Paper chromatography and polarography as a tool for the study of histidine metabolism in skin. Estimation of histidine and urocanic acid in human sweat

The occurrence of urocanic acid (imidazole-acrylic acid) (UA) along with histidine in human sweat might be due to a peculiarity of the metabolism of the latter in sweat glands as first suggested by HAIS, KRÁL AND ŽENÍŠEK[1,2].

To obtain more information about this phenomenon it was necessary to develop simple rapid methods for the direct estimation of these compounds in sweat. For simultaneous estimation of histidine and UA by paper chromatography, a method of standard addition described by CAIN AND BERRY[3] and applied by KRÁL AND ŽENÍŠEK[4] to amino acids in sweat was used. This method takes into account distortions produced in chromatograms by other constituents.

Undesalted samples of sweat were applied on paper alternatively in 25 μl and 50 μl amounts. A standard mixture containing known concentrations of histidine and UA was added to each of 25 μl samples, so that 0.5 μg was added to the first sample, 1 μg to the second, 2 μg to the third, etc.

Chromatograms were run in the top layer of the *n*-butanol:water:acetic acid mixture (4:5:1) for 5 hours and after the detection by the Pauly reagent a visual comparison was made to see which pairs of two adjacent spots are of the same intensity. For such a pair of spots, following equation can be written: $x = n$, where x is the amount of histidine or UA in 25 μl of sweat and n the known amount of either substance added to the spot. If, *e.g.*, 2 μg of standard is added to a 25 μl sample of sweat and the spot in this mixed sample is of the same intensity as in 50 μl of sweat alone, the concentration is 8 mg % (2 μg/25 μl). Below 2 mg % and beyond 16 mg % the accuracy of the estimation of either substance is not satisfactory.

Our experience that on electrolytic desalting UA is transformed quantitatively to imidazole propionic acid suggested the use of its reducibility for direct estimation of UA in sweat by means of polarography.

The polarographic behaviour of UA has not yet been studied. According to our experiment UA gives a cathodic reduction wave on the dropping mercury electrode. The relation between the height of the wave and the pH of the solution was studied (buffers from pH 2 to pH 8 were examined). Up to pH 5.5 the height of the wave was practically constant, then it showed a decrease with the increase of pH. For the polarographic determination of UA in sweat the acetate buffer of pH 4.7 is suitable. In this buffer, after elimination of oxygen, UA gives a very well developed diffusion wave. The limiting current is proportional to the concentration of UA (Fig. 1). For the direct estimation of UA a sample of sweat was added to 0.5 *M* acetate buffer (pH 4.7) containing approximatively 0.5 *M* KCl. After elimination of oxygen in the stream of pure nitrogen the polarographic curve was registered. This curve shows usually two waves. The more positive not well developed wave belongs to an unknown substance, the second one, 250 mV more negative (—1.3 V S.C.E.), belongs to UA.

Fig. 1. Concentration dependence of the UA wave. 0.5 *M* acetate buffer pH 4.7: UA concentrations from right to left 1.7; 3.36; 6.56; 9.6; 12.5; 18.0; 23.0 mg %.

This was ascertained in two ways. (1) After adding UA to sweat, only the negative wave increased (Fig. 2). (2) After the isolation of the UA fraction by means of paper chromatography and its elution and evaporation to dryness, UA was dissolved in acetate buffer (pH 4.7) and polarographed. The resulting wave corresponded by its height, shape and half-wave potential to the wave of UA in the original sample. As may be seen, the waves of UA in sweat (Fig. 2) are not as well developed as in buffers with pure UA (Fig. 1). However, they can be measured reliably. The accuracy of the determination is limited by the accuracy of the measurement of the height of the polarographic waves. Using the method of standard addition[5,6] six analyses of the same sample of sweat (6 mg %) were made with a maximum relative error of $\pm$ 2%;

under less favourable conditions this error was ± 10%. The time necessary for one analysis is 12 minutes and may be reduced to one half in routine analysis, when a calibration curve is applied. In combination with paper chromatography the proposed method can be used for other biological material where direct polarographic estimation is not feasible. An alternative method for the determination of UA to the spectrophotometric method most commonly used appears important in view of the present interest centering around this substance as an intermediate in the conversion of histidine to glutamic acid and as a precursor of available formyl group[7,8].

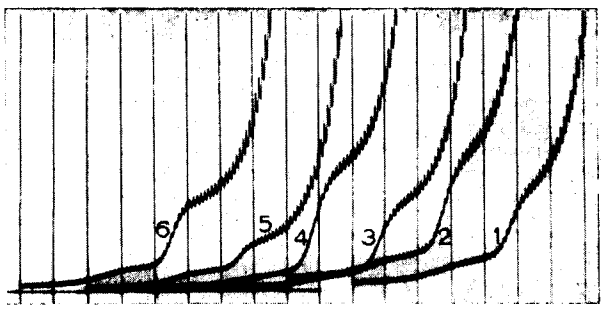

Fig. 2. The wave of UA in sweat samples. 1 ml 0.5 *M* acetate buffer pH 4.7, 0.5 *M* KCl; 1 ml sweat: Curve 1 corresponds to 13.4 mg%, curve 3 to 14.0 mg %, curve 5 to 6.5 mg % UA in sweat. Curves 2, 4, 6 represent the curves of the respective sweat samples after 0.05 ml 138 mg % UA was added.

25 Pairs of results obtained by both methods (average 6.66 mg % for the polarographic, 6.60 for the chromatographic method) were analysed by the method of paired *t*-test. The difference between both methods, which are based on widely different structural features (imidazole ring in chromatographic detection and acrylic side chain in polarographic reduction), was found to be due to chance. This can be taken as evidence for the absence of systematic error in either of them.

By means of both methods the UA content in sweat of subjects with rheumatoid arthritis and of sportsmen before and after muscular effort has been studied. Histidine was estimated by paper chromatography. The results will be published elsewhere.

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1 A. ŽENÍŠEK AND J. A. KRÁL, *Biochim. Biophys. Acta*, 12 (1953) 479.
2 J. HAIS, J. A. KRÁL AND A. ŽENÍŠEK, *Časopis Lékařů Českých*, 92 (1953) 974.
3 L. CAIN AND H. K. BERRY, *Univ. Texas Publ.*, 5109 (1951) 77.
4 J. A. KRÁL AND A. ŽENÍŠEK, *Časopis Lékařů Českých*, 93 (1954) 1155.
5 J. HEYROVSKÝ, *Polarographie*, Springer, Wien, 1941, p. 265.
6 I. M. KOLTHOFF AND J. J. LINGANE, *Polarography*, Interscience Publishers, New York, 1952, p. 375–381.
7 K. SLAVÍK AND V. MATOULKOVÁ, *Chem. Listy*, 48 (1954) 765.
8 A. MILLER AND H. WAELSCH, *Biochim. Biophys. Acta*, 17 (1955) 278.

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On the mechanism of the intestinal absorption of sugars[1]

The current hypotheses on the mechanism of "active" transport of sugar in the intestine postulate an enzymic modification of the sugar molecule at either carbon atom 1 or 6 by phosphorylation[2] or at carbon atom 1 by mutarotation[3]. With respect to the hypothesis of phosphorylation, the recent experiments of SOLS[4] on the substrate specificity of the hexokinase of intestinal mucosa clearly demonstrate that this enzyme cannot participate directly in "active" transport. However, by the methods used, the participation of a different enzyme of phosphorylation could not be disproved.

It occurred to us that direct test of the validity of these hypotheses could be made by the use in absorption experiments of model compounds lacking the hydroxyl function at carbon atoms 1 or 6 and incapable of undergoing phosphorylation or mutarotation. Accordingly, 1-deoxy glucose, 1-deoxy mannose, and 6-deoxy glucose were chosen as test compounds. Glucose and

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