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.

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The Ultraviolet Absorption Spectrum of Human Cerebrospinal Fluid

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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

warrant any conclusion relating the spectrum to the pathologic condition, consequently this report is concerned only with the interpretation of the form of the spectrum.

The c.s.f. was diluted with equal parts either of 0.85% NaCl solution or of Sørensen phosphate buffer solution of pH 7.6 and compared with an appropriate blank in a Beckman quartz spectrophotometer at wave-lengths from 230 to 300 $m\mu$. in steps of 2 $m\mu$. The curves obtained by dilution of the c.s.f. with NaCl solution were identical with those diluted with phosphate buffer.

Fig. 1. Absorption spectra of c.s.f. from six patients. Each sample was diluted with an equal part of 0.85% sodium chloride solution.

RESULTS

The protein precipitated from 2 ml. of c.s.f. by 2 ml. of 10% trichloroacetic acid and dissolved in 4 ml. of saline showed maximum absorption at 280 $m\mu$. (Fig. 2, *E*). These precipitates contained no phosphorus (Ma, Haas & Horecker, 1940) and consequently no nucleoprotein, nucleic acid or nucleotide. The globulin precipitated from 2 ml. of c.s.f. by 2 ml. of a saturated aqueous ammonium sulphate solution and dissolved in 4 ml. of saline, had the same spectrum except that all extinctions were somewhat less. The wave-length of the maximum of these spectra was independent of the pH, except when the solution was very alkaline. Then a maximum, attributable to tyrosine (Stenstrom & Reinhard, 1925), appeared at 290 $m\mu$.

c.s.f. filtered at a pressure of 100–140 mm. Hg through Bechhold-Koenig porcelain crucibles (Alexander, 1926) impregnated with Parlodion (Mallinckrodt) and diluted with an equal part of phosphate

Fig. 2. Absorption spectrum: *A*, of c.s.f. diluted with an equal part of phosphate buffer solution of pH 7.6; *B*, of fresh ultrafiltrate (shown by titration to contain 1.3 mg. of ascorbic acid/100 ml.); *C*, of *D* to which ascorbic acid had been added to make a concentration of 1.1 mg./100 ml.; *D*, of ultrafiltrate *B* after exposure to room air for 120 min.; *E*, of trichloroacetic acid precipitate from 2 ml. of *A* dissolved in 4 ml. of 0.85% sodium chloride solution; *B + E*, arithmetic addition of *B* and *E*.

Fig. 3. Absorption spectra: *A*, of ascorbic acid in 0.2N-HCl; *B*, of ascorbic acid in phosphate buffer solution of pH 7.6.

buffer of pH 7.6 gave an absorption spectrum similar to that of ascorbic acid at the same pH (Figs. 2, *B* and 3, *B*). This spectrum disappeared com-

pletely, usually within 60 min., when the ultrafiltrate was exposed to air at room temperature (Fig. 2, *D*). When to such a preparation an appropriate quantity of ascorbic acid was added, its spectrum was restored to the form of the original ultrafiltrate (Fig. 2, *C*, *B*). The amount of ascorbic acid required was that determined by titration of the original ultrafiltrate with 2:6-dichlorophenol-indophenol.

The arithmetic addition of the protein spectrum (Fig. 2, *E*) and of the ultrafiltrate spectrum (Fig. 2, *B*) gives a curve (Fig. 2, *B* + *E*) which reasonably approximates the c.s.f. curve (Fig. 2, *A*) if allowance is made for the fact that the spectrophotometric examination requires time, during which the ascorbic acid is being oxidized and consequently disappears from the spectrum.

Fig. 4. *A*, absorption spectrum of an oxidized ultrafiltrate of c.s.f. (of *D*, Fig. 2) to which uric acid had been added to a final concentration of 0.5 mg./100 ml.; *B*, typical pyrimidine absorption spectrum obtained from 1.2 mg. aneurin hydrochloride in 100 ml. of a phosphate buffer solution of pH 7.6.

It has been reported (Spiegel-Adolf, Henny, Wycis & Spiegel, 1945; Spiegel-Adolf & Spiegel, 1945; Spiegel-Adolf, Wycis & Spiegel, 1946) that the normal c.s.f. of dogs show 'end absorption only', whereas the c.s.f. of dogs after electrically induced convulsions and of dogs, cats and men after concussion show spectra apparently identical with those reported here. The oxidation of ascorbic acid may account for the reported lack of maxima in c.s.f. of the control animals. Because the maximum at

265 mμ. reported in the experiments of Spiegel-Adolf *et al.* (1945, 1946) had been attributed to the purine-pyrimidine moiety of thymonucleic acid on the basis of traces of colour obtained by the diphenylamine method (Dische, 1930), the effect of ascorbic acid on this reaction was investigated. The addition of ascorbic acid in amounts similar to those found in c.s.f., to an extract of lymphatic tissue (cat) suppressed the reaction with the diphenylamine reagent, if the extract by itself gave only a trace of colour. Moreover, the production of colour in controls was prevented by such concentrations of ascorbic acid.

Uric acid in concentrations up to 1 mg./100 ml. is said to be a normal constituent of c.s.f. (Katzenelbogen, 1935). Such concentrations of uric acid readily passed through the ultrafilter and were demonstrated in the filtrate. Fig. 4, *A* shows the spectrum of a c.s.f. ultrafiltrate, minus the labile substance (ascorbic acid), to which sufficient uric acid had been added to give a concentration of 1 mg./100 ml. The resulting spectrum is that of uric acid and is different from any so far obtained from c.s.f. ultrafiltrates.

All patients received sodium pentothal intravenously not more than 15 min. prior to the withdrawal of the c.s.f. Since the barbiturates have an ultra-violet absorption spectrum (Stuckey, 1941) indistinguishable from that of uric acid, it is apparent that the sodium pentothal cannot be considered an interfering substance.

SUMMARY

1. Ascorbic acid and phosphorus-free protein preponderate in the absorption of ultra-violet light by human cerebrospinal fluid.
2. Ascorbic acid was shown to prevent the colour reaction of Dische's reagent with thymonucleic acid.
3. The presence of uric acid could not be demonstrated in the spectra of c.s.f. ultrafiltrates.
4. The use of intravenous sodium pentothal as an anaesthetic did not influence the spectrum of the c.s.f.

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