CXCII. THE MICRO-DETERMINATION OF PENTOSEs, FREE AND COMBINED. I.

By SAMUEL ANDREWS AND JOHN ALEXANDER MILROY.

*From the J. C. White Department of Biochemistry, The Queen's
University of Belfast.*

(Received July 21st, 1933.)

RECENT investigations have shown the widespread distribution of pentoses in combined form within the animal organism. In many cases the amount of material available for analysis is small and as it is frequently necessary to carry out rapidly a large number of analyses, it is advisable to have at one's disposal a simple and reliable micro-method. The majority of modern methods depend upon the conversion of pentoses into furfuraldehyde on distillation with 12–20 % hydrochloric acid [Gans *et al.*, 1888] and the determination of the amount of furfuraldehyde, either gravimetrically after condensation with phloroglucinol in acid solution or colorimetrically with aniline and acetic acid. The colorimetric method has the great advantage of detecting furfuraldehyde in extreme dilutions. Youngburg and Pucher [1924] examined carefully the conditions under which the reaction between furfuraldehyde and aniline acetate requires to be carried out in order to obtain accurate quantitative results. Pervier and Gortner [1923] had already directed attention to the advantages of steam-distillation in the removal of furfuraldehyde from the reaction mixture, and Hoffman [1927] employed this method with certain modifications, along with the colorimetric technique of Youngburg and Pucher. Steudel and Wohinz [1931] and many others have adopted, with slight modifications, the Hoffman method. Youngburg [1927] later replaced hydrochloric with phosphoric acid, removing the furfuraldehyde in the usual way by steam-distillation, and obtained the theoretical yield from *D*-xylose, 78.5 % of the theoretical from *D*-ribose, 64.9 % from *L*-arabinose and *D*-arabinose and 52.2 % from *D*-lyxose. He obtained very small yields (1–6 %) from various hexoses, disaccharides and polysaccharides, glucose furnishing 4.84 %.

The recent literature shows that even when all precautions are taken as regards removal of furfuraldehyde from the reaction mixture the yields obtained by different investigators from the same pentoses, free or combined, are far from uniform. All are agreed that the yield from the pyrimidine nucleotides is extremely small. The most recent results of furfuraldehyde yields from various purine nucleotides and nucleosides of yeast and of animal origin are those given by Steudel [1933].

The great difference in the furfuraldehyde yields between the yeast and the animal nucleotides is very striking and has naturally attracted a great deal of attention. The rise in the furfuraldehyde yield after deamination and removal of the phosphoric acid from the muscle adenylic acid has naturally been interpreted as due to a difference in the structure of the ribosephosphoric acid in the yeast and the muscle nucleotides. Embden and Schmidt [1929] were the first to show that there are striking differences between the muscle adenylic acid and the yeast

adenylic acid, among which may be mentioned solubility, optical rotation, rates of acid hydrolysis of the phosphoryl linkage, deamination (chemical and enzymic) as well as furfuraldehyde yields. Recently Klimek and Parnas [1932] have drawn attention to a difference as regards the formation of a complex copper salt with muscle adenylic acid and not with the yeast derivative.

That such differences are due in part to the position of the hydroxyl groups and the phosphoryl linkage of the pentose is extremely probable, but it is difficult to ascribe the very great disparity in the furfuraldehyde yields to such a cause.

The principle of the micro-method which we have employed during the last two years is briefly the production of furfuraldehyde from the action of acetic acid on pentoses, free and combined, in evacuated and sealed tubes kept at 170° for some hours, and the subsequent direct determination of the furfuraldehyde colorimetrically with aniline acetate. Numerous experiments were carried out in order to determine the conditions which led to maximum yields of furfuraldehyde.

(1) *Acids*. Many acids in different concentrations were tested, *e.g.* oxalic, phosphoric, acetic, acetic *plus* trichloroacetic and others. Finally it was found that acetic acid alone gave the best yields and that the most favourable concentration was approximately 50 %. This may not be the exact optimum, but such a concentration is more convenient to work with than one slightly stronger or weaker.

(2) *Temperature*. The optimum temperature was found to be about 170° in order that the maximum yield should be obtained within 2–4 hours.

(3) *Evacuation of the tubes*. Good evacuation (water- or oil-pump) is absolutely essential in order to avoid destruction of furfuraldehyde. Furfuraldehyde solutions heated under the standard conditions show no sign of any destruction, the recovery being complete.

(4) *Concentration of solutions*. Different concentrations covering a wide range were tested. Dilute solutions (1.0 cc. = 0.05 mg. furfuraldehyde) were found on the whole to be most suitable.

The technique of the method.

The aqueous solutions of the pentoses and of the purine nucleotides and their derivatives are usually made of such a concentration that 1.0 cc. of each solution should give a theoretical yield of 0.1 mg. furfuraldehyde. 50 cc. of the solution are then run into a 100 cc. flask and made up to 100 cc. with glacial acetic acid. The theoretical yield of furfuraldehyde from this 50 % acid solution is therefore 0.05 mg. per cc. Three to 4 cc. of the acid solution are then transferred with a capillary pipette into glass tubes 1 cm. in diameter and 16–18 cm. in length. The closed ends of the tubes are previously blown into small bulbs to facilitate evacuation. The tubes are then drawn out, evacuated and sealed. They are now covered with asbestos paper, placed in iron or lead tubes and heated in an electric oven at the temperatures and for the times found to be most suitable. The tubes are finally allowed to cool and opened with the usual precautions. The colorimetric determination is then made in the usual way, using as a standard the one employed by Youngburg and Pucher, the solutions for comparison being made up as follows:

I. The reaction mixture.	II. Series of fractions of standard furfuraldehyde solution (1.0 cc.–0.05 mg. furfuraldehyde).
1.00 cc. of the solution.	(1) 0.70 cc., (2) 0.80 cc., or (3) 0.90 cc. of standard furfuraldehyde.
0.5 cc. aniline.	0.5 cc. aniline.
3.5 cc. glacial acetic acid.	4.0 cc. glacial acetic acid.
Distilled water up to 10 cc.	Distilled water up to 10 cc.

After the reaction mixture and series of standards have stood for 15 minutes in the dark, they are compared, and the standard (1), (2) or (3), which most closely approximates to the colour of

the unknown is matched with it in the colorimeter. When dealing with reaction mixtures of widely varying furfuraldehyde concentrations, the final procedure of Youngburg and Pucher [1924, p. 745] was adopted in its entirety. All measurements and reaction mixtures were made with standard 1.0 cc. pipettes divided into 0.01 cc.

The stock furfuraldehyde solution is made up in water saturated with toluene so that the strength is 5.0 mg. furfuraldehyde per cc. and is stored in the ice-chest. From this the dilute standard can be prepared as required.

A few examples from a very large number are given in Table I in order to show why a concentration of 50 % acetic acid at 170° for a period of 3–4 hours has been chosen as most satisfactory.

Table I. *Yields of furfuraldehyde from pentoses, nucleotides and nucleosides under various conditions.*

Substance	Conc. of acetic acid	Temp. ° C.	% yield of furfuraldehyde after								
	%		1	1.25	1.5	1.75	2	3	4	5	6 hrs.
<i>d</i> -Xylose (a)*	50	170	66.5	—	—	—	84.5	86.7	92.9	93.1	80.6
„ (b)	50	170	—	—	—	—	86.0	88.8	91.8	—	—
„ (b)	50	180	76.4	85.1	88.9	82.2	80.5	—	—	—	—
„ (a)	25	170	—	—	—	—	—	72.1	77.1	63.1	—
„ (a)	75	170	50.3	—	—	—	64.5	—	—	52.7	—
<i>l</i> -Arabinose (a)	50	170	—	—	—	—	62.3	73.4	81.3	68.6	—
„ (b)	50	170	—	—	—	—	69.8	70.2	70.2	—	—
„ (b)	50	180	—	—	76.4	—	63.8	—	—	—	—
Yeast adenylic acid (a)	50	170	65.9	—	—	—	84.0	85.5	95.1	83.5	76.9
„ (b)	50	170	—	—	72.6	—	81.1	82.7	81.6	71.4	—
„ (b)	50	180	67.0	84.5	74.4	—	—	—	—	—	—
Yeast adenosine (a)	50	170	74.5	—	—	—	—	89.2	—	—	82.5
„ (b)	50	170	—	—	—	—	75.0	85.6	82.1	72.6	—
„ (b)	50	180	72.4	76.3	71.4	—	—	—	—	—	—
Yeast guanosine (a)	50	170	—	—	—	—	64.4	—	—	—	—
„ (b)	50	170	—	—	—	—	70.9	75.5	—	68.2	—
„ (b)	50	180	—	72.4	—	—	68.2	—	—	—	—
Yeast guanylic acid (b)	50	170	—	—	—	—	67.7	78.9	72.6	—	—
„ (b)	50	180	72.6	72.9	71.4	—	—	—	—	—	—
Muscle adenylic acid I (b)	50	170	60.2	—	—	—	73.0	62.4	59.8	—	—
„ II (b)	50	170	—	—	—	—	67.5	75.1	61.6	—	—
Ba inosinate (b)	50	170	—	—	—	—	76.0	—	—	—	—
Ca adenosinetriphosphate (heart muscle)	50	170	62.5	—	—	—	55.5	55.5	62.5	—	—
Ditto (rabbit's skeletal muscle)	50	170	53.8	—	—	—	53.1	49.1	—	—	—
Ditto (frog's skeletal muscle)	50	170	44.6	—	—	—	—	—	—	—	—

* (a) and (b) signify concentrations of substance yielding theoretically 0.1 and 0.05 mg. furfuraldehyde per cc. of solution.

Note. We are indebted to Dr Steudel for the adenylic acid (I), to Dr Henning for the same substance (II), to Dr Levene for the Ba inosinate and to Dr Strain for the specimen of adenosinetriphosphate from the heart muscle of the horse; to all of whom we offer our best thanks.

The furfuraldehyde yields from the muscle nucleotides are very much higher than those which have been obtained by others using the distillation method [cf. Steudel, 1933], so that differences between the nucleotides of yeast and of muscle origin are by no means so evident as one had been led to expect. Further work on this subject is being carried out.

Furfuraldehyde yields from the pyrimidine nucleotides.

A specimen of cytidylic acid supplied by the British Drug Houses Ltd., gave the following yields on heating with 50 % acetic acid at 170°.

3 hours, 27.5 %; 5 hours, 29.4 %; 7 hours, 28.2 %.

Furfuraldehyde yields from hexoses and sucrose.

In dilute solutions there was practically no furfuraldehyde production, the colour development being so slight as to make the readings impossible. In much more concentrated solutions than those used for pentoses, glucose and sucrose gave apparently small yields, 4.18 and 4.56 % respectively. It is probable however that the colour development is due to the presence of hydroxymethyl-furfuraldehyde. Slight humin formation also masks the colour change and renders colorimetric comparison unsatisfactory.

It is evident that there are variations in the maximum yields of furfuraldehyde obtained by this method, depending as always on the difficulty of obtaining maximum production of furfuraldehyde with minimum destruction, but in our opinion the same is true of all acid distillation methods. This is shown by a comparison of the yields obtained by Hoffman and Steudel respectively, using approximately the same procedure. The method here described can be readily carried out and one may easily compare and determine with accuracy the yields under exactly the same conditions from a pure pentose of known concentration and pentose in some form of combination. With the qualifications which follow from the above remarks, one may give provisionally as approximate maximum yields of furfuraldehyde from pentoses, *etc.* which have been examined the following values.

	%
<i>d</i> -Xylose	90-93
<i>l</i> -Arabinose	75-81
Yeast adenylic acid	85-95
Yeast adenosine	80-90
Yeast guanosine	70-75
Yeast guanylic acid	70-80
Muscle adenylic acid	70-75
Muscle inosinic acid	75
Muscle (skeletal) adenosinetriphosphoric acid ...	50-53
Muscle (cardiac) adenosinetriphosphoric acid ...	55-63
Cytidylic acid	27-29
Hexoses (in dilute solution)	0

SUMMARY.

A micro-method for the determination of pentoses, free and combined, is described, and the conditions under which maximum yields may be obtained are discussed.

By this method the furfuraldehyde yields from the purine nucleotides of muscle are found to be much higher than those obtained by the usual steam-distillation method from a hydrochloric acid reaction mixture.

REFERENCES.

- Embden and Schmidt (1929). *Z. physiol. Chem.* **181**, 130.
 Gans, Stone and Tollens (1888). *Ber. deutsch. chem. Ges.* **21**, 2148.
 Hoffman (1927). *J. Biol. Chem.* **73**, 15.
 Klimek and Parnas (1932). *Biochem. Z.* **252**, 392.
 Pervier and Gortner (1923). *Ind. Eng. Chem.* **15**, 1167, 1255.
 Steudel (1933). *Z. physiol. Chem.* **216**, 77.
 — and Wohinz (1931). *Z. physiol. Chem.* **100**, 82.
 Youngburg (1927). *J. Biol. Chem.* **73**, 599.
 — and Pucher (1924). *J. Biol. Chem.* **61**, 741.