of the phylloerythrin absorption bands and the dependence of *R* on small wavelength differences require accurate calibration of the spectrophotometer and the use of narrow slit widths.

The highest plasma phylloerythrin level so far found with the method described has been 0.3 mg./100 ml., in a sheep poisoned by *Panicum miliaceum*, although Clare (1944) estimates that levels up to at least 1.6 mg./100 ml. occur in severe cases of facial eczema in sheep.

The method appears to be applicable to other biological materials, especially urine. Phylloerythrin has been detected in the urine of humans on vegetable diets (Kemer, 1924; Fischer & Hilmer, 1925), indicating that absorption of chlorophyll-degradation products from the gut can take place in humans. Whether phylloerythrinaemia occurs in human-liver dysfunctions and porphyrias is not at present known, but the method described makes feasible the examination of blood from affected persons. In addition to its application to photosensitivities of unknown origin it also enables information to be obtained on the concentrations of phylloerythrin involved in photosensitization in sheep and cattle.

SUMMARY

1. Details are given of a method for estimating phylloerythrin in blood, consisting in extraction into ether-acetic acid solution, distribution between ether and hydrochloric acid solutions, and spectrophotometric measurement.

2. The inflexion at 4260 Å in the absorption spectrum of phylloerythrin provides a quantitative check on the purity of the material being measured.

3. The method is applicable over the range 0.05–3.5 mg. of phylloerythrin/100 ml. of plasma, but a modification giving approximate values down to 0.005 mg./100 ml. is described. Proto-porphyrin and other porphyrins do not cause significant interference.

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APPENDIX

The Form in which Phylloerythrin Occurs in Bile and Blood

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Although phylloerythrin is extremely insoluble in water it occurs as a normal constituent of sheep and ox bile, from which it is not thrown down on centrifuging. Similarly, although it can be quantitatively extracted into ether from bile acidified with acetic acid, phylloerythrin is not readily extracted

from fresh bile with ether alone. A similar observation was made with plasma from a congenitally photosensitive Southdown sheep. Dialysis of bile in collodion tubes led to the deposition of most of the phylloerythrin on the walls, with very little present in the diffusate. The effect of pH on

extractability of phylloerythrin from bile was tested by adding fresh ox bile to equal volumes of buffer solutions and shaking with ether. In solutions below pH 5 almost all the phylloerythrin was extracted into the ether. Above pH 7.5 less than 5% of the phylloerythrin was present in the ether phase. Between pH 5 and 7.5 intermediate values were obtained. This behaviour could be imitated by adding phylloerythrin (dissolved in a small volume of dilute hydrochloric acid) to solutions of sodium tauroglycocholate, and similar results were also obtained with strong soap solutions. On the other hand, with phosphate buffer solutions of pH 7.5 distribution of phylloerythrin was strongly in favour of the ether phase. As soaps and bile salts in aqueous solution are colloidal electrolytes (Roepke & Mason, 1940) it is concluded that

phylloerythrin is present in bile in the free state and is probably maintained in solution by adsorption on the colloidal micelles.

Results from the study of bile suggest that in blood phylloerythrin is dispersed on colloidal micelles such as the bile salts and the plasma proteins. In support of this hypothesis is the observation (Rimington & Quin, 1934) that in sheep affected with geeldikkop phylloerythrin was present entirely in the plasma, only traces being found in the well-washed corpuscles.

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Studies on the Structure of Brain Ganglioside

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The brain gangliosides are macromolecular glycolipids first described by Klenk (1942). They are known to contain neuraminic acid, sphingosine, fatty acids, hexose and hexosamine, although little is known of the manner in which these constituents are combined. The present study reports detailed structural evidence obtained by stepwise hydrolytic cleavage of a highly purified preparation of brain ganglioside leading to the isolation and characterization of a constituent glucocerebroside, and the formulation of the structure of the repeating unit.

METHODS

Nitrogen. The digestion (Gunning, 1889; Arnold & Wedemeyer, 1892) was continued for 45 min. after the solution had become colourless. Water (14 ml.) and one drop of 1% gum ghatti were then added. After 6 min., 8 ml. of Nessler's reagent (Koch & McMeekin, 1924) was added to successive tubes at 45 sec. intervals, the colour allowed to develop for 9 min. and then read at 435 m μ (M. Lees, unpublished work).

Phosphorus. Phosphorus was determined spectrophotometrically (Sperry, 1942).

Neuraminic acid. The humin reaction (Folch, Arsove & Meath, 1951), the direct Ehrlich's reaction (Odin & Werner, 1952) and Bial's orcinol reaction (Bohm, Dauber & Baumeister, 1954) were employed. The Bial's reaction was found to be the most reliable.

Reducing sugar. The methods of Hagedorn & Jensen

(1922) and of Hanes (1929) were adapted. Results were expressed as equivalents of galactose or glucose.

Hexose. Hexose was estimated by a modified orcinol-H₂SO₄ method (Sorensen & Haugaard, 1933) and expressed as equivalents of glucose or galactose. Since the absolute values differed slightly for the two sugars, identification of the hexose by paper chromatography indicated which curve was to be used. When glucose and galactose were together in a single fraction, the hexose was expressed as equivalents of galactose, since galactose was always in two- to three-fold excess.

Determination of hexosamine. The following modifications of the Elson & Morgan (1934) reaction were employed: 1 ml. of acetylacetone (6% in 2N-carbonate buffer) was added to a 2 ml. aqueous sample. The covered tube was heated at 100° for 20 min. Colour was developed with Ehrlich's reagent (2.6%) for 45 min. and read at 530 m μ . The standard curve, based on a purified sample of galactosamine hydrochloride, was verified for each determination. This modified method was suitable for estimation of 5–40 μ g. of galactosamine (maximum error 3%). Maximum values were obtained after the subfractions were hydrolysed with N-HCl in a sealed tube for 16 hr.

Identification of the hexosamine. The method of Stoffyn & Jeanloz (1954), Gardell, Heijkenskjold & Roch-Norlund (1950) was used. The reaction with ninhydrin was carried out in a sealed capillary tube. The solvent system was modified (see chromatography methods). The amino sugar fractions used were first freed from neutral sugars and other contaminants by chromatography on a column of Dowex 50.

Amino nitrogen. Primary amino nitrogen was determined by the method of Van Slyke (1929).