

chlorophyll *a* is much less strongly adsorbed and can be separated from chlorobium chlorophyll by development with light petroleum/ethyl ether (9:1:12) by chromatography of their phaeophytins prepared according to the method of VAN NIEL AND ARNOLD⁹. Although the absorption maxima of chlorobium phaeophytin (Fig. 2) (maxima at 660, 694, 549, 515 and 412 $m\mu$ in ether) are very similar to those of chlorophyll *a*, the two compounds can again be easily separated on a sugar column.

The positions of these maxima also agree well with those recorded by LARSEN³ for chlorobium chlorophyll phaeophytin but differ in their relative intensities (possibly because he was dealing with a mixture) and in the fact that he did not observe the 412 $m\mu$ band.

The chlorophyll fraction extracted from *Rsp. rubrum*, using the same method as for *Chlorobium* spp., yields on chromatography one major zone, bacteriochlorophyll (maxima at 770, 708, 574 and 396 $m\mu$ in ether); adsorbed just below bacteriochlorophyll is a small green band, fluorescing red in ultraviolet light, with main maxima at 438 and 675 $m\mu$ in ether. This appears to be SEYBOLD AND HIRSCH'S⁵ bacteriochlorophyll *b* and is an oxidative artifact¹, which under our experimental conditions and those of HOLT AND JACOBS⁴ is produced only in traces.

It is obvious from the absorption spectra of chlorobium chlorophyll and bacteriochlorophyll and their respective phaeophytins (Figs. 1 and 2) that the two compounds are very different. Their non-identity can further be demonstrated by chromatography on sugar; they possess somewhat similar adsorptive properties but prolonged development with light petroleum containing 30% ethyl ether (v/v) will separate them; the chlorobium chlorophyll (upper) zone being bluish-green and the bacteriochlorophyll (lower) zone being bluish-grey. The phaeophytins can be similarly separated.

As we agree with HOLT AND JACOBS⁴ that bacteriochlorophyll is quite stable and as we cannot reproduce SEYBOLD AND HIRSCH'S⁵ claim that it is rapidly converted into bacteriochlorophyll *b*, it must be concluded that chlorobium chlorophyll is the naturally occurring chlorophyll in *Chlorobium* spp., and that it is not a degradation product of bacteriochlorophyll produced during experimental procedures. The further claim of SEYBOLD AND HIRSCH⁵ that chlorobium chlorophyll and bacteriochlorophyll *b* are identical can easily be disproved although they have similar absorption spectra: (a) they can be separated chromatographically on sugar, chlorobium chlorophyll being more strongly adsorbed, (b) chlorobium chlorophyll is almost completely insoluble in light petroleum whilst bacteriochlorophyll *b* is easily soluble.

The individuality of the various pigments discussed can readily be demonstrated by chromatographing a mixture of them on a sugar column when they will separate in the following order of decreasing adsorptive power: chlorobium chlorophyll; bacteriochlorophyll; bacteriochlorophyll *b*; chlorophyll *a*.

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Galactose 1-phosphate in galactose cataract*

Galactose 1-phosphate (Ga 1-P) accumulates in the red blood cells of galactosaemic infants on a milk diet¹. At the same time the O₂ uptake of these cells is partially inhibited compared with that of cells taken before galactose feeding². While no causal relationship has as yet been established between these two findings, it seems possible that Ga 1-P acts as, or gives rise to, an inhibitor of glucose metabolism. Since glucose is the main source of energy of the lens and since inhibition of glucose metabolism leads to cataract formation *in vitro*³, an occurrence of Ga 1-P in the lenses of galactose-fed rats may be of considerable interest.

Experimental: Young male albino rats (initial weight 50–60 g) were fed a diet containing 30% of galactose; controls were given the same diet without galactose. After varying intervals the animals were killed and the lenses excised. Either whole lenses or the capsules were used,

the latter freed as far as possible from lens fibres, without scraping off the epithelium. The lenses or capsules were homogenised in 5 ml of ice-cold 5% trichloroacetic acid, and the precipitate was spun down and digested with H_2SO_4 for determination of protein by micro-Kjeldahl. The supernatant was fractionated with Ba into water soluble and insoluble ester phosphates according to the method of LE PAGE *et al.*⁴. Aliquots of the water soluble Ba salt fraction were chromatographed on acid-washed paper, using isopropanol: NH_3 : H_2O (60:30:10) as solvent⁵. Standard amounts of glucose 1-phosphate or galactose 1-phosphate were run simultaneously. The chromatogram was developed with acid molybdate reagent⁶. On a similar chromatogram, the hexose 1-phosphate area was eluted with water and the eluate was hydrolysed with 0.1 *N* HCl at 100° for 7 min, neutralised and evaporated in vacuo. The residue was extracted with pyridine and the extract chromatographed in *n*-butanol:pyridine: H_2O (3:2:1.5)⁷ together with suitable amounts of galactose for comparison. The chromatogram was developed with benzidine reagent and the amount of galactose estimated visually in u.v. light by comparison with the standards. Less than 0.5 μg galactose was detectable in this way.

TABLE I

Concentration of galactose added to diet, %	Days on diet	Appearance of lens (naked eye examination)	Tissues analysed		Galactose total (μg)	1-Phosphate mg per 100 g tissue*
			Lenses	Capsules		
0	—	Normal	24		0	0
30	46	Opaque	8 + 4 decapsulated		7.2	2.2
0	—	Normal		9	0.7	1.7
30	88	Opaque		14	16.3	17
30	92	Opaque		18	23.0	17
30	92	Clear		7	0.7	1.6

* Weight of tissue calculated from Kjeldahl protein $\times 4$.

The results are presented in the accompanying table. The following facts emerge from these findings:

(1) Control lenses or capsules contain a maximum of 0.08 μg Ga 1-P per capsule.

(2) Whole cataractous lenses contain approximately the same amount of Ga 1-P as do separated capsules, i.e. approximately 1 μg per capsule.

The Ga 1-P seems to be confined to the capsule or epithelium, where the concentration is at least 17 mg per 100 g tissue. The actual concentration is likely to be rather greater, since a considerable proportion of protein estimated must be derived from lens fibres adhering to the capsule.

(3) Capsules of non-cataractous lenses from galactose-fed animals contain amounts of Ga 1-P similar to those found in normal lenses. (The lenses were not examined histologically, but there were no opacities visible to the naked eye).

The amount of Ga 1-P present in cataractous capsules is of the same order as that found in galactosaemic red cells¹. If the reduction of the metabolic rate observed in these cells should prove to be linked with the accumulation of Ga 1-P, a similar mechanism may be operative in the lens.

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