THE COMPOSITION OF OCTOPUS RENAL FLUID
II. A CHROMATOGRAPHIC EXAMINATION OF THE ORGANIC CONSTITUENTS **

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With 2 Figures in the text

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Introduction

The previous communication of this series has outlined the inorganic chemistry of octopus nephridial fluid (EmanuEl and Martin 1956). In the present report, a chromatographic examination of the organic constituents is presented for purposes of future orientation. By means of conventional chromatographic procedure, it was possible to determine the individual variability and relative complexity of the urine samples. At the same time, information concerning specific reactivities of the separated compounds was obtained.

The relatively small amount of organic material to be found in octopus urine indicated that rather considerable volumes would be required for the subsequent isolation of the individual constituents in the crystalline state necessary for their identification. Fortunately, it has been possible to obtain two or three liters of urine during each yearly field trip. Such quantities, collected over a period of about eight years, have sufficed for the isolation of most of the principal constituents revealed on the chromatograms described in this paper.

Methods

The cephalopods [O. hongkongensis (Hoyle)] used for the work reported here were captured in the intertidal regions of the San Juan Archipelago in the spring of the years 1948 to 1950, inclusive, and to a lesser extent up to 1956. Some specimens were also taken in the Tacoma Narrows (Puget Sound) during the winter of 1954—1955.

The paired nephridial sacs of the octopus periodically empty into the mantle cavity (and thence into the sea) through a pair of small papillae which are externally accessible to the investigator. These sacs are normally capable of great distention, and a simple ligation of the papillae suffices to retain a relatively, if not entirely normal urine sample of 100 to 500 milliliters (total) per 24 hour period. Actual ligation and subsequent removal of the urine by means of a large syringe was accomplished under anaesthesia in 2.0 to 2.5 per cent ethanol (HARRISON 1954) or 4 per cent magnesium sulfate solutions in sea water (v. Furtth 1900). The urine thus obtained was filtered (gravity) through Schleicher and Schuell sharkskin paper to remove the large mezozoan population.

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Before proceeding with the description of the urine fractionation, brief mention should be made concerning the outcome of some general qualitative tests which had been carried out upon the crude (filtered) urine. The absence of carbohydrate or mucoid material was indicated on the basis of negative anthrone tests (Dreywood 1946) as well as the negative outcome of chromatographic procedures using aniline phthalate (Partridge 1949) or ammoniacal silver nitrate spray reagents. The urine gave positive Molisch tests (carbohydrate or organic acids), a positive lactic acid test (p-hydroxydiphenyl reagent, Eggew 1933), a positive or negative test (depending on the urine sample at hand) for protein when mixed with trichloroacetic acid or heated, a negative murexide test for uric acid (Hawke et al. 1947), negative tests for creatine and creatinine (Hawke et al. 1947), and a negative test for urea (urease). Steam distillation from acid, neutral, or alkaline urine solutions yielded little or no organic material in the distillate.

The filtered urine samples were concentrated to powdery dryness over sulfuric acid. The concentration process was performed under sufficient vacuum to keep the liquid nearly frozen. Complete dryness was attained over P2O5 and NaOH at 0.4 mm of Hg. The hygroscopic residue from each two liters of urine was mixed with several grams of Hyflo Super Cel (Johns-Manville Products) and quickly transferred to a large Soxhlet apparatus.

The Soxhlet extraction of the dry powder was carried out consecutively with three different solvents of increasing dielectric strength. Dry, peroxide-free ether was used for the first 24 hours of extraction. This was followed by equal periods of extraction with dry acetone and finally with absolute methanol. These three extracts contained all of the organic constituents of the urine1 and were designated as Fractions I, VII and VIII, respectively. Because the methanol tends to extract considerable amounts of inorganic salts, particularly those of ammonia and magnesium, it was later found advisable to omit the alcoholic extraction and to prolong the acetone extraction step for three or four days. The acetone extracts almost no inorganic material but does eventually remove all of the organic substances except quaternary salts.

During the acetone extraction, a considerable amount of yellow precipitate formed on the walls of the extraction vessel. This residue was filtered, washed with water, and finally air dried. This "acetone precipitate" will be described in the third communication of this series.

Each of the above extracts was concentrated to a syrup by means of a surface impinging air stream and then stored at 3°C. The unextractable residue left in the Soxhlet thimble consisted of inorganic material which was often intermixed with a small amount of denatured protein.

Ascending paper chromatography (Williams and Kirby 1948) was used to determine the general complexity of each fraction and, in the case of the major constituents, these results were compared chromatographically with the unfractiated urine. The most effective chromatographic solvents, of the many that were tried, were 65 per cent isopropyl alcohol which was 2 normal in hydrochloric acid (Wyatt 1951) and the ammoniacal-ethanol solution devised by Cheffet et al. (1953). For detection, the most useful agents were diazotized sulfanilic acid, ninhydrin, ultraviolet fluorescence and quenching, aqueous ammonium reineckate, dimethylaminobenzaldehyde, alkaline nitroprusside, and bromocresol green2. A modified Dragendorff reagent was useful for indicating tertiary and quaternary amines as well as

1 The absence of organic material in the extracted residue was indicated by the failure of the residue to char when ignited. If protein were present in the urine sample, it would, of course, still be present in the final residue.

2 For the preparation of these reagents, see Williams et al. (1951).