XXIII. THE CRITICAL SOLUTION POINT OF URINE.

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In order to determine the efficiency of a kidney as an excretory organ, the most exact method is the determination of the total solid content of the urine (neglecting colloids), as shown by the depression of its freezing-point below that of pure water. The examination of urine and blood by this method has proved very useful and reliable, but the time required for carrying it out constitutes a serious drawback when only one or two specimens are to be examined.

The following method was brought forward by one of us [Atkins, 1908] in the hope that it might be of service in cases where great accuracy is not essential and saving of time an advantage. Since no special apparatus is required and ice is not employed the usefulness of critical solution point determinations as a method of clinical examination is much increased especially for work in warm climates.

The present paper gives an outline of the method of such determinations, as the subject is a comparatively recent one. The data of the former paper are extended and the influence of the various constituents of the urine upon the critical solution point is traced out.

OUTLINE OF CRITICAL SOLUTION POINT THEORY.

(a) *Two pure substances.* Consider two liquids not completely miscible at room temperatures, such as phenol and water. If small quantities of these two substances are shaken together a white emulsion is obtained; this on standing separates into two layers, a lower one, consisting of phenol with some water dissolved in it, and an upper one of water with some dissolved phenol. If the temperature be now raised, the quantity of each dissolved in

the other increases till a temperature is reached at which the two become completely miscible; on cooling even a tenth of a degree separation again occurs, a white emulsion is formed, and on standing the two layers separate out as before. The temperature, at which the two layers become homogeneous, depends upon the proportions of water and phenol initially present, and rises in the case of water as the amount of phenol is increased until a mixture is reached which remains in the two phases at a higher temperature than any other mixture of the two pure components.

The composition of this mixture is termed the "critical concentration," and the temperature at which the liquid becomes homogeneous is called the "critical solution temperature." This temperature is accordingly that at which the two phases have the same composition and the same properties. At the critical concentration and critical solution temperature the volumes of the two phases, water in phenol and phenol in water, are equal.

At the critical temperature the two phases have the same surface tension, and are therefore completely miscible; at a very slightly lower temperature the surface tensions of the two phases are almost equal, consequently the meniscus between them is flat. As the homogeneous mixture cools, at a temperature very little above the critical temperature a beautiful opalescence appears, which is blue by reflected light and brown by transmitted.

If now a series of mixtures of water and phenol be taken and the temperatures at which they become homogeneous be determined, and the values thus obtained be plotted as ordinates, taking the corresponding percentage compositions of the mixtures as abscissae, a curve is obtained, the highest point of which is the critical solution point [Fig. 1, Schreinemakers, 1899]. This curve is somewhat flat at the top, hence the composition of the mixture may be slightly altered to one side or the other of the critical concentration without altering the temperature of miscibility more than about 0.1°C . The blue opalescence is still obtained, but it is not as beautiful as at the critical solution point itself.

(b) *Effect of a third substance.* The addition of a very small quantity of a third substance to any mixture of water and phenol raises the temperature at which the two phases become homogeneous, provided the added substance be freely soluble in water and almost or quite insoluble in phenol, or *vice versa*. If the added substance be freely soluble in both, then the temperature at which a homogeneous mixture is obtained is lowered. Thus by adding a small quantity of sodium oleate to a mixture of phenol and water, it is possible to obtain a very strong solution of phenol, homogeneous at room temperatures. Lysol, the disinfectant, is prepared in a similar way.

Fig. 1. In part after Schreinemakers. The dotted line shows the data for urine given in Table I.

In the case of a substance much more soluble in water than in phenol, if curves (Fig. 1) constructed as described be plotted when one of the pure liquids is replaced by a dilute solution of the third substance in that liquid, the general outline of the original curve is followed, the ordinates of temperature being everywhere greater and markedly so in what is termed the crest region. In each of these curves there is a critical point, but it no longer coincides with the maximum temperature as is the case when two pure components are concerned; accordingly it must be recognised by the beautiful opalescence and equality of volume of the layers.

The rise of critical temperature for dilute solutions, say one per cent., is roughly proportional to the number of gram-molecules of the substance added, with regard to its own solvent. This rise is very considerable, for an amount of solid sufficient to depress the freezing point of water 0.5° may raise the critical temperature of a mixture of water and phenol by as much as $4-5^{\circ}$. This rise therefore which can readily be determined to 0.1° gives about the same accuracy as a freezing point measured to 0.01° with greater facility and simple apparatus.

APPLICATION OF THE METHOD TO URINE.

If in the preceding experiments urine, which is an aqueous solution of certain compounds, be substituted for water, a considerable rise in the critical solution temperature is found. This varies with the concentration of the urine.

A specimen of normal urine was examined throughout the whole curve, which was found to be similar to that given by a single substance when added to a water and phenol mixture, as shown by the broken line in Fig. 1. The method of procedure was as follows:—Weighed quantities of phenol and urine were mixed in test tubes, and the temperatures at which they became homogeneous were noted. A thermometer graduated in tenths from $50-100^{\circ}$ is suitable, but for clinical work one graduated in degrees is sufficiently accurate. It is not so rough a measurement as it may appear to those accustomed to Beckmann thermometers, for the temperature differences to be measured are nearly ten times as great as those met with in freezing point work. The data from which the broken line in Fig. 1 was constructed are given below.

Inspection of the above table shows that the critical solution point of the phenol-urine mixture is at 79.7° , the critical concentration being 36 per cent. phenol. The critical solution point is by no means at the crest of the curve,

but is recognised by the characteristic opalescence. The critical solution temperature of the phenol-water mixture similarly determined is 66·5°. Thus the rise produced by the solids dissolved in the urine amounts to 13·2°.

TABLE I.

Percentage weight, phenol	Percentage weight, urine	Temperature of homogeneity	Notes
22·42	77·58	73·4	—
31·31	68·69	78·0	—
33·99	66·01	78·3	Opalescence.
34·28	65·72	78·7	Good opalescence.
35·93	64·07	79·7	Best opalescence.
36·48	63·52	80·0	Good opalescence.
39·78	60·22	81·2	No opalescence.
48·45	51·55	84·3	—
54·22	45·78	84·7	Maximum temperature = crest
60·06	39·94	82·2	point.

Having established the similarity between the curve afforded by urine and by a single added substance it is quite sufficient to determine the critical solution point of any urine with phenol in the following manner. Dry crystalline phenol is placed in a test tube to a depth of about 1 cm., and

TABLE II.

Normal Urine.

No.	Δ	R_c	$\frac{R_c}{\Delta}$
1	0·98°	7·7°	7·86
2	1·07	9·4	8·78
3	1·07	9·7	9·06
4	1·16	8·5	7·34
5	1·41	10·4	7·39
6	1·45	11·6	8·00
7	1·47	11·3	7·68
8	1·47	12·5	8·50
9	1·63	11·9	7·28
10	1·67	12·3	7·36
11	1·71	14·0	8·19
12	1·73	12·5	7·21
13	1·79	15·0	8·38
14	1·85	16·3	8·80
15	1·87	11·8	6·31
16	1·87	15·9	8·71
17	1·87	15·5	8·28
18	1·87	16·0	8·55
19	1·92	15·3	7·96
20	2·48	17·4	7·00
Mean			7·93

slightly more than enough urine to cover it is added from a pipette. The mixture is warmed and, if the critical opalescence does not appear on cooling, urine is added in small quantities till a good opalescence is shown. In this manner one proceeds from a too concentrated mixture of phenol to one of the critical composition without the trouble of weighing the components. These determinations can be very rapidly made, the time depending on how nearly the critical concentration has been reached at the first trial.

A number of normal urines were examined by this method, their freezing points being also obtained in the usual manner, and opposite the depression Δ the rise in critical solution temperature R_c has been tabulated. A third column gives the ratio R_c/Δ , as shown in Table II.

It is clear from the above figures that there is a roughly constant ratio between the two sets of numbers. The fluctuations however are considerably beyond the range of any likely experimental error, though with highly coloured urines it is not easy to decide as to the most characteristic opalescence. (In practice it is permissible, within limits, to dilute such specimens, but in obtaining the above results this was avoided.) Thus in Nos. 15-18, which have the same freezing point, it is seen that in three cases the critical solution points are within 0.5° , whereas the fourth is widely divergent. As it seemed probable that such cases were to be explained by some appreciable differences in the relative proportions of the constituents of the urines in question, the most important of these were estimated in a number of specimens. Furthermore, in order to study the effect of one constituent alone, this was added in varying quantities to the analysed urine. Table III shows the analyses of Nos. 4, 5, 6, 10, 15 of Table II, expressed as grams per 100 cc.

TABLE III.

No.	Cl'	PO ₄ '''	SO ₄ ''	NH ₄	Urea	Uric acid	Total N	Δ	R_c	$\frac{R_c}{\Delta}$
4	0.351	0.225	0.173	0.059	1.612	0.015	0.909	1.16	8.5	7.34
5	0.270	0.289	0.238	0.056	1.902	0.021	1.074	1.41	10.4	7.39
6	0.721	0.227	0.138	0.049	1.542	—	0.843	1.45	11.6	8.00
10	0.756	0.222	0.236	0.078	1.854	—	1.080	1.67	12.3	7.36
15	0.596	0.270	0.251	0.097	2.252	—	1.397	1.87	11.8	6.31
Dextrose										
10 a	0.453	0.133	0.141	0.047	1.113	2.02	0.648	1.21	10.0	8.26
10 b	0.226	0.066	0.070	0.023	0.556	3.53	0.324	0.86	6.8	7.86

The analyses were made in the usual manner as described by Neubauer-Huppert, *Analyse des Harns*. The first urea estimations were made by the method of Benedict and Gephart [1908] while in the later ones Folin's new method was employed [1912]. The latter gives slightly lower results than the former, owing probably to the fact that in it uric acid is not hydrolysed.

In considering what effect the various constituents will have upon the critical solution point, it must be remembered that whereas the salts are far more soluble in water than in phenol, urea is soluble in both. It has been shown, moreover, that urea forms a compound with phenol; this, however, is very largely dissociated [J. C. Philip, 1903]. Accordingly, though an increase of urea causes an increase in the depression of freezing point, it causes a decrease in the critical solution point temperature as shown later on.

The analyses recorded show that Nos. 4, 5, 10 are very similar in composition, the only notable difference being the larger chloride content of No. 10. It may be seen that the ratio R_c/Δ is for these specimens quite constant. In No. 6, while the chloride percentage is high, that of urea is relatively low and R_c/Δ has increased; whereas, in No. 15, the diminished amount of chloride and the large rise in urea and total nitrogen combine to lower R_c/Δ very considerably.

To study the effect of increase in chloride, the sodium salt was added in various proportions to No. 4, as shown in Table IV.

TABLE IV.

Chloride alone varied.

No.	Cl'	Δ	R_c	$\frac{R_c}{\Delta}$
4	0.351	1.158°	8.5°	7.34
4x	0.703	1.472	12.8	8.70
4y	1.055	1.797	18.0	10.02
4z	2.111	2.780	29.7	10.69

Here the effect of the chloride in raising the critical solution point is well brought out by the increased values of R_c/Δ . Separate studies of the other salts were not made, as, owing to their solubility relations, their effects must be much the same as that of the chloride. Also they seem to vary less and occur in smaller quantities.

The addition of pure urea was next studied by adding weighed quantities to No. 5. This, of course, alters the amount of total nitrogen also.

TABLE V.

Urea and total nitrogen varied.

No.	Urea	Total nitrogen	Δ	R_c	$\frac{R_c}{\Delta}$
5	1.902	1.074	1.408°	10.4°	7.39
5x	2.446	1.328	1.527	10.2	6.68
5y	2.992	1.583	1.721	9.5	5.52
5z	4.628	2.345	2.157	7.8	3.62

Thus while urea alone lowers the critical solution temperature, its effect in urine is to lessen the rise produced by the saline bodies. Consequently a specimen which contains more urea than is usual, will have a smaller value for R_c/Δ . Thus the fluctuations in this ratio, as seen in Table II, are explained by the mutually counteracting effects of the salts and urea upon the critical solution point.

PATHOLOGICAL URINES.

It remains to consider to what extent pathological conditions of the urine may alter the constancy of the ratio R_c/Δ . The simplest case is, perhaps, that in which the urine is normal in its constituents, but dilute. Table VI shows the results obtained by examining both normal urines after dilution and pathological urines.

TABLE VI.

Normal diluted and pathological dilute urines.

No.	Nature of specimen				Δ	R_c	$\frac{R_c}{\Delta}$
21.	Normal diluted	...	...	...	0.33°	3.2°	9.70
22.	" "	...	...	...	0.44	4.7	10.70
23.	" "	...	...	...	0.87	7.6	8.74
24.	Pathological	...	...	...	0.70	7.0	10.00
25.	From right kidney of A, by catheterisation				0.47	4.5	9.58
26.	From left kidney of A, " "				0.73	7.0	9.58
27.	From both kidneys of B, morning; nothing but water since previous evening				0.63	5.5	8.73
28.	From right kidney of B, by catheterisation at 1 p.m. Conditions as above. Albumin present				0.24	2.5	10.42
29.	From left kidney of B, by catheterisation. Conditions as above. No albumin				0.30	3.0	10.00
						Mean	9.72

These figures show that in very dilute urines the salts have a somewhat greater effect in raising the critical solution temperature than they have in those of average concentration. It is, however, to be noted that with such small values of R_c , an error of 0.1° affects the value of R_c/Δ very considerably. The similarity of R_c/Δ in Nos. 25 and 26, and also in Nos. 28 and 29, is noteworthy, and gives reason for supposing that the two kidneys were in each case excreting fluids almost identical in composition though not in concentration. The identity in composition of the urine from the right and left kidney is now completely established as a normal occurrence, though the earlier work seemed to point to the opposite conclusion [Casper and Richter].

A small number of other pathological urines were examined, as shown in Table VII.

TABLE VII.

No.	Nature of specimen	Δ	R_c	$\frac{R_c}{\Delta}$
30.	Chronic parenchymatous nephritis, albumin present ...	1.12°	9.3°	8.30
31.	Specimen with albumin, which was removed by boiling	1.04	9.3	8.94
32.	Alkaline, much albumin	1.27	11.0	8.66

In the above three instances, high values of R_c/Δ were obtained, probably due to a preponderance of salts in the urines. Albumin, being a colloid, can hardly be expected to have any perceptible effect either in raising or lowering the critical solution temperature. In the presence of the phenol in the critical concentration (36 %), the albumin in any specimens met with was not precipitated, even by boiling for five minutes.

DIABETIC URINES.

As the presence of dextrose has a great effect upon the freezing point it was thought well to see what alteration in the critical solution temperature its presence in urine would cause. As it is somewhat soluble in phenol, its addition to a phenol-water mixture was first studied. Results are shown in Table VIII.

TABLE VIII.

Dextrose added to phenol-water system.

Dextrose, grams per 100 cc. water	Δ	R_c	$\frac{R_c}{\Delta}$
5.05	0.56°	1.7	3.04
4.04	0.42	1.0	2.38
2.02	0.21	0.4	1.90
1.01	0.10	0.3	3.00
0.50	0.05	-0.1	-2.00

It will be seen that the addition of dextrose causes a relatively small rise in the critical solution temperature, the last figure being obviously due to experimental error. As before pointed out, with low values of R_c small errors cause the ratio R_c/Δ to fluctuate greatly. The effect of dextrose in a urine will, therefore, be to lower the value of R_c/Δ , for though it causes a rise in R_c yet this is not so great as is the case with the salts. To test this point further, No. 10 was diluted by the addition of dextrose solution, so as to have the

composition recorded in Nos. 10 *a* and 10 *b*, in Table III. Also dextrose was weighed out and made up to 100 cc. with No. 15, so that the resulting solutions had very closely the composition of No. 15, but not exactly on account of the volume occupied by the dissolved dextrose. The figures obtained are shown in Table III and in Table IX.

TABLE IX.

Dextrose added to phenol-urine system.

No.	Dextrose, grams per 100 cc. urine	Δ	R_c	$\frac{R_c}{\Delta}$
10	—	1.67°	12.3°	7.36
10 <i>a</i>	2.02 (No. 10 diluted)	1.21	10.0	8.26
10 <i>b</i>	3.53 (No. 10 diluted)	0.86	6.8	7.86
15	—	1.87	11.8	6.31
15 <i>a</i>	1.08	1.98	11.3	5.71
15 <i>b</i>	5.40	2.43	12.3	5.06

Here it is seen that in 10 *a* and 10 *b* the dilution effect has more than counterbalanced the addition of dextrose, so that R_c/Δ is slightly raised. In 15 *a* and 15 *b*, however, it is evident that the already low value of R_c/Δ afforded by No. 15 is depressed still further.

In Table X are shown the values given by diabetic urines, not intentionally altered in any way. They had not been heated.

TABLE X.

Diabetic urines.

No.	Dextrose, grams per 100 cc.	Δ	R_c	$\frac{R_c}{\Delta}$
33	1.87	1.17°	8.0°	6.84
34	5.62	1.56	10.0	6.41
35	?	1.75	11.0	6.30
36	0.19	1.94	15.3	7.88
37	2.34	2.03	16.0	7.88
38	3.38	2.27	16.5	7.56
Mean				7.14

Here again the mean value of R_c/Δ is lower than with normal urines, though, owing to the variations of the other constituents of the urine, the decrease is not proportional to the sugar concentration.

We hope to make further experiments with pathological urines, taking into account other abnormal constituents of diabetic specimens.

SUMMARY AND DISCUSSION OF RESULTS.

The examination of a considerable number of normal urines has shown that the rise in critical solution temperature is roughly eight times as great as the depression of freezing point. This holds good for urines of normal concentrations, but with very dilute urines the critical solution temperature rise is somewhat greater, consequently R_c/Δ has a value greater than eight. This is also the case in specimens which, for any cause, contain an unusually large quantity of salts. On the other hand, the presence of an exceptionally great amount of urea occasions a smaller rise of critical solution temperature; glucose, when present in quantity, has the same effect, but it is far less marked. In the last two cases accordingly the ratio R_c/Δ is somewhat below eight.

This relation between the freezing point depression and the rise in critical solution temperature enables one to transfer the conclusions established by the former method to the latter. Thus A. v. Koranyi [1897, 1899: Quoted from Casper and Richter] gives $1.3^\circ - 2.3^\circ$ as the usual limits for the freezing point of urine, from a normal kidney under ordinary conditions. Taking $R_c/\Delta = 8$, this range corresponds to a rise in critical solution temperature of $10.4^\circ - 18.4^\circ$.

Lindemann [1899: Quoted from Casper and Richter] gives a more extended range, $0.9^\circ - 2.7^\circ$, for values of Δ , and states that kidney patients almost always excrete urine for which Δ is less than 1.0° . Accordingly, taking Lindemann's limit, 0.9° , and allowing for experimental error and fluctuations due to the composition of the sample, it seems safe to take a rise of 8° C. in the critical solution temperature as indicating that the kidney supplying the sample was functioning satisfactorily. It must be remembered, however, that the converse is not always true; thus a specimen giving a rise of 7° might really be normal, as, owing to the ingestion of a large amount of liquid, or to the presence of much urea, the value of R_c could very well be lowered.

In the latter limiting case, it would be well to make a freezing point determination or rough urea determination by the hypobromite method, to see if there was an unusual quantity of this substance. Ordinary values of R_c lie between 11° and 16° C.

In conclusion, we wish to thank Dr J. Timmermans, of Brussels, for putting his extensive knowledge of critical solution point determinations

entirely at our disposal during his visit to Trinity College. A detailed treatment of the subject will be found in his paper [1907]¹.

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¹ See also Findlay, *The Phase Rule*, and Young, *Stoichiometry*. London, Longmans, Green & Co.