The Tautomeric Conversion of *p*-Hydroxyphenylpyruvic Acid

By H. A. PAINTER AND S. S. ZILVA (Member of the Scientific Staff, Medical Research Council)
Lister Institute, London

(Received 7 February 1947)

It is well known that *p*-hydroxyphenylpyruvic acid in aqueous solution undergoes tautomeric change. According to Bougault & Hemmerlé (1915) the chemically related phenylpyruvic acid exists in the solid state in the enolic form, but in aqueous solution it is gradually converted into the ketonic tautomer. In our attempt to prepare standards for the determination of *p*-hydroxyphenylpyruvic acid in connexion with another investigation (Painter & Zilva, 1947) we soon realized that a similar change was taking place in the case of this acid, since the colour given by the Penrose & Quastel (1937) keto test became more intense as time progressed, whilst at the same time the indophenol titration values, indicating the amount of the enolic form, fell. This rendered it advisable to study the tautomeric conversion in some detail in order to ensure a more intelligent interpretation of the results obtained in our main inquiry. As will be seen, various observations were made in this connexion which invited further investigation but these were not pursued as they had no bearing on our immediate purpose.

METHODS

Reagents. (1) *p*-Hydroxyphenylpyruvic acid. The acid was prepared according to the method of Neubauer & Fromherz (1910) as modified by Raper & Wormald (1925). The standard solutions of *p*-hydroxyphenylpyruvic acid (0.01%, w/v) were made up immediately before use.

(2) 2:4-Dinitrophenylhydrazine reagent, see Penney & Zilva (1943).

(3) *N*-NaOH (A.R.).

Methods. The Penrose & Quastel test was carried out by diluting 5 ml. of the test solution to 10 ml. with distilled water. 1 ml. of the hydrazine solution was then added and the mixture shaken. The reaction was allowed to proceed for 30 min. at room temperature, after which time 5 ml. of *N*-NaOH were added and the solution diluted further to 25 ml. with distilled water. On the addition of the NaOH a brown colour was produced which rapidly disappeared. The measurement of the permanent red colour was carried out as described by Penney & Zilva (1943).

The Briggs test was carried out according to the procedure of Medes (1932). Our standard solution, however, was prepared by dissolving *p*-hydroxyphenylpyruvic acid in normal guinea-pig urine and was allowed to stand for 1 hr. The solution was then diluted 1 in 10 and after the addition of the reagents the colour was measured by the photoelectric colorimeter after 5 min.

RESULTS

Effect of pH on the tautomeric conversion of p-hydroxyphenylpyruvic acid in aqueous solution. These results are summarized in Fig. 1, from which it will

Fig. 1. Effect of pH on enol-keto conversion in aqueous solution at 15°. ○—○ pH 9.0, adjusted in this and all subsequent experiments with 0.01 *N*-NaOH. ○—○ Brought to pH 1.0 with HCl immediately after adjusting to pH 9.0 with 0.01 *N*-NaOH. □—□ pH 7.4, adjusted with 0.01 *N*-NaOH. ×—× pH 4.0, aqueous solution of the keto acid. ◇—◇ pH 1.0, adjusted with HCl. (A) Solution adjusted to pH 1.0 at this point. In this and all subsequent experiments each test solution contained 0.5 mg. of solid *p*-hydroxyphenylpyruvic acid.

be seen that the change from the enolic to the ketonic form proceeds rapidly in alkaline solution (pH 9.0) and very slowly in acid and neutral media. At pH 7.4 the velocity of the conversion is of the same order as that at lower pH values. Although the points on the curves in Fig. 1 are those of the ketonic tautomer as determined by the Penrose & Quastel reaction, corresponding points could be obtained when the enolic form was determined in the same solution by titration with dichlorophenolindophenol and the results subtracted from the total content, thus confirming the values (Fig. 2). The results obtained by the Penrose & Quastel reaction can lay claim to higher accuracy since there was a lag in the end-point of the indophenol titrations and consequently figures for the enolic form tended to be on the low side. It may be pointed out that whilst the indophenol titration indicated the presence of

only 1–2 % of the enolic form at pH 9.0, when the solution was adjusted to pH 1.0 and allowed to stand for several days this value was raised to 10 %. The reconversion is evidently slow since it could not be demonstrated after 40 min. (see the interrupted line in Fig. 1, *A* to *B*). When however the solution at pH 9.0 was brought up immediately to pH 1.0 by the addition of HCl, bringing it up approximately to the pH under which the Penrose & Quastel test is carried out, the rate of conversion was simultaneously reduced to that which is characteristic for this particular pH.

Fig. 2. Enol-keto conversion at pH 9.0 at 12°. □—□ By indophenol titration. ×—× By the Penrose & Quastel test.

Fig. 3. Effect of temperature on enol-keto conversion at pH 9.0 as determined by the Penrose & Quastel reaction. △—△ 25°. ○—○ 15°. □—□ 10°. ◇—◇ 3°.

Effect of temperature on the tautomeric conversion of p-hydroxyphenylpyruvic acid at pH 9.0. It is seen from Fig. 3 that, as was to have been expected, the

rate of conversion increases with increasing temperature and that experiments can conveniently be carried out at room temperature. It was further found that when the log. of the percentage of the enolic tautomer present was plotted against the time the solution had stood, the curves approximated to straight lines thus showing that it was a reaction of the first order.

Tautomeric conversion of p-hydroxyphenylpyruvic acid in urine and tissues. Crystalline *p*-hydroxyphenylpyruvic acid was added to normal undiluted guinea-pig urine which was previously adjusted to pH values of 4.0, 7.4 and 9.0, and the acid was then determined by the Penrose & Quastel reaction after 30 min. At the two highest pH values the maximum formation of the ketonic form was already reached by that time. At pH 4.0 the maximum conversion value was reached after 100 min., i.e. at a much higher rate than in aqueous solution in which the maximum value was reached only after 1–2 days (see Fig. 1). It is, therefore, evident that the conversion from the enolic to the ketonic tautomer proceeds at a very much higher rate in neutral and acid urines than it does in the corresponding aqueous solutions and consequently *p*-hydroxyphenylpyruvic acid may usually be expected to be present in the ketonic form.

In another experiment when the blood, liver, urine, stomach and large intestine contents from guinea-pigs, in which *p*-hydroxyphenylpyruvic acid was expected on dietetic grounds to be present, no difference in the Penrose & Quastel values could be observed when the metaphosphoric acid extracts of the material and the untreated urine were adjusted to pH 4.0 and 9.0. It would appear that *p*-hydroxyphenylpyruvic acid is also present in the tissues as the ketonic tautomer.

In view of the fact that the Briggs reaction is very often used in the determination of *p*-hydroxyphenylpyruvic acid it was advisable to compare the results obtained by this method with those obtained by the Penrose & Quastel procedure and by titration with indophenol. Determinations by the three methods were therefore carried out on urines diluted with water to concentrations which were found to be convenient for our main investigation and to which 0.01 % of *p*-hydroxyphenylpyruvic acid was then added. It will be seen from Table 1 that the Briggs and the indophenol titration procedures were not a reliable index of the relative quantities of the two tautomers present. Whilst the Penrose & Quastel method showed a progressive conversion to the ketonic form which was found to be complete after 19 hr., the Briggs reaction, which on theoretical grounds should have indicated the enolic form, accounted during the first hour for almost the entire *p*-hydroxyphenylpyruvic acid added and fell only after 19 hr. to about half the original value.

Table 1. *p*-Hydroxyphenylpyruvic acid found in diluted guinea-pig urine containing 0.01 % (w/v) of the compound
(0.5 mg. present in each test)

Dilution	1/10			1/100		
	By indophenol titration (mg.)	By Briggs test (mg.)	By Penrose & Quastel test (mg.)	By indophenol titration (mg.)	By Briggs test (mg.)	By Penrose & Quastel test (mg.)
0 min.	0.25	0.41	0.14	0.25	0.40	0.03
20 min.	0.24	0.47	0.19	0.25	0.44	0.05
40 min.	0.23	0.47	0.25	0.25	0.44	0.07
60 min.	0.21	0.44	0.31	0.25	0.44	0.10
19 hr.	0.01	0.22	0.50	0.05	0.25	0.38

Comparative experiments on the determination of *p*-hydroxyphenylpyruvic acid by the Briggs method in aqueous solution with and without the addition of salts have shown that the problem required a detailed investigation before the method could be used with any degree of reliance. The same applied to the indophenol titration procedure except, as already mentioned, in aqueous solutions in the absence of salts, when satisfactory results could be obtained in the assessment of the enolic form. We did not, however, investigate the matter further, as we had convinced ourselves that the Penrose & Quastel reaction was reliable enough for our purpose and supplied us with the necessary information.

The discrepancy between our results and those of Medes (1932), who was unable to isolate the phenylhydrazone, semicarbazone, or bisulphite compound from the urine of a patient excreting *p*-hydroxyphenylpyruvic acid cannot at present be explained.

SUMMARY

1. In aqueous solution the conversion of *p*-hydroxyphenylpyruvic acid from the enolic to the ketonic form was greatly accelerated in alkaline solution.

2. In guinea-pig urine, the conversion to the ketonic form took place more rapidly than in water at all values of pH investigated. Probably the conversion proceeds in guinea-pig tissue at a rate similar to that in urine. In both cases approximately 99 % of the *p*-hydroxyphenylpyruvic acid was found to exist in the ketonic form.

3. The determination of *p*-hydroxyphenylpyruvic acid in guinea-pig urine by the Briggs reaction or by indophenol titration does not yield satisfactory results, but the Penrose & Quastel reaction is reliable.

One of us (H.A.P.) is indebted to the Medical Research Council for a whole-time grant.

REFERENCES

- Bougault, J. & Hemmerlé, R. (1915). *C.R. Acad. Sci., Paris*, **160**, 100.
 Medes, G. (1932). *Biochem. J.* **26**, 917.
 Neubauer, O. & Fromherz, K. (1910). *Hoppe-Seyl. Z.* **70**, 326.
 Painter, H. A. & Zilva, S. S. (1947). *Biochem. J.* **41**, 511.
 Penney, J. R. & Zilva, S. S. (1943). *Biochem. J.* **37**, 39.
 Penrose, L. & Quastel, J. H. (1937). *Biochem. J.* **31**, 266.
 Raper, H. S. & Wormall, A. (1925). *Biochem. J.* **19**, 84.

The Ultraviolet Absorption Spectrum of Human Cerebrospinal Fluid

By M. H. HACK, *Department of Psychiatry, University of Illinois College of Medicine, Illinois Neuropsychiatric Institute, Chicago, Illinois*

(Received 21 February 1947)

Ultra-violet absorption spectra for specimens of human cerebrospinal fluid are illustrated in Fig. 1. The curves differ in shape and in the intensity and wave-length of maximum absorption. This suggests that there are at least two absorbing constituents. The data to be presented indicate that they are predominantly protein and ascorbic acid.

EXPERIMENTAL

All samples were portions of the whole of the clear and colourless cerebrospinal fluids (C.S.F.) withdrawn for pneumoencephalography, and the examination of each sample was completed within 30 min. The C.S.F. was obtained from an unselected group of patients (34 cases) from the neurological service. Our data are insufficient to