Table 1. Percentage Estimation of Fœtal Hæmoglobin in Mixtures of Carboxyhæmoglobin *A* and *F* estimated by the Two Chromatographic Methods at 0° C.

Sample No.	Calculated	Elution method	Cuvette method
1	83·5*	85·5; 84·0†	91·5; 91·5
2	72·5	75·0; 73·0	79·0; 77·5
3	59·0	56·5; 61·5	61·0; 61·0
4	42·5	48·5; 43·5	49·5; 47·5
5	24·0	23·0; 29·5	22·0; 19·5
6	12·0	17·0; 12·5	12·5; 12·5

* Estimated by the alkali denaturation method of Brinkman and Jonxis (ref. 8); the percentages of carboxyhæmoglobin *F* in the other five samples were obtained by dilution with normal adult carboxyhæmoglobin.
† Duplicate estimations.

method can be carried out in a normal refrigerator with a larger number of cuvettes simultaneously.

As regards quantitative results, it is found (Table 1) that in general they are in good agreement with the calculated values. With the elution method, there is a deviation of about 2 per cent, whereas the cuvette method shows a slightly higher error, especially at low concentrations of one of the components.

The methods described here may be of value for the determination of relatively low percentages (less than 10 per cent) of fœtal hæmoglobin, as the separation of this pigment from the other hæmoglobins is good even at small concentrations. Moreover, the elution method has the advantage that an abnormal hæmoglobin can be eluted free of other pigments and used for further research.

A full account of this work will be given elsewhere. We wish to thank the Rohm and Haas Company (Philadelphia 5, Pennsylvania) for a gift of 'Amberlite *IRC*-50 (*XE*-64)'. The investigations were supported by a grant of the Nutricia Co., Zoetermeer, The Netherlands.

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Nature of the Cuticle of Pycnogonida

Recent work has tended to show that, in spite of differences in structural features, the cuticle of insects conforms to a basic pattern in consisting of an inner protein-chitin endocuticle and an outer non-chitinous lipoprotein layer bounded externally by a very thin lipid membrane[1]. Although the cuticle of other groups of Arthropoda are not known in such detail as in insects, previous work indicates that in general the pattern in them may not be unlike that in insects. However, in the mode of hardening of the cuticle marked differences have been noted. While in insects the hardening is by phenolic tanning, in crustaceans, though the cuticle is initially hardened to some extent by phenolic tanning, the prime cause of hardening is by calcification[2]. In arachnids such as *Limulus*[3] and *Palamneus*[4] there is evidence of the occurrence of hardening by sulphur linkages. Although the significance of such a mode of hardening in the above-mentioned types is not clear, it appears that it may be more common than has hitherto been suspected. It is therefore of interest to find in the cuticle of *Propallene kempi*, a common pycnogonid of Madras, evidence to suggest the occurrence of disulphide linkages which are probably involved in the hardening of the cuticle.

Transverse sections of the cuticle show an inner layer marked by horizontal lamellations and traversed by dermal gland ducts, and a very thin, apparently homogeneous, outer layer bounded by a very thin dark membrane. These two layers correspond to the epi- and endo-cuticle of insects. With Mallory's triple stain, the endocuticle turns a blue colour while the epicuticle is unstained. With Heidenhain's hæmatoxylin also the epicuticle did not take up the stain; but the endocuticle took up a faint brown coloration. From the chitosan and Schultze's tests it is inferred that the endocuticle contains chitin and the epicuticle is devoid of it. By prolonged treatment with chlorated nitric acid the epicuticle may be separated from the endocuticle, which does not survive the above treatment. Both the epicuticle and endocuticle are not positive to Millon's and the xanthoproteic tests, while only the outer dark membrane gives a positive reaction to the argentaffin test. This membrane is also positive to sudan black *B*. The chemical features indicated by the above tests strongly recall those of the epicuticle of *Palamneus*[4,5]. The similarity is further emphasized by the positive reaction to tests for organic sulphur. The unstained region of the epicuticle is reactive to the thioglycollate test, which is further confirmed by the positive reaction to alkaline lead acetate; this turns the uncoloured inner epicuticle to a deep brown or black. With alkaline sodium sulphide the cuticle undergoes marked softening, and sections of such material when stained with Mallory's triple stain show that the inner epicuticle, which in untreated sections is non-staining, now becomes reactive to stains. But unlike the epicuticle of other arthropods, which invariably stains red with acid fuchsin of the Mallory, the epicuticle of the pycnogonid takes up a blue colour like the endocuticle. The absence of a differential staining of the epi- and endo-cuticle in Mallory by which the two layers are usually distinguished in other arthropods is remarkable.

From the observations reported above, it may be inferred that the epicuticle is hardened by disulphide bonds as in *Palamneus swammerdami*, and treatment with alkaline sodium sulphide results in a breaking up of the bonds and the consequent resumption of the ability to take up stains, similar to the effect produced by diaphanol on cuticle hardened by phenolic tanning. A fuller account of this work will be published elsewhere.

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