Dartnall states "that when one quantum of light is absorbed one chromophore is altered, but there is no evidence for the statement, often occurring in the literature, that the secondary process following activation of the chromophore is a loosening, that is, a dissociation of the chromophore-protein bond". A similar conclusion[6] was reached on the basis of a comparative study of the squid visual purple, cephalopsin[7]. This substance is a retinene$_1$ compound which is chemically almost identical with vertebrate visual purple, but is not normally dissociated by light, either in solution or in the most sensitive preparations of living retinas[8]. An increase in retinene release from illuminated squid retinas has been reported[9], but has been shown to be an artefact[8].

Ball, Collins and Morton[10], in their answer to Dartnall, state that there are three materials in bleached solutions of rhodopsin: retinene, and the acid and alkaline forms of indicator yellow. However, according to Lythgoe[3] there are two forms of acid indicator yellow: a stable form with adsorption maximum at 450 mμ below *p*H 4 and an unstable form with absorption maximum near 445 mμ between *p*H 4 and 6. The unstable form of acid indicator yellow readily releases retinene when its solutions are shaken with petroleum ether[4], and thus probably corresponds to the "loosely bound retinene" mentioned by Ball, Collins and Morton.

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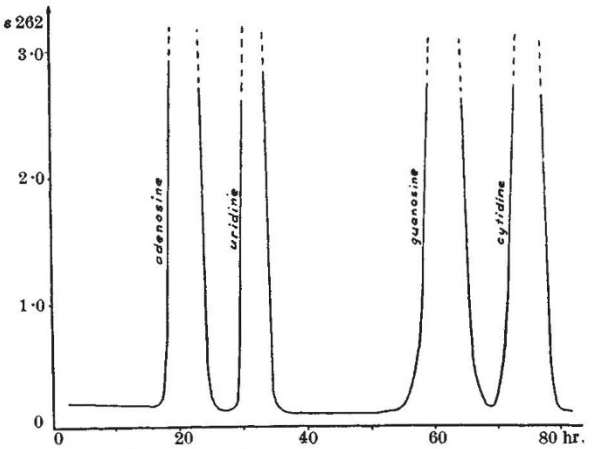
[1] Ball, S., Collins, F., Morton, R., and Stubbs, A., *Nature*, **161**, 424 (1948).
[2] Dartnall, H., *Nature*, **162**, 222 (1948).
[3] Lythgoe, R., *J. Physiol.*, **89**, 331 (1937).
[4] Bliss, A., *J. Biol. Chem.*, **172**, 165 (1948).
[5] Lythgoe, R., *J. Physiol.*, **94**, 399 (1938).
[6] Bliss, A., *J. Gen. Physiol.*, **26**, 361 (1943).
[7] Bliss, A., *J. Biol. Chem.*, **176**, 563 (1948).
[8] Bliss, A., *Biol. Bull.*, **93** (October 1948).
[9] Wald, G., *Amer. J. Physiol.*, **193**, P479 (1941).
[10] Ball, S., Collins, F., and Morton, R., *Nature*, **162**, 222 (1948).

Partition Chromatography on Starch of Ribonucleosides

DURING work on ribonucleic acid turnover with nitrogen-15 in this Department, we found it necessary to isolate the different nitrogen-containing substances from small amounts of ribonucleic acid. It was thought that partition chromatography on starch as introduced by Elsden and Synge[1] might give a possible solution to our problem. Vischer and Chargaff[2] have applied partition chromatography on paper to the purine-bases; Edman, Hammarsten, Löw and Reichard[3] have worked out a method for separating guanine and adenine on a starch column. Tinker and Brown[4] have recently applied Craig's counter-current distribution technique to purines and pyrimidines and related compounds.

In regard to the pyrimidine bases, it seemed to be difficult to prepare them in a good yield from ribonucleic acid without destruction and conversion of cytosine to uracil during hydrolysis. The preparation of the ribonucleosides, however, was thought to be a simpler task, and a method for separating them from a mixture of the pure compounds on a starch column has been worked out.

The solvent chosen was butanol saturated with water. The starch was prepared by extracting ordinary commercial potato starch with methanol for 24 hours in a Soxhlet apparatus. The starch column was 200 mm. long and had an inner diameter of 23 mm. About 5 mgm. of each of the four pure ribonucleosides were dissolved in about 3 ml. of butanol–water and added to the top of the column. The butanol was run continuously for about three days; a sufficient head of liquid was kept in the column so that 5–7 ml. of fluid ran through the column per hour. The effluent was collected each hour by a simple apparatus constructed by Edman[5], and analysed in the Beckman spectrophotometer at 262 mμ. In this way the nucleosides may be easily localized (see graph). The different nucleosides have specific light absorption maxima around 262 mμ and may be characterized by comparing the light absorption at 262 mμ (maximum for uridine and adenosine), 248 mμ (guanosine) and 270 mμ (cytidine). Under these conditions, the values of R, according to Martin and Synge[6], for the different nucleosides were as follow: adenosine, 0·80; cytidine, 0·21; guanosine, 0·28; uridine, 0·52.



Separation of 4 mgm. of adenosine, uridine, guanosine and cytidine on a starch column 200 mm. long with butanol–water as the mobile phase

Samples containing the same nucleoside were combined, the solvent evaporated off and the residue dissolved in 0·1 N hydrochloric acid. The purity of the different compounds after chromatography was established by determining the ratio ε max./γ nitrogen per ml. These values obtained were: adenosine 0·196 (ε = 257 mμ); cytidine, 0·295 (ε = 280 mμ); guanosine, 0·164 (ε = 255 mμ); uridine, 0·332 (ε = 262 mμ).

The yield with about 5 mgm. of each nucleoside was 90–100 per cent. This method has been successfully applied in tracer experiments to hydrolysates of ribonucleic acid. The hydrolysis of ribonucleic acid to nucleosides was carried out with a pyridine–water mixture by a modification of Bredereck's method[7]. The results of these experiments will be published elsewhere.

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[1] *Biochem. J.*, **38**, *Proc.* ix (1944).
[2] *J. Biol. Chem.*, **168**, 781 (1947).
[3] *J. Biol. Chem.* (in the press).
[4] *J. Biol. Chem.*, **173**, 585 (1948).
[5] *Acta Chem. Scand.* (in the press).
[6] *Biochem. J.*, **35**, 1358 (1941).
[7] *Ber. dtsch. chem. Ges.* **74** B, 694 (1941).

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