

layer being in the left-hand flask (Fig. 1), whilst the aqueous layer was in the right-hand flask. After chromatography, the strips were air dried and the sugar spots revealed with aniline hydrogen phthalate (Partridge, 1949). After removal from the oven the strips may be treated with a saturated solution of 'Perspex' polymethacrylate resin in chloroform. This serves to retard the development of background colour which would otherwise obscure the spots after a day or so. This treatment does not hinder the spots from fluorescing strongly in ultraviolet light. It does not, however, prevent ninhydrin-amino-acid spots from fading.

RESULTS

The results of thirty tests are summarized in Table 1, which gives the range of R_F values for each individual sugar. Comparison of the bridge-unit values with

those recorded in the literature (Horrocks & Manning, 1949) show very close agreement, and the inference may be drawn that the modification in technique and the change in the position and nature of the filter-paper strip in no significant way affect R_F values.

SUMMARY

1. An all-glass bridge unit for filter-paper strip partition chromatography is described.
2. The unit can be used for any of the standard methods of one-dimensional partition chromatography; examples for carbohydrates are quoted.
3. The unit is supplied with ventilators, allowing a method of analysis in which continuous evaporation takes place from the paper.

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The Effect of Temperature on the Interference of Potassium in the Determination of Sodium

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In most of the microchemical methods for the determination of sodium in biological material the metal is precipitated as sodium zinc or magnesium uranyl acetate, and subsequently the uranium is estimated colorimetrically with potassium ferrocyanide or the acetic acid by titration. Phosphates are also precipitated by uranium salts, and must therefore first be removed with lime or ethanolic zinc sludge (McCance & Shipp, 1931*a*). Potassium as well as sodium forms a sparingly soluble zinc uranyl acetate. McCance & Shipp (1931*a*) considered that this would not interfere at 0°, provided the solution in which sodium was to be determined contained less than 0.3 mg. potassium/ml. Using the method of McCance & Shipp (1931*a, b*) for the determination of sodium in liver, unexpectedly high values were obtained. These could not be accounted for by sodium in the reagents, and the same high values were obtained however the phosphates had been removed. Since, moreover, no phosphate could be detected in the solutions after treating them with lime, it seemed improbable that phosphate was interfering.

As these high results were obtained both by colorimetry and titration, it was concluded that some potassium was being precipitated with sodium as the triple salt. Although the concentrations of potassium were well within the limits laid down by McCance & Shipp (1931*a*), the effect of temperature and time upon the precipitation of potassium was therefore reinvestigated using both acid extracts of liver tissue and pure solutions containing known amounts of sodium and potassium.

METHODS

For the precipitation and colorimetric determination of the triple salt, the method of McCance & Shipp (1931*a*) was used, with the following modifications. The precipitate of triple salt was dissolved in 5.0 ml. 10% (v/v) acetic acid, transferred to a 100 ml. flask and diluted almost to volume before adding 2.0 ml. 20% (w/v) $K_4Fe(CN)_6 \cdot 3H_2O$. The optical density of the coloured, colloidal solution of uranium ferrocyanide was determined in the Spekker Absorptiometer using a 1 cm. cuvette and Ilford filter 604.

The amount of K present in the triple acetate precipitate was determined by dissolving the triple salt in water and

Table 1. *Effect of temperature on the precipitation of potassium with sodium as the zinc uranyl acetate triple salt*

| Temperature (°) | Amounts taken | | Apparent Na found ($\mu\text{g.}$) | K recovered | | |
|--------------------|--------------------------|-------------------------|---|--|---|---|
| | Na ($\mu\text{g.}$) | K ($\mu\text{g.}$) | | (a) From precipitate | | (b) From supernatant by precipitation at -4° after Na had been removed ($\mu\text{g.}$) |
| | | | | (i) By calculation* ($\mu\text{g.}$) | (ii) By analysis ($\mu\text{g.}$) | |
| -4 | 40 | 120 | 82 | 71 | 86 | 0 |
| | 100 | 300 | 230 | 219 | 243 | 0 |
| 0 | 40 | 120 | 67 | 49 | — | — |
| | 100 | 300 | 216 | 197 | — | — |
| 18 | 40 | 120 | 39 | 0 | — | 60 |
| | 100 | 300 | 100 | 0 | — | 170 |

* K was calculated from the increase in 'Na' content and expressed as $\mu\text{g. K}$.

removing K by precipitation with sodium cobaltinitrite (Eden, 1943), and then estimating the nitrite by the sulphoamide method of Bratton & Marshall (1939). (See Hawk, Oser & Summerson, 1947.)

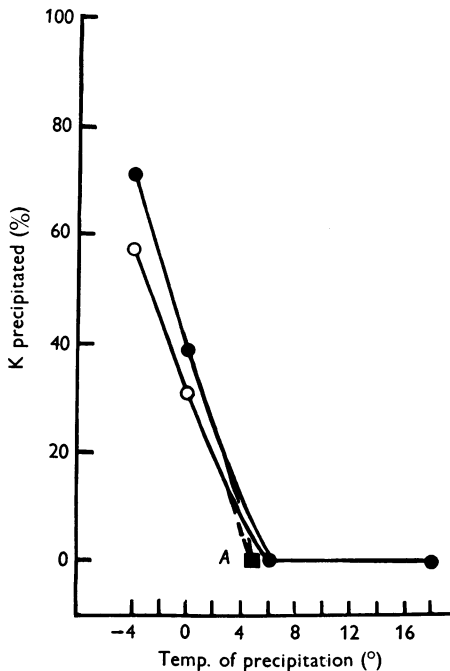


Fig. 1. The effect of temperature upon the precipitation of K: (a) from acid extract of liver tissue, \circ — \circ ; (b) from solution of NaCl and KCl of similar composition to (a), \bullet — \bullet . ■, Point A, see text, p. 285.

For the determination of Na in liver tissue, the phosphate present in the acid extract of the ashed liver was removed with $\text{Ca}(\text{OH})_2$. A sample containing 200–300 $\mu\text{g. Na}$ was evaporated, and to it, when dry, were added 10.0 ml. water and sufficient $\text{Ca}(\text{OH})_2$ (about 0.05 mg.) to make the solution alkaline to phenolphthalein. After 1 hr. it was filtered and the determinations were carried out on 2.0 ml. of the extract.

RESULTS

Effect of temperature. The results for the precipitation of potassium from solutions of sodium chloride and potassium chloride are summarized in Table 1.

These results show that, at 0 and -4° , the high values for sodium were caused by the simultaneous precipitation of potassium triple acetate. This was confirmed by the analyses of precipitates from solutions containing potassium alone.

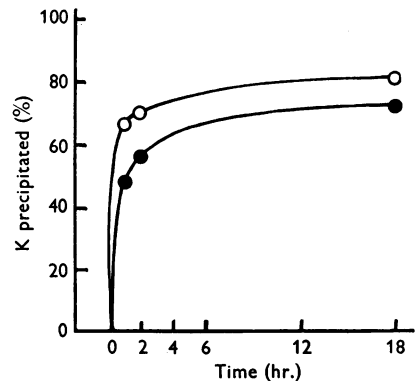


Fig. 2. The effect of time upon the precipitation of K from standard solutions of NaCl and KCl at -4° containing per 2.0 ml. sample: (a) 40 $\mu\text{g. Na}$ and 120 $\mu\text{g. K}$, \bullet — \bullet ; (b) 100 $\mu\text{g. Na}$ and 300 $\mu\text{g. K}$, \circ — \circ .

Similar results were obtained for liver. Between 18 and 6° , the quantity of sodium found was always 2.87 m-equiv./100 g. wet tissue, but at 0 and -4° the sodium found increased to 5.83 and 8.17 m-equiv./100 g. wet tissue respectively. This interference of potassium is graphically represented in Fig. 1 where the temperature of the reactants is plotted against percentage potassium precipitated (a) from acid extract of ash of liver tissue, and (b) from standard solutions containing 40 $\mu\text{g. sodium}$ and 120 $\mu\text{g. potassium}$ /2.0 ml. sample taken for analysis which was equivalent to the sodium and potassium present

in the liver extract. Similar curves were obtained, and when the lines joining the points at 0 and -4° were extrapolated, they converged and cut the x-axis between 4 and 6° (point marked *A* in Fig. 1).

By cooling solutions containing sodium and potassium, and potassium alone, it was also found experimentally that precipitation of potassium began within this range of temperature. The precipitation of potassium at -4° was also demonstrated by warming to room temperature precipitates and supernatant solutions which had been at -4° overnight. The potassium salt dissolved and the sodium 'found' in the tubes containing sodium and potassium was then equal to that found in the tubes containing sodium alone.

Effect of time. (a) Precipitation of potassium (Fig. 2).

In 1 hr. at -4° , 40–70 % of the potassium was precipitated, which caused an apparent increase of 70–115 % in the sodium. Within 2 hr. at the same temperature, over 50 % potassium was precipitated from the dilute solutions, and within 18 hr. 70–80 % potassium.

(b) Precipitation of sodium.

Sodium was precipitated quantitatively within 30 min. at room temperature (18°) from standard solutions of sodium and potassium, and from the liver extract.

DISCUSSION

These results show that under the conditions laid down by McCance & Shipp (1931*a*) some potassium was always precipitated with the sodium from a solution of the two ions. Salit (1932), also following the method of McCance & Shipp (1931*a*), observed an increase in precipitation of the triple salt at low temperatures but not at room temperature. Prof. R. A. McCance has informed me that in practice he and his collaborators always placed their tubes in a cool chamber, the temperature of which was always loosely referred to as ' 0° ', but which was certainly not below 4° , or more recently in a modern refrigerator, set to maintain a temperature of 4° . There seems, therefore, no reason to suspect that any of

the figures published by them are wrong, and a considerable number have been verified as correct within the last few months (McCance & Shipp, 1931*a, b*; McCance & Widdowson, 1940).

The present results confirm that precipitation of sodium is complete at room temperature (18°) (Peters & Van Slyke, 1932; Salit, 1932; Stone & Goldzieher, 1949), whereas McCance & Shipp (1931*a*) found precipitation incomplete at 20° . There is, however, little agreement between investigators about the exact formula of the crystalline precipitate of sodium triple acetate, some considering that it is $\text{NaZn}(\text{UO}_2)_3 \cdot (\text{CH}_3\text{COO})_9 \cdot 6\text{H}_2\text{O}$ (Barber & Kolthoff, 1928; Butler & Tuthill, 1931; Hald, 1933; Peters & Van Slyke 1932), and others



(Blanchetière, 1923). Perhaps both forms exist and have different solubilities. Hence different workers, using different media and techniques to precipitate the sodium triple salt, may be precipitating different crystalline forms of sodium triple acetate. The test of this hypothesis would require a complicated piece of research, and has not been attempted.

Quantitative precipitation of potassium has not been observed at the temperatures employed in this investigation. It might be possible, however, to separate the two cations and determine them both with the same reagents by precipitating the sodium at room temperature, and subsequently the potassium at a much lower one.

SUMMARY

1. When sodium is determined in the presence of potassium, precipitation of the triple acetate should be carried out above 4 – 6° . Below this temperature potassium is precipitated, and at 0° and -4° the apparent sodium content of liver tissue increases to two and three times the value at 6° respectively.

2. Between 6 and 18° there is quantitative recovery of sodium in 30 min.

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