A large number of substances containing the indole ring are known to occur in plants, some of them having striking effects on the processes of growth (cf. Ruhland 1961). It is however somewhat difficult at present to distinguish clearly between "free" and "bound" indoles.

In a previous paper (Tandler 1962) we reported in the giant unicellular green alga Acetabularia the presence of intracellular crystals whose indole nature was indicated by intense Ehrlich and Salkowski positive reactions. Alkaline hydrolysis of ethanol-extracted algae liberated, besides tryptophane, substantial amounts of Salkowski-positive indoles.

It is the purpose of the present paper to study further (A) the nature of the indoles released by hydrolysis and (B) the chemical and cyto logical properties of the Acetabularia crystals.

A. Chromatographic analysis

Material and Methods

The algae, Acetabularia crenulata and A. mediterranea, were cultured in "Erd schreiber" medium (Hämmerling 1944; Beth 1953). The cells (3—6 cm long, with and without caps) were killed by immersion in Carnoy's ethanol:chloroform: acetic acid, 6:3:1 (v/v) mixture for 3 h and extracted for at least 3 weeks with 96 and 80% ethanol, all steps being carried out at 2—4°C in darkness.

Chromatography. Using Whatman paper No 1, linear and two-dimensional (ascending) chromatograms were obtained. The solvents used were: isopropanol—25% ammonia—water = 8:1:1 (Sen and Leopold 1954), isopropanol—glacial acetic acid—water = 4:1:1 (Kaper and Veldstra 1958) and distilled water. The spots were developed by spraying with the reagents according to Ehrlich (660 mg of p-dimethylaminobenzaldehyde dissolved in a mixture of 36 ml of ethanol and 8 ml conc. HCl) (Kaper and Veldstra 1958) or Salkowski (0.02 M FeCl₃ in 20% HClO₄); a more diluted reagent is 5% HClO₄—0.05 M FeCl₃ = 50:1. The Ehrlich reagent was employed in the following manner: after spraying, the chromatogram is left for 5—15 min at room temperature (~22°C) and then is heated at 60°C for another 5—15 min. This procedure was adopted as it was observed that some spots

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developed a colour immediately, others required a few minutes and still others showed up only upon heating the chromatogram.

The "tryptochrome" spray was employed as described by Egelhaaf (1957). Preparation of hydrolyzate: 50 to 200 ethanol-extracted algae were heated for 24 h with 5—10 ml of a 4% barium hydroxyde solution in a sealed glass tube at 100°C and in darkness. A 14% Ba(OH)₂ solution for 72 h was sometimes employed with essentially the same qualitative results. The excess of Ba(OH)₂ was removed by solid CO₂ (over dry ice) and the hydrolyzate was evaporated to dryness in a desiccator over CaCl₂ at reduced pressure. The residue is taken up in a small amount of 40% isopropyl alcohol or in the isopropanol-ammonia-water solvent mixture, and centrifuged. This leaves a clear yellow supernatant. The slight quantity of Ba⁺⁺ remaining in the hydrolyzate does not interfere in the chromatograms. Another procedure was also employed: the clear Ba(OH)₂ hydrolyzate was directly concentrated on the paper by alternatively spotting a drop of the solution and hanging the paper in CO₂ atmosphere (over dry ice). In this way Ba(OH)₂ is converted to Ba-carbonate which remains at the start point and does not interfere appreciably with the Rᵣ values in the ammoniacal mixture. No significative differences were obtained in this manner on the chromatograms and thus the earlier method was generally adopted.

Tryptophane-1⁻¹⁴C labelled in the side chain (alanine 3⁻¹⁴C), specific activity 8 mc/mM was purchased from Calbiochem (California, USA). Radioactivity was measured in a windowless, gas flow G-M counter. For autoradiography the chromatograms were attached to Agfa X-ray film by means of paper clips and kept in the dark in the original packing envelopes.

**Results**

We were immediately confronted with the difficulty of establishing whether spots in the chromatogram are genuine or are formed as artefacts during the hydrolysis procedure, a rather general experience with this type of experiments (Ruhland 1961; Gordon and Weldman 1943; Schocken 1949). It became undoubtedly important to determine if all the spots are artefacts derived from tryptophane, a normal constituent of proteins.

Hydrolysis of tryptophane: under our conditions this aminoacid (20—50 mg) gives rise to very small amounts (although biologically important, cf. Schocken 1949) of Ehrlich (purple) and Salkowski (pink) positive substances which are visualized as two or three faintly stained spots. One runs near the solvent front and the other has an Rᵣ similar to indole-3-acetic acid in the ammoniacal mixture. No such spots are formed by chromatography of the unheated aminoacid. Quantitative measurements — optical density at 280 mµ after elution of the spots in 50% ethanol — indicated that less than 0.1% of the original tryptophane can be found in the indoleacetic acid zone.

Hydrolysis of two tryptophane-containing proteins, casein and egg albumin (Merck), yielded similarly very small quantities of indoles different from tryptophane.