3. Calcification occurs only in places in the zone of provisional cartilage at a pH of 7.1 to 7.2.

4. Slight calcification may occur at a pH of 7.0 to 7.1.

5. No calcification occurs in solutions more acid than pH 7.0, and the sections appear swollen and tenacious.

6. In solutions more alkaline than pH 7.35, calcification occurs, but the initial pH usually changes to the optimum pH of 7.25 or 7.3 over night.

7. Sections left in acid solutions for from 2 to 6 hours and later transferred into solutions in which the concentrations of calcium, inorganic phosphorus and hydrogen ions are optimal, fail to calcify; whereas sections transferred from solutions of low Ca × P product into solutions of a higher product may calcify if the pH is optimal.

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1 Shipley, P. G., Kramer, B., and Howland, J., *Biochem. J.*, 1926, xx, No. 2, 379.

2 Howland, J., and Kramer, B., *Monatsschr. f. Kinderheilk*, 1923, xxv, 279.

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The Gasometric Determination of Potassium.

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We have used the constant volume apparatus of Van Slyke and Neill in the estimation of potassium in solutions whose inorganic composition approximates that of serum. The potassium is precipitated as the potassium-sodium cobalti-nitrite compound, essentially as in the original method of Kramer and Tisdall.[1] The precipitate is decomposed with sodium hydroxide as suggested by Clausen,[2]* and the nitrogen of the sodium and potassium nitrite is liberated by adding a solution of urea and sulphuric acid.[3]† The amount of nitrogen is then determined in the constant volume apparatus. It is possible to determine from 0.07 to 0.45 mg. potassium, with an error not exceeding 0.01 mg.

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* $K_2NaCo(NO_2)_6 + 3\ NaOH = 2\ KNO_2 + 4\ NaNO_2 + Co(OH)_3$.

† $CO(NH_2)_2 + 2\ HNO_2 = 2\ N_2 + CO_2 + 3\ H_2O$

One cc. of the potassium solution is measured into a 15 cc. graduated centrifuge tube, containing 1 cc. of water. One cc. of the sodium-cobalti-nitrite reagent is added slowly, drop by drop, and the solution thoroughly mixed after each drop. At the end of 45 minutes, the volume is made up to 5 cc. with water and the contents mixed again and centrifuged for one-half hour at a speed of about 1800 revolutions per minute.

All but 0.3 cc. of the supernatant fluid is pipetted off so as not to disturb the precipitate. Five cc. of water are allowed to run in slowly. The stream is controlled in such a way that the added water is thoroughly mixed with the residual reagent and the precipitate is not disturbed. The tube is centrifuged for 5 minutes. The procedure is repeated twice, making three washings in all. In the third washing, the water is added in such a way as to gently agitate the upper portion of the precipitate. It is emphasized that the supernatant fluid from the last washing must be colorless. After this has been pipetted off, 0.7 cc. of approximately normal sodium hydroxide is added and the precipitate is thoroughly mixed with a fine glass rod. The tube is heated in a boiling water bath for about 5 minutes, after which a brownish flocculent precipitate of cobalt hydroxide settles on the bottom of the tube. The solution is cooled and transferred to the cup of the Van Slyke gas apparatus. The remainder of the precipitate is washed into the cup, with 2 cc. of 10 per cent urea solution. One cc. of approximately 8 N sulphuric acid is led in, after which a vigorous evolution of gas takes place. The mercury is lowered almost to the bottom of the 50 cc. bulb, the apparatus is sealed, and the level of the mercury then lowered to the 50 cc. mark. After shaking for about 5 minutes, the mercury is raised again so that a space of several cc. is left between the water meniscus and the 2 cc. mark. Three cc. of approximately 10 N sodium hydroxide is permitted to flow gradually into the chamber. The apparatus is sealed again and the carbon dioxide is absorbed. The gas volume is reduced to the 2 cc. mark and the pressure and temperature are read. To obtain the pressure which is exerted by the nitrogen liberated, subtract a blank pressure which is obtained on 1 cc. of approximately normal NaOH treated in the same manner as the unknown.

The pressure is converted into millimols of nitrogen by using the last column of Van Slyke's Table III.[1] Since 1 cc. of solution was taken for analysis, and the factors are for 2 cc. samples, the resulting value is multiplied by 2 to obtain the millimols per liter of solution. Since the quantity of nitrogen evolved in the reaction is twice the amount present in the nitrite, this value is divided by 2. The number of milligrams of nitrogen so calculated is multiplied by 0.9286 (the ratio of potassium to nitrogen in the $K_2NaCo(NO_2)_6$ precipitate) in order to obtain the value for potassium in milligrams per 100 cc.

[1] Kramer, B., and Tisdall, F. T., *J. Biol. Chem.*, 1921, xlvi, No. 2, 339.
[2] Clausen, S. W., *J. Biol. Chem.*, 1918, xxxvi, 479.
[3] Frankland, P., *J. Chem. Soc.*, 1888, liii, 364.

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The Metabolism and the Respiratory Quotient of Excised Renal Tissue.

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The respiratory quotient of the excised renal tissue of the rat was determined by the Warburg-Barcroft manometers. The accuracy of the method was judged by duplicate quotients, performed with the same tissue, at the same time. The results are recorded in Table I, columns 1 and 2. The average of each pair is given in the third column, and the numerical difference in the fourth. The series was consecutive, and only those observations discarded in which technical error could be demonstrated. Observation 7 is excluded from the averages; a technical error was probable, but not proved. With this exception, the mean difference was 0.027, and the error ±0.014.

The data include observations on well-fed, as well as fasting rats, with or without glucose in the fluid. Variations in the results would therefore be expected. The lowest observation was 0.705, which happens to be the theoretical quotient for fat. The highest was 0.949, which does not exceed the level of the exclusive oxidation of carbohydrate. In 19 out of 25 observations the quotients were between 0.750 and 0.899. Thus all the experiments fall within the limits which we should expect to find in the intact organism, even when there is no reason to suspect the formation of fat from carbohydrate, or the reverse. The results imply that excised tissue,

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