Murphy & Schiff (1960).

By comparing the rates at which C-1 and C-6 of glucose were converted into carbon dioxide, Freinkel (1960) also concluded that the hexose monophosphate pathway operates in human skin. Using a similar technique, Gilbert (1962) was unable to demonstrate the hexose monophosphate pathway in cattle skin: he attributed this difference to species variation. However, the observations of Freinkel (1960) were confirmed by Pomerantz & Asbornsen (1961) with rat skin. These authors interpret their findings with caution, however, bearing in mind the fact that not all of the carbon atoms of glucose are converted into carbon dioxide (Wood, 1955) and the uncertainty concerning the fate of the triose phosphate (Katz & Wood, 1960). The evidence for the existence of the hexose monophosphate pathway in skin is thus conflicting and indirect. It therefore seemed desirable to demonstrate in skin the presence of some of the intermediates involved in the hexose monophosphate pathway.

MATERIALS AND METHODS

Preparation of skin extracts. Slices of guinea-pig-ear skin were cut by hand from the dorsum of the ear of the adult animal after cervical fracture. Skin slices weighing between 5 and 10 mg. were labelled with ^{32}P by incubating at 37° for 4 hr. in 0.5 ml. of Krebs-Ringer phosphate (Krebs, 1933) containing $[\text{P}^{32}\text{O}_4]^{3-}$ (approx. $100\text{ }\mu\text{C}/\text{ml.}$), glucose (0.02 M) and streptomycin sulphate ($50\text{ }\mu\text{g.}/\text{ml.}$). In some experiments, ^{14}C was also incorporated by adding uniformly labelled glucose [$\text{U-}^{14}\text{C}$]glucose ($3.6\text{ }\mu\text{C}/\mu\text{mole}$ of glucose) to the medium: for reasons of economy, the volume of medium used in these experiments was 0.1 ml.

After incubation the skin was frozen in liquid nitrogen and extracted in a glass tissue-grinder with ten times its weight of cold 10% (w/v) trichloroacetic acid. To the clear supernatant obtained after centrifuging, EDTA (approx. 5 mg.) and sufficient ammonia to make the solution alkaline were added. This extract was used for chromatography.

Chromatographic methods. For chromatography of sugar phosphates, Whatman no. 1 chromatography paper was soaked for 30 min. in a 0.2% solution of EDTA adjusted to pH 8.5 with sodium hydroxide. After washing in six changes of water, the sheets were hung up to dry at room temperature. For chromatography of sugars, untreated Whatman no. 1 paper was used.

Descending chromatograms were run in the following solvents at 27° : I, propan-1-ol-aq. ammonia (sp.gr. 0.88)-water (6:3:1, by vol.) for 40 hr.; II, 2-methylpropan-2-ol-pyridic acid-water (20:1:5, v/w/v) for 30 hr.; III, phenol-water (4:1, w/v) for 24 hr.; IV, butan-1-ol-propionic acid-

water (10:5:7, by vol.) for 24 hr.; V, ethylacetate-pyridine-water (12:5:4, by vol.) for 10 hr.

Phosphates were detected on paper by dipping the chromatogram in a solution containing ammonium molybdate (1 g.), water (8 ml.), conc. hydrochloric acid (3 ml.), 60% (w/v) perchloric acid (3 ml.) and acetone (85 ml.) (Burrows, Grylls & Harrison, 1952), followed by exposure to unfiltered light from a mercury-vapour lamp. Aldoses were detected by the method of Partridge (1949). Sedoheptulose was detected by dipping the paper twice in a solution of orcinol (0.5 g.) and trichloroacetic acid (15 g.) in propan-2-ol (100 ml.), drying in air and heating for 1 min. at 105° (Bevenue & Williams, 1960).

Localization of ^{32}P on chromatograms was effected by pinning the chromatogram to an envelope containing a fast no-screen X-ray film for about 1 week and developing in the usual way. ^{14}C was detected by placing the chromatogram in contact with the film for periods up to 2 months.

Co-chromatography and rechromatography. Selected radioactive compounds located by radioautography were cut from chromatograms in mitre-shaped pieces and eluted with water into silicone-treated vessels. Eluates were concentrated to a convenient volume over conc. sulphuric acid in a vacuum desiccator. Compounds contaminated with picric acid from solvent II were rechromatographed in solvent I (Wawszkiewicz, 1961). Reference compounds (1 μmole) were added at this stage before co-chromatography. The reference compounds sedoheptulose 7-phosphate and sedoheptulose diphosphate were gifts from Dr B. L. Horecker, New York University School of Medicine, New York, U.S.A. Ribose and ribose 5-phosphate were purchased from British Drug Houses Ltd. Sedoheptulosan was purchased from L. Light and Co. Ltd.

Hydrolysis of sugar phosphates. Sugar phosphates labelled with ^{14}C and ^{32}P were eluted from chromatograms, freed from picric acid and incubated overnight with human seminal plasma as described by Moses (1960). The reaction mixture was then subjected to chromatography or co-chromatography without further treatment.

RESULTS

Labelled skin extracts chromatographed in two dimensions in solvents I and II produced radioautographs similar to those described by Brooks, Lawrence & Ricketts (1959). However, these authors did not describe a faint spot which travels with glucose 1-phosphate in solvent I, but which moves a little faster in solvent II. Typical R_{F} values (i.e. movement relative to inorganic phosphate) for this compound are 1.35 in solvent I and 0.55 in solvent II. This radioactive compound coincided exactly with added sedoheptulose 7-phosphate when subjected to two-dimensional chromatography in solvents I and II and also in III and IV. The identity of this compound was further confirmed by chromatographing a skin extract labelled with ^{32}P and ^{14}C in solvents I and II, eluting the spot in question and hydrolysing with seminal plasma after removal of picric acid. The reaction mixture was co-chromatographed with acid-treated sedoheptulosan (Bevenue & Williams, 1960)

in solvent V and a radioautograph prepared. The ^{14}C labelled compound corresponded exactly with the sedoheptulose spot. A slower-moving ^{14}C -labelled spot travelled with glucose that had been chromatographed alongside: this had probably been derived from the stronger glucose 1-phosphate spot that runs close to sedoheptulose 7-phosphate in solvents I and II.

In a similar way, sedoheptulose diphosphate coincided exactly with a feebly labelled compound appearing on a two-dimensional chromatogram developed in solvents I and II. Sedoheptulose diphosphate travelled with fructose 1,6-diphosphate in the alkaline solvent, but moved less quickly in the acid solvent; typical R_{F} values were 0.60 in solvent I and 0.45 in solvent II. When the ^{14}C -labelled spot under consideration was treated with acid phosphatase, labelled sedoheptulose was not detected in the reaction mixture: this was probably due to the very small amount of activity present.

Though a R_{F} value for ribose 5-phosphate in solvent I has been reported (Wood, 1961), under our conditions this compound is destroyed by this solvent. However, when a labelled skin extract was co-chromatographed with ribose 5-phosphate in two dimensions in solvents III and IV, good superposition was obtained with a radioactive spot having a R_{F} value of 1.2 in solvent III and 0.65 in solvent IV. The identity of this spot was confirmed by eluting the spot and demonstrating that the sugar released on hydrolysis co-chromatographed with ribose in solvent V.

DISCUSSION

None of the phosphorus compounds described were noted by Brooks *et al.* (1959). Their failure to find ribose 5-phosphate is explained by the fact that this compound is decomposed by one of their chromatographic solvents. The use by Brooks *et al.* (1959) of water as a tissue-extracting fluid coupled with the relatively short exposure used in the production of their radioautographs probably accounts for their failure to observe the faint spots due to sedoheptulose 7-phosphate and sedoheptulose diphosphate.

In agreement with Olley & Dawson (1956) individual phosphorus-containing compounds have shown extremely variable R_{F} values, particularly in solvents I and III. However, the spatial relationship of spots comprising the general pattern is very reproducible. We therefore conclude that the position of a spot in relation to neighbouring spots is a better guide to its identity than its R_{F} value.

Our results show clearly that, in guinea-pig skin, ribose 5-phosphate, sedoheptulose 7-phosphate and sedoheptulose diphosphate are present. The evidence for the last-mentioned compound is less

strong than for ribose 5-phosphate and sedoheptulose 7-phosphate in that the sugar moiety was not recovered after hydrolysis: this is likely to be due to the very small amount of this compound present in skin. We thus conclude that the hexose monophosphate pathway operates in skin.

SUMMARY

1. Skin slices were incubated in a medium containing uniformly labelled [^{14}C]glucose or [^{32}P]orthophosphate or both. The phosphate esters present in a trichloroacetic acid extract of such skin were separated by two-dimensional chromatography.

2. Of the labelled phosphates present, sedoheptulose 7-phosphate, sedoheptulose diphosphate and ribose 5-phosphate were identified by co-chromatography.

3. The identity of sedoheptulose 7-phosphate and ribose 5-phosphate was confirmed by co-chromatography of their labelled hydrolysis products with the appropriate sugar.

4. The demonstration of these compounds in guinea-pig-ear skin provides direct evidence that the hexose monophosphate pathway operates in this tissue.

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The Metabolism of Carbohydrate by Liver of the Domestic Fowl

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Although there exists a considerable literature on the carbohydrate metabolism of mammalian liver, by contrast that relating to avian liver is relatively small. Avian liver, and in particular that of the domestic fowl, undergoes marked changes in gross composition when the bird comes into lay, the composition returning to the previous state when laying ceases. Information on the carbohydrate metabolism of the liver, and its ability or otherwise to use various carbohydrates as substrates under these two conditions, has not been recorded. The present experiments were therefore undertaken as part of a study of the metabolism of the liver of the domestic fowl as it is affected by the onset of laying.

MATERIALS AND METHODS

Birds. The domestic fowls were Single Comb White Leghorns of an inbred line purchased from Moncrieff's Animal Station, Godstone, Surrey. They were maintained

under artificial daylight at a constant temperature of 60–62° F. Birds in lay were exposed alternately to 14 hr. of light from 6 a.m. to 8 p.m. followed by 10 hr. of darkness. Birds not in lay were exposed alternately to 10 hr. of light from 8 a.m. to 6 p.m. followed by 14 hr. of darkness. Moulting was induced in a few birds by decreasing the light to a minimum of 6 hr./day. This was not uniformly successful, some birds continuing to lay with an unimpaired performance. The birds were fed either on 'Intensive growers pellets' or on 'Battery layers pellets' (British Oil and Cake Mills Ltd.) *ad libitum* as required and were allowed free access to water.

Pigeons were obtained from O. Kershaw, 22 Hope Street, Manor Road, Ossett, Yorkshire, and were maintained on a diet of corn, maple peas and limestone grit. The physiological state of the birds, i.e. in lay or not in lay, was determined by daily egg records, by the presence or absence of lipophosphoprotein in the plasma (McIndoe, 1959) and by examination of the ovaries at autopsy.

Preparation of tissue slices. Birds were killed by rapidly severing the neck vessels and were allowed to bleed as much as possible. The liver was rapidly excised and placed