dence confirms that the natural dyes present in crude blackberry juice are of the anthocyanin type (Harbourne, 1967). For this reason the stain has been named anthocyanin BB—for blackberry. Extraction and purification of the stain from the crude juice was undertaken but the effort involved was not judged worthwhile as the purified extract was no better a nuclear stain than the crude juice.

Anthocyanins from a variety of sources such as red cabbage, elderberry, and dahlia were used fitfully as histological stains in the late 19th and early 20th centuries. These findings have been reviewed by Harms (1965). Blackberries were apparently first used by Claudius (1899) in Copenhagen and more recently by Gruber (1949). Reports in the literature of haematoxylin substitutes have recently been extensively surveyed (Lillie *et al.*, 1975). Anthocyanins featured prominently in that review but the authors, at that time, had not tested any of them.

The domestic availability, ease of preparation and use, resistance to fading, and, above all, its good nuclear staining properties strongly commend anthocyanin BB as an alternative to haematoxylin in routine histological procedures. The results are so comparable that an unsuspecting observer does not usually question whether the section under his microscope is stained with haematoxylin and eosin or anthocyanin BB and eosin. Nuclear contrast and clarity are particularly striking in the van Gieson

procedure and so it is probable that anthocyanin BB staining can be further improved by introducing a deliberate acid differentiation stage.

The technical, bibliographic, and secretarial assistance of Mrs S. Stewart, Miss L. H. Wain, and Mrs L. J. Richardson is gratefully acknowledged.

References

- Claudius, M. (1899). Ueber die Anwendung einiger gewöhnlicher Pflanzenfarbstoffe in der mikroskopischen Färbungstechnik. *Zentralblatt für Bakteriologie*, 1. Abt., **5**, 579-582.
- Gruber, G. B. (1949). Zur Frage des Haematoxylinersatzes, insbesondere über Holunderbeerensaft als histologisches Färbemittel. *Mikroskopie*, **4**, 187-188.
- Harbourne, J. B. (1967). *The Comparative Biochemistry of Flavonoids*, pp. 13-36. Academic Press, London.
- Harms, H. (1965). *Handbuch der Farbstoffe für die Mikroskopie*. Volume 2, pp. 116-118. Staufen, Kamp-Lintfort.
- Lamb, R. A. (1973). Commercial communication.
- Lillie, R. D. (1974). The haematoxylin shortage and the availability of synthetic substitutes. *American Journal of Medical Technology*, **40**, 355-362.
- Lillie, R. D., Pizzolato, P., and Donaldson, P. T. (1975). Haematoxylin substitutes: A survey of mordant dyes tested and consideration of the relation of their structure to performance as nuclear stains. *Stain Technology*, **51**, 25-41.

Measurement of plasma volume using ^{59}Fe -labelled transferrin

C. RICKETTS AND I. CAVILL *Department of Haematology, Welsh National School of Medicine, University Hospital of Wales, Cardiff, UK*

The measurement of plasma volume is usually based on the dilution of an intravenously injected labelled protein. The recommended method uses radioiodinated human serum albumin (ICSH, 1973) but there are a number of occasions when other labelled proteins are being used, in particular ^{59}Fe -labelled transferrin in the ferrokinetic investigation of blood disorders, which could also provide an estimate of the plasma volume. Intravascular proteins of similar

size and shape would be expected to have similar distributions, and there is no a priori reason why ^{59}Fe -labelled transferrin and ^{125}I -labelled albumin should not give equivalent plasma volumes. However, it has been suggested that there is a difference between the two measurements (Najean *et al.*, 1967) and that the ^{59}Fe plasma volume may be unreliable (Dacie and Lewis, 1975). These observations may have been due to non-specific labelling with ^{59}Fe ferric chloride (Cavill and Ricketts, 1974). We have therefore compared the dilution volumes of specifically labelled ^{59}Fe transferrin and ^{125}I albumin in patients with a variety of haematological disorders to see if the two are equivalent.

Subjects and methods

Fully informed consent was obtained from the 25 patients who took part in this study. One patient had haemochromatosis, one had a refractory anaemia, one had polycythaemia rubra vera, and two patients

Technical methods

had sideroblastic anaemia; the remaining 20 patients either were iron deficient or had been recently treated for iron deficiency anaemia.

Ten millilitres of venous blood was collected from each patient and the transferrin in the defibrinated plasma was labelled specifically with ^{59}Fe by the method of Cavill (1971). Four millilitres of the labelled plasma, containing 3–10 μCi ^{59}Fe , was taken up into a 5-ml syringe. The radioactivity present in this was counted with the syringe and needle supported in a fixed position over a well-type scintillation counter. The contents of the syringe were then injected intravenously, and the activity remaining in the syringe and needle was counted as before. Similarly, the ^{125}I activity in a syringe containing a commercially prepared 1-ml dose of labelled human serum albumin (Radiochemical Centre, Amersham) containing 3–5 μCi was counted before and after injection. Both injections were made using the same intermittent infusion set and the mid-time of each injection was noted.

Venous samples were obtained, avoiding the vein used for injection, at approximately 10, 30, and 60 minutes after the ^{59}Fe injection. The elapsed time after injection for each sample was calculated separately for each isotope. The plasma was separated by centrifugation and the activity of ^{59}Fe and ^{125}I was counted in a 4-ml aliquot. ^{125}I activity was corrected for interference from ^{59}Fe by means of standards.

The $\log_e$ ^{59}Fe and $\log_e$ ^{125}I activities above background in each sample were fitted by a least squares method to a linear function of time:

$$\log_e \text{ activity} = \log_e \alpha - \lambda t$$

The initial activity (α) of each isotope after injection was calculated from the intercept of this regression line. The dilution of each isotope was determined by relating this initial activity to the activity injected using appropriate standards.

Results

There was a significant positive correlation (Figure) between the plasma volume estimated using [^{125}I] albumin and that estimated from [^{59}Fe] transferrin (Spearman rank correlation coefficient, $\rho = 0.84$, $P < 0.001$). The least squares regression line between the two sets of data was ^{59}Fe plasma volume = $0.092 + 0.99$ ^{125}I plasma volume, where the plasma volume is in litres. The intercept was not significantly different from zero ($t = 0.37$, $P > 0.70$) nor was the slope different from unity ($t = 0.10$, $P > 0.90$). The mean ^{59}Fe plasma volume was 2.53 l and this was not significantly different from the mean ^{125}I plasma volume of 2.46 l (Wilcoxon's matched pair signed-rank test, $T = 155$, $P > 0.35$).

Figure Relationship between plasma volume measured using [^{125}I] albumin and that measured using [^{59}Fe] transferrin. The solid line describes the relationship. ^{59}Fe plasma volume = ^{125}I plasma volume.

Discussion

This study has demonstrated that when transferrin is solely and specifically labelled with ^{59}Fe it has the same distribution volume as albumin over a wide range of values. The fractional clearance rate of ^{59}Fe from the plasma was between four and 40 times faster than that of ^{125}I . There was, however, no significant correlation between the fractional clearance rate of ^{59}Fe and the difference between the ^{125}I and ^{59}Fe plasma volumes ($\rho = 0.33$, $P > 0.10$). Even in iron deficient patients with extremely rapid clearance of ^{59}Fe the plasma volume can be estimated using [^{59}Fe] transferrin.

In ferrokinetic studies the additional use of [^{125}I] albumin to measure plasma volume is unnecessary. [^{59}Fe] transferrin may also be used in other circumstances provided that the transferrin is specifically labelled and that the initial activity is calculated from at least three samples taken during not more than 60 minutes after injection. Two microcuries of either ^{59}Fe or ^{125}I is sufficient for the measurement of plasma volume, and the lower cost of ^{59}Fe may have advantages for routine investigations. The use of the patient's own plasma may also be more acceptable, and measurements of plasma volume in this laboratory are now made using [^{59}Fe] transferrin.

References

- Cavill, I. (1971). The preparation of ^{59}Fe -labelled transferrin for ferrokinetic studies. *Journal of Clinical Pathology*, **24**, 472-474.
- Cavill, I., and Ricketts, C. (1974). The kinetics of iron metabolism. In *Iron in Biochemistry and Medicine*, edited by A. Jacobs and M. Worwood, pp. 613-647. Academic Press, London.
- Dacie, J. V., and Lewis, S. M. (1975). *Practical Haematology*, 5th edition. Churchill Livingstone, Edinburgh and London.
- International Committee for Standardization in Haematology (ICSH) (1973). Standard techniques for the measurement of red-cell and plasma volume. *British Journal of Haematology*, **25**, 801-814.
- Najean, Y., Dresch, C., Ardaillou, N., and Bernard, J. (1967). Iron metabolism—study of different kinetic models in normal conditions. *American Journal of Physiology*, **213**, 533-546.

Letters to the Editor

The study of Kendeel and Ferris in your May 1977 issue reports no significant increase of muscle in the pulmonary arterioles and arteries of victims of the sudden infant death syndrome (SIDS). This is counter to a previous study from our laboratory which found an increase of such muscle in the SIDS victims (*New England Journal of Medicine*, 1973, **289**, 1167). The methods used by Kendeel and Ferris would not likely demonstrate the vascular abnormality. Our original study found that pulmonary arterioles were dilated in SIDS victims. Kendeel and Ferris will have excluded the largest arterioles in SIDS victims from their analyses, those with the most muscle, because dilatation gave these largest arterioles a diameter greater than the 100 micron limit established for arterioles. Dilatation would have had a similar effect on measurements from arteries which were limited to 101-200 microns diameter and on the number of medial nuclei in both arterioles and arteries. If the arterioles and arteries dilated over their normal size limits had been included in the analyses, the SIDS victims would almost certainly have had more muscle in these vessels than the non-hypoxic and acute hypoxic controls.

The relative size of arteriolar and arterial muscle cells is less affected by vessel dilatation, and the data of Kendeel and Ferris show the SIDS victims with somewhat larger muscle cells in their pulmonary arterioles than the acute hypoxia controls ($P < 0.01$). This difference between the SIDS victims and the controls

would have been substantially greater if the comparison had used cytoplasm/muscle cell rather than total cell size. It has been shown repeatedly that the size of muscle cell nuclei in pulmonary arterioles and arteries is not affected by chronic hypoxia.

The acute hypoxic controls used by Kendeel and Ferris are suspect, because they died with respiratory tract infections. Children who have repeated episodes of sleep apnoea have recently been found to have an excessive death rate in early life from respiratory tract infections. Monitoring has shown that many children with repeated episodes of sleep apnoea have chronic hypoxaemia between apnoeic episodes. Thus children who die with acute respiratory infections in the early months of life cannot be assumed to have been free of chronic hypoxia during sleep before the onset of the fatal illness.

Victims of accidents and homicide used as non-hypoxic controls by Kendeel and Ferris cannot be used uncritically for this purpose. We have found that a high proportion of homicides and a smaller proportion of accident victims have evidences of brain damage incurred before the final traumatic event. There is no way to be certain that such brain damage does not influence alveolar ventilation and therefore a very thorough postmortem study of such brains must be undertaken before such cases can be used as non-hypoxic controls. In our original study of pulmonary vessels in SIDS victims, more than half of the homicide and accident victims considered as possible controls were

excluded from the analyses because brain abnormalities, often subtle.

RICHARD L. NAEYE

Department of Pathology,
The Miltons, Hershey Medical Center,
The Pennsylvania State University,
Hershey, Pennsylvania, USA

The authors have commented as follows:

Thank you for allowing us the opportunity to reply to Dr Naeye's comments on our paper in the *Journal of Clinical Pathology*, **30**, 487, 1977.

It was not the purpose of our study to investigate vessels of all sizes in the lungs of our cases. This study was stimulated by the findings of Dr Naeye (the *New England Journal of Medicine*, 1973, **298**:1167). However, unlike his reported results, which were restricted to vessels of less than 100 microns, we expanded our investigations to include arteries and arterioles of up to 200 microns.

We agree that the problem of vascular dilatation or contraction renders the quantitative assessment of arterial muscle tissue difficult, and our studies showed that dilatation of pulmonary arterioles was not a constant finding in all cases of sudden infant death syndrome (SIDS). In addition, various degrees of dilatation, and sometimes contraction, were found in cases from all groups under study. As we reported, the problem is largely compensated by dividing the medial muscular area by the number of medial muscle