standard is replaced in the instrument at regular intervals and the setting readjusted to maintain the standards at the desired L values. Marked change in readings or lack of consistency in the color developed by the standards would preclude use of the simplified method, although the same objection would apply to results obtained by conventional procedures.

The accuracy obtained will depend on the extent to which the readings of the standards deviate from the desired scale values and the consequent extent of the shutter or galvanometer adjustment. It has been possible to select filters or alter sample sizes so that this deviation was kept to less than 5 mg per 100 ml for blood sugar, 1 mg per 100 ml for blood urea N, and 0.1 g per 100 ml for serum protein in a range of values ordinarily encountered. If the instrument adjustment required is appreciable, error will increase in proportion (1) as zero is approached, and (2) as the difference between concentration of the unknown and that of the standard increases.

Application of this method of calculation does not necessarily require that standards be included with each run. In clinical laboratory procedure, however, this is desirable regardless of the system of calculation employed, particularly when equilibrium reactions like those involved in blood sugar determinations are utilized.

The method described deviates partially from the accepted principles of photometry and any new applications should be thoroughly tested. Furthermore, it deliberately introduces an approximation and thus should be used with discrimination. However, it is suitable for many purposes and has proven to be of great convenience where large numbers of analyses are required in a minimal period as in the clinical laboratory. Here it has expedited analyses and decreased the number of errors arising in calculation of results.

The simplified procedure has been applied to determinations of blood sugar, urea nitrogen, serum total protein, and free and esterified cholesterol. The results in each instance have agreed closely with those obtained by the standard method of readings. Differences have been small and without statistical significance.

The Separation of Purine and Pyrimidine Bases and of Nucleotides by Ion Exchange

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As a prelude to tracer studies on the enzymatic formation and degradation of nucleic acids, we have undertaken to develop ion exchange techniques for the separation and analysis of the various bases, nucleosides, and nucleotides at the milligram level. Although this technique is finding increasing application in biochemical studies for separation of these compounds (*8, 9*) and others (*1, 2, 6, 7*), we were led independently to its use through our earlier experience with radioisotope separation by ion exchange chromatography (*4, 11*). The present note describes briefly methods we have developed for the separation of each of the purine and pyrimidine bases and of the ribose nucleotides.

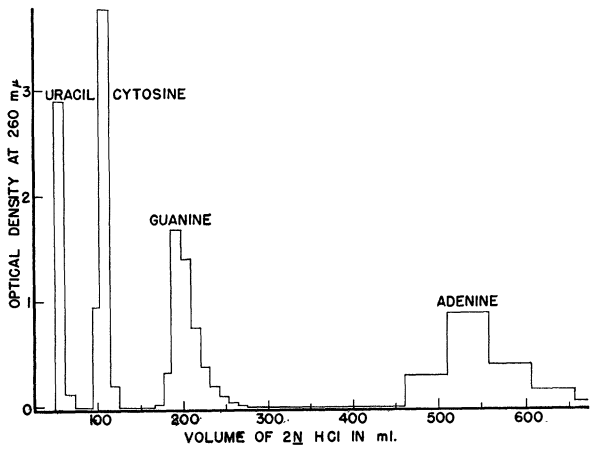

FIG. 1. Separation of purine and pyrimidine bases by cation exchange. *Exchanger*: 8.1 cm × 0.74 cm² Dowex-50, ca. 300 mesh, H⁺ form. *Eluting solution*: 2*N*-HCl at 0.6 ml/min. *Test material*: 0.5 mg uracil, 1.0 mg each of adenine, guanine, and cytosine, in 7.5 ml 2*N*-HCl. *Recoveries*: 98.5–101.5% (based on optical density at 260 mμ).

Of the five naturally occurring purine and pyrimidine bases, three (cytosine, guanine and adenine) exist as cations in solution of pH < 4 and hence are able to combine with cation exchangers. Since their affinities for the cation exchanger differ, they may be eluted successively with such simple reagents as HCl or NaCl; this is demonstrated in Fig. 1. This procedure does not separate

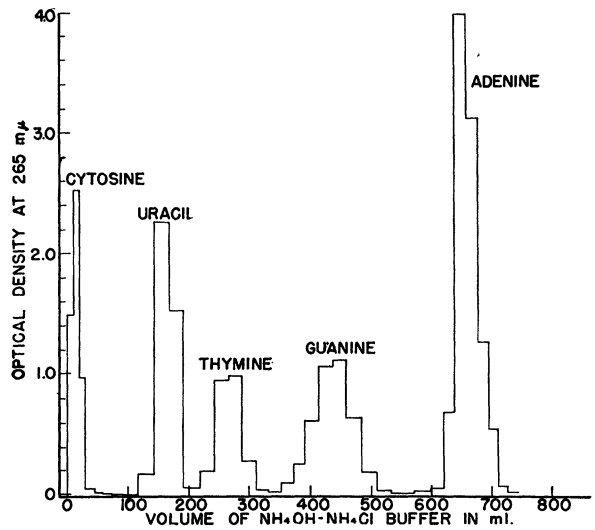

FIG. 2. Separation of purine and pyrimidine bases by anion exchange. *Exchanger*: 8.5 cm × 0.74 cm² Dowex-Al, ca. 300 mesh, Cl^- form. *Eluting solution*: 0.2*M*-NH_4OH-NH_4Cl buffer, pH 10.6, 0.025 *M*-Cl^- (at 575 ml, pH changed to 10.0, Cl^- to 0.1 *M*), at 0.25 ml/min. *Test material*: 1 mg cytosine, 2 mg each of other bases, in 10 ml eluting solution. *Recoveries*: 97.5–99% (based on optical density at 265 mμ).

the non-ionized bases, thymine and uracil. Since these will not usually be encountered together, no disadvantage attaches to this fact, but it is easy to separate them, as well as the other three substances, by resorting to anion exchangers[1] and alkaline solutions as shown in Fig. 2.[2] Recoveries by both procedures, based on spectrophotometric absorption in the ultraviolet (260–265 mμ) are essentially quantitative. Identification of components was made by the shape of the ultraviolet absorption curve and by partition chromatography (*3*).[3]

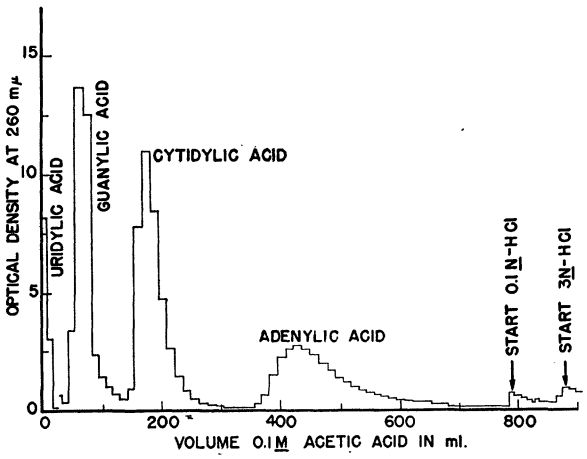

FIG. 3. Separation of mononucleotides of yeast nucleic acid by cation exchange. *Exchanger*: 24 cm × 0.74 cm² Dowex-50, ca. 300 mesh, H^+ form. *Eluting agent*: 0.1 *M* acetic acid at 0.15 ml/min. *Test material*: 100 mg mixed nucleotides, recovered from their Ba salts following 0.6*N*-$Ba(OH)_2$ hydrolysis of yeast nucleic acid (conversion of nucleic acid to nomonucleotides not quantitative), in 10 ml 0.1 *M* acetic acid. *Recoveries*: 85–100% (based on optical density at 260 mμ).

The nucleotides of yeast nucleic acid (uridylic, cytidylic, guanylic and adenylic acid) exhibit to some degree the basic properties of the purine or pyrimidine constituent; this is modified by both size and the negative phosphate group with the result that the exchanger-to-ion bond is considerably weaker. Weak acids have been found to be the most practical eluting agents and a separation by means of dilute acetic acid is demonstrated in Fig. 3. Recovery of the starting material, based upon absorption at 260 mμ, varies from 85 to 100%. In the absence of pure starting materials (the commercial preparations showing 5–50% impurity by these methods), it is difficult to do better than this without an extensive series of preparations of pure compounds. It is possible that there is a significant amount of acid hydrolysis of purine nucleotides occurring during their stay in the exchanger bed but we have not been able to prove this conclusively. Regardless of this possibility, the method as it stands will isolate the bulk of each constituent in spectrophotometrically pure form.

Concentration and metathesis of the large volumes collected in the separations are easily achieved (*5*, *10*), where evaporation is undesirable, by recycling the effluent through the same column, washing with water to remove residual acid or base, and then eluting with a reagent which removes the adsorbed ion rapidly (e.g., NH_4OH in the acid separations).

Although the separations reported are all at or near the milligram level, we have been able to effect practical separations on the same size columns on up to nearly 100-mg amounts. The lower limit is, of course, set by the limit of detection. At the higher levels, overlapping of bands becomes more prominent because of the relationship between the concentration of an ion and its distribution coefficient (*12*).

The details of these and related separations, with examples of their application to specific problems, will be reported elsewhere.

[1] The Dowex-A1 anion exchanger, a strong base, was kindly furnished us by Dr. L. Matheson of the Dow Chemical Company, Midland, Michigan.

[2] These experiments were carried out by Mr. J. X. Khym.

[3] We are indebted to Dr. C. E. Carter not only for the chromatographic analyses but also for continued encouragement and assistance during the course of these experiments.

References

1. ARCHIBALD, R. M. *J. biol. Chem.*, 1944, **156**, 121.
2. CANNAN, R. K. *J. biol. Chem.*, 1944, **152**, 401.
3. CARTER, C. E. (Unpublished data).
4. COHN, W. E., and KOHN, H. W. *J. Amer. chem. Soc.*, 1948, **70**, 1986.
5. COHN, W. E., PARKER, G. W., and TOMPKINS, E. R. *Nucleonics*, 1948, **3**, 22.
6. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, 1948, **42**, 443.
7. DRAKE, B. *Nature, Lond.*, 1947, **160**, 602.
8. ELMORE, D. T. *Nature, Lond.*, 1948, **161**, 931.
9. HARRIS, R. J. C., and THOMAS, J. F. *Nature, Lond.*, 1948, **161**, 931.
10. KOCHOLATY, W., and JUNOWICZ-KOCHOLATY, R. *Arch. Biochem.*, 1947, **15**, 55.
11. TOMPKINS, E. R., KHYM, J. X., and COHN, W. E. *J. Amer. chem. Soc.*, 1947, **69**, 2769.
12. TOMPKINS, E. R., and MAYER, S. W. *J. Amer. chem. Soc.*, 1948, **69**, 2859.

A Simplified Procedure for the Analytical Extraction of Lipids[1]

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Recently Hsiao (*3*) described a procedure for the extraction of lipids from fish tissues. When this procedure was applied to pulverized, moist, mouse muscle tissues certain difficulties were encountered. The tissues clumped, adhered to the walls of the extraction tube, and did not disperse during the extraction period, thus interfering with complete extraction.

In order to circumvent these difficulties, to expedite the extraction of comparatively large numbers of samples, and at the same time to attain the fine state of division recommended by Bloor (*1*) the following procedure was developed.

The method of preparation of the tissue for extraction is that of Floyd (*2*) and Singher (*6*). A glass

[1] Aided by a grant in memory of Mary Frances Sarkisian.