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# The determination of small quantities of pentose, especially in derivatives of adenylic acid.

by Wanda Mejbaum.

Z. f. Physiol. Chem. 258: 117-120 (1939).

The increasing importance of pentose derivatives in biochemical research requires the utilization of methods for their analysis. Pentose analysis based on transfer to furfurol and the color reaction of distilled furfurol fails in compounds in which the phosphoric acid group at carbon 5 of the ribose has been esterified (1). The yields in furfurol are low and inconstant with these compounds, they depend on the amounts of pentose; the cause of this phenomenon is unknown.

However, it is possible to find conditions for color reactions in the same reaction milieu, where pentose is transferred to furfurol by heating with HCl, under which a proportionality is obtained between the pentose concentration on one hand and the color intensity on the other. No color reaction is as well suited for this purpose as the green Bial reaction which appears when a pentose derivative is heated with orcin and hydrochloric acid containing $FeCl_3$. Bial's reaction has already been used by G. Embden and M. Lehnartz (2) as well as by Z. Dische and K. Schwarz (3) in the quantitative analysis of pentoses. Milligrams of arabinose, xylose and adenylic acid were determined in studies by Dische and Schwarz.

The work performed at our laboratory has for some time required a method for the analysis of very small quantities of the derivatives of ribose-5-phosphoric acid. Studies dealing with the transfer of phosphorus with the aid of radioactive phosphorus (4) showed the extent to which organic phosphoric compounds may adhere among themselves as well as to inorganic precipitates; e.g. $NH_4MgPO_4 \cdot 6\ H_2O$ invariably carries the nucleotides from a solution containing inosinic acid or adenylic acid. In this connection, Bial's reaction was always used for the testing of phosphoric preparations and its quantitative evaluation, especially of very small amounts of pentose derivatives, seems highly desirable. Following a suggestion by Prof. Parnas, I have undertaken to establish the conditions for colorimetric evaluation of Bial's reaction, particularly in its application to derivatives of pentose-5-phosphoric acid.

I have investigated the analysis of pentose in quantities of 1-25 γ, i.e. in amounts representing about 1/10 of those utilized by Dische and Schwarz. These quantities do not require the fixation of the indicator by addition of alcohol and ether or by cooling in ice water. On the other hand, heating to 100°C proved necessary for a much longer duration; Dische and Schwarz heated for 3 minutes, I heat for 20 minutes and obtain a constant green color. The principle of this method is very simple: It is most important that the concentration of HCl and $FeCl_3$ is kept constant, that the orcin reagent is freshly prepared and that the period of heating is sufficiently long.

Reagents. Concentrated hydrochloric acid is mixed with a concentrated solution of $FeCl_3$, so that the $FeCl_3$ amounts to 0.1%. This reagent must be stored in flasks with glass stoppers; cork stoppers make the reagent completely useless, merely yielding a positive Bial reaction. Orcin in the ratio of 10 mg to 1 cc solution is added to small amounts of the reagent prior to its use; the reagent may be used only within a few hours. Small test tubes with a diameter of 10 mm, calibrated at 2, 4 and 6 cc, are most practical for analytical purposes. They serve in the determination of small as well as larger quantities of pentose. The determination of small amounts is conducted as follows: 0.5 cc of the test solution and 0.5 cc of the reagent (the test liquid must be precisely measured!) are mixed and heated in an open beaker with boiling water. The tube is then filled to the 2 cc mark and, after cooling, measured in the Pulfrich photometer in a microcuvette with a stratal thickness of 5 cm, utilizing filter S61. This rule applies to amounts of from 1 to 3 γ of pentose. For larger quantities, 1 cc each of the test solution and the reagent are mixed, and the solution is filled to the 4 cc mark; measurements are made in normal cuvettes of the Pulfrich photometer with a stratal thickness of 1 cm. The calibration of 6 cc serves in the dilution of the final solution by 1½, in the event it proves too concentrated for analysis in a volume of 4 cc. After some experience, a preliminary test is indicated in order to ascertain whether a microcuvette or a normal cuvette should be used; and furthermore, which volume should be measured. It must be stressed particularly that turbidness, paling and variation in hue should not be feared in connection with quantities and concentrations indicated here; if larger amounts are to be analysed, the test solution must be diluted to that 1 cc does not contain more than 20 γ pentose.

I have tested the method with the following well-defined substances: Arabinose, barium of inosinic acid, and muscle adenylic acid. In the light of filter S61 (the second beam of the Pulfrich photometer passes through distilled water), extinction for concentrations 1-18 γ pentose in 1 cc of test solution is exactly proportional to free or bound pentose: It amounts to 0.0536 for 2 γ pentose in 1 cc of test liquid, with 1 cm stratal thickness. In one series (35 determinations), using pure, analysed barium of inosinic acid as standard substance, I found values

of 0.0536 ± 0.0008; with higher concentrations it drops and amounts to 0.049 at 24 γ , provided the test solution is heated to 100°C for 20 minutes with an equal volume of the reagent, then diluted to a twofold volume. If a few γ pentose are to be treated in 0.5 cc of a pentose solution, filled to 2 cc, photometric measurements are made in micro cuvettes with a stratal thickness of 5 cm; with higher contents of pentose the original solution is diluted with a doubled volume of water and treated identically; if larger amounts are available, 1 cc test solution and 1 cc reagent are taken, diluted to 4 cc and photometrically measured in cuvettes with a stratal thickness of 1 cm, or 0.5 cm if the color is intense.

Bial's reaction is disrupted by various factors; the presence of glucose, lead and nitrates is especially disturbing. Barium, often used as precipitant of nucleotides and nucleotide polyphosphoric acid, has no derogatory effect (*).


## Summary.

A simple method for the photometric evaluation of Bial's reaction in connection with pentoses and soluble pentose derivatives is described, suited for the determination of 1 to 20 of free or bound pentose, particularly for the determination of pentose in derivatives of adenosine-5-phosphoric acid.


## Literature.


(1) Cf. J.K. Parnas and B. Umschweif, Sur le dosage des pentoses dans les nuclectides, Bull. Soc. Chim. biol. (Fr.) 29, 325-335 (1937).
(2) G. Embden and M. Lehnartz, this journal 201, 149 (1931).
(3) Z. Dische and Schwarz, Mikrochim. Acta (Oe.) 11, 13-19 (1938).
(4) G. Hevesy, T. Baranowski, A.J. Guthke, P. Ostern and J.K. Parnas, Acta Biol. exper. (Pol.) 12, 34 (1938).


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(*) Footnote: The method described here may also be utilized if no Pulfrich photometer is available; a colorimeter may be used. A standard solution if produced, and Bial's reaction is developed in the test and standard solutions as indicated, then compared colorimetrically. A suitable standard substance which may be maintained in a pure and well-defined state, is offered by barium of inosinic acid. It may be extensively purified and tested for purity by the determination of Ba, P and N contained therein.