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]

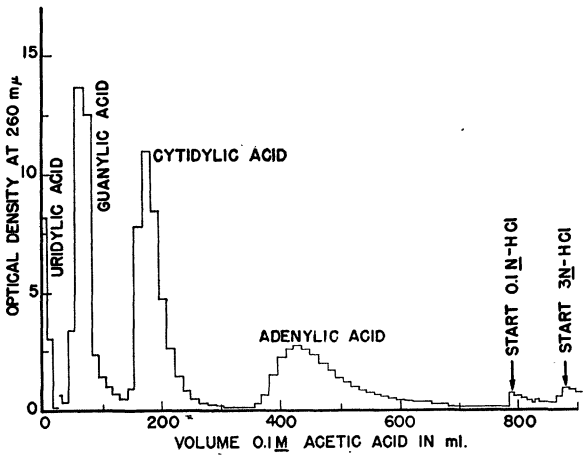

FIG. 3. Separation of mononucleotides of yeast nucleic acid by cation exchange. *Exchanger*: 24 cm × 0.74 cm² Dowex-50, ca. 300 mesh, H^+ form. *Eluting agent*: 0.1 *M* acetic acid at 0.15 ml/min. *Test material*: 100 mg mixed nucleotides, recovered from their Ba salts following 0.6*N*-$Ba(OH)_2$ hydrolysis 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.

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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.

mortar with pestle is placed in an evaporating dish, and surrounded by and filled with solid carbon dioxide; after thorough cooling of the mortar and pestle, the dry ice is removed from the mortar. The excised tissue, which has been stored in vials over dry ice, is thawed just enough so as to be transferable to the mortar, and dry ice equal to about three times the volume of the tissue is then added. When the tissue is again frozen solid, the mixture is ground until a fine homogeneous powder is obtained. Small bits of dry ice are, if necessary, added during the grinding. The ground powder is then transferred to a vial and the carbon dioxide allowed to evaporate until a pasty mass is obtained. As soon as this change has taken place the tissue is again frozen solid.

Samples of approximately 200 mg of the partially thawed ground tissue are weighed into 50 ml conical centrifuge tubes equipped with a 24/12 ⅋ outer joint.[2] The tube is hung on a balance by an easily made support (*4*). Five ml of 95% ethanol is added and the tube is stoppered and allowed to stand until the entire series of samples have been weighed (usually 6 or 12 samples). The samples are then thoroughly dispersed with the aid of a stirring rod, and sufficient alcohol is added to each tube to wash down the walls and the stirring rod so that a total of 10 ml of solvent is used for each 200 mg of moist sample.

Pyrex #2480 Allihn condensers (200-mm jacket, 19/38 ⅋ joints) are connected to the centrifuge tubes by special adapters. These adapters are provided with an inner 24/12 ⅋ joint and an outer 19/38 ⅋ joint.[3]

The heat source is an electric digestion furnace as described by Miller and Miller (*5*). Rate of boiling may be controlled by using either a variable transformer or by adjusting the height of each tube above the heating element. When the 10-min boiling period is completed, the extraction unit is raised from the heater. After cooling to room temperature, contents of the tubes are swirled to wash down solids that creep up the walls during extraction. After centrifuging 10 min at 3,000 rpm, the clear supernatants are decanted into tared aluminum weighing dishes (Fisher Scientific Co. #8-732). The dishes with contents are placed in a desiccator over calcium chloride under moderate vacuum. The extraction is repeated with an additional 10 ml of ethanol and the resulting supernatants are added to the dishes.

In order to insure complete dryness, the residues are placed on the following day into another desiccator over fresh calcium chloride under high vacuum.

If dispersion during boiling is thorough, the second extraction does not yield more than about 3% of the weight of residue resulting from the first extraction.

Eighteen samples in duplicate of normal and cancer tissue of mice were extracted, with an average difference of 2% between the results of pairs. The average sample weight used was 224 mg, and the evaporation residues ranged from 9.5 to 14.9 mg.

The following additional information may be obtained from a series of extractions. If the insoluble residue is dried in the centrifuge tube at 100° C overnight and weighed, the sum of the weights of the soluble and insoluble portions will give a value which agrees with that of independent drying experiments on the whole tissue ($100 \pm 3\%$). When this insoluble residue is ashed overnight in the centrifuge tube at 450°, and the resulting ash weighed, a correction for soluble ash can be obtained provided the total ash is known. The soluble ash in our series was $58 \pm 5\%$ of the total ash or 14% of the evaporation residue.

[2] These versatile centrifuge tubes may be obtained by special order from either Ace Glass, Vineland, New Jersey, or Corning Glass Works, Corning, New York.

[3] Made by J. F. Uhrig, 4104 North Fifth Street, Philadelphia 40, Pennsylvania.

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Evidence that Amputation of Bacterial Flagella Does Not Affect Motility

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In previous papers (*5, 6, 8, 10, 11, 12*) the somewhat revolutionary theory was proposed that the motile bacteria which are commonly credited with flagella do not move by means of such flagella, but by means of undulating gyrating contortions of their spiral-shaped bodies. Bacterial flagella would then have to be regarded, not as motor organs, but as mucous twirls peeled off from the mucous capsule, through the very movement of the bodies.

Acceptance of this theory would have far-reaching consequences. When applied to bacteria, the word "flagellum" (which means "whip") would have to be invested with quite a new meaning, or else be given up and replaced by something like "mucous twirl." "Bacillus" and "bacterium" (meaning "rod") would become misnomers in many instances, because although a rod need not be straight, an object which definitely has the shape of a spiral, or rather coil, cannot reasonably be termed a rod. Apart from nomenclature, the new theory would obviously affect classification in a fundamental manner. This might, however, at the same time lead the way to a phylogenetic classification. Teachers might like the new theory, for with less emphasis on the appearance and importance of "flagella," irksome "flagella staining" might be given up!

Notwithstanding my several publications on the subject, and the production of a cinemicrographic film in