Preliminary Communications

Dipyrrolic Urinary Pigments in Congenital Heinz-body Anaemia due to Hb Köln and in Thalassaemia

Brit. med. J., 1966, 2, 396

Dark urine is a feature of the syndrome known as congenital Heinz-body anaemia, and where identification of the urinary pigment has been carried out it has consistently proved to be a dipyrrole of the bilifuscin-mesobilifuscin type (see Hutchison *et al.*, 1964; Sansone and Pik, 1965). Furthermore, it is becoming apparent that this syndrome is a result of having an unstable haemoglobin which is unduly liable to conversion to methaemoglobin with the formation of Heinz bodies (Dacie *et al.*, 1964; Hutchison *et al.*, 1964; Huehns and Shooter, 1965; Sansone and Pik, 1965). The precise source of this unusual urinary pigment and its relation to the abnormal haemoglobin fraction are unknown.

A similar urinary pigment has been found in a patient with severe thalassaemia intermedia (Bannerman *et al.*, 1966). In a recent study of this patient with ^{14}C -glycine *in vivo* it was found that the label appeared very early in the urinary dipyrrole, within hours of administration (Kreimer-Birnbaum and Bannerman, 1966), so that it seems to be associated with the early-labelled bile pigment and "ineffective erythropoiesis" seen in this condition (Grinstein *et al.*, 1960; Bannerman and Kreimer-Birnbaum, 1966).

Recently, we have had the opportunity to compare directly the dipyrroles from the thalassaemic patient with those from a subject with congenital Heinz-body anaemia due to Hb Köln (Hutchison *et al.*, 1964).

The behavioural characteristics of the urinary pigments from these two individuals have proved to be closely similar to one another and to those reported for dipyrroles in the literature. Table I shows the results of elemental analysis of the dipyrrolic pigments from the patient with thalassaemia intermedia, together with those obtained by others; and Table II presents data on solubility in various solvents; very close agreement is seen throughout. In addition, the findings on column and paper chromatography of the pigments from our two patients are identical to those described for dipyrroles by Siedel and Möller (1939) and Gilbertsen *et al.* (1959).

Thus we conclude that the urinary pigment in patients with thalassaemia intermedia and congenital Heinz-body anaemia due to Hb Köln are at least closely similar, if not identical, and their dipyrrolic nature is confirmed.

The very early labelling of the dipyrrolic pigments in thalassaemia intermedia suggests that its origin is in the break-

down of redundant haem or haem precursors in the bone-marrow, in association with "ineffective erythropoiesis," and it is hoped that a similar study of the dipyrroles in congenital Heinz-body anaemia due to unstable haemoglobin may throw further light on the source of these unusual urinary pigments.

TABLE II

Reference (see Table I)	Unesterified Pigment			Esterified Pigment				
	a	b	c	d	e	f	g	h
1 (all three sources)	—	—	—	—	++	—	++	—
3	—	+	++	—	+	—	++	—
4	—	+	++	—	+	—	++	—
Present study:								
Thalassaemia intermedia ..	—	+	++	—	++	—	++	—
Congenital Heinz-body anaemia	—	+	++	—	++	—	++	—

++ Soluble. + Partly soluble. — Insoluble.
a = Water, chloroform, dilute acid, ethyl ether. b = Pyridine, ethanol, methanol. c = Dilute alkali. d = Water. e = Chloroform. f = Petroleum ether. g = Hot glacial acetic acid. h = Ethyl ether.

This work was supported in part by grants from the United States Public Health Service (AM-05581) and the United Health Foundation of Western New York (G-65-BGH-10).

MARTHA KREIMER-BIRNBAUM, PH.D.,

P. H. PINKERTON, M.C.PATH., M.R.C.P.ED.,

R. M. BANNERMAN, D.M., M.R.C.P.,

Department of Medicine, State University of New York at Buffalo.

H. E. HUTCHISON, M.D., F.C.PATH., F.R.C.P.GLASG.,

University Department of Haematology, Western Infirmary, Glasgow.

REFERENCES

- Bannerman, R. M., Keusch, G., Kreimer-Birnbaum, M., Vance, V. K., and Vaughan, S. (1966). In preparation.
— and Kreimer-Birnbaum, M. (1966). Proceedings of the 7th Annual Meeting of the British Society for Haematology. Abstract, *Brit. J. Haemat.* In press.
Dacie, J. V., Grimes, A. J., Meisler, A., Steingold, L., Hemsted, E. H., Beavan, G. H., and White, J. C. (1964). *Brit. J. Haemat.*, 10, 388.
Gilbertsen, A. S., Lowry, P. T., Hawkinson, V., and Watson, C. J. (1959). *J. clin. Invest.*, 38, 1166.
Grinstein, M., Bannerman, R. M., Vavra, J. D., and Moore, C. V. (1960). *Amer. J. Med.*, 29, 18.
Huehns, E. R., and Shooter, E. M. (1965). *J. med. Genet.*, 2, 48.
Hutchison, H. E., Pinkerton, P. H., Waters, P., Douglas, A. S., Lehmann, H., and Beale, D. (1964). *Brit. med. J.*, 2, 1099.
Kreimer-Birnbaum, M., and Bannerman, R. M. (1966). *Fed. Proc.*, 25, 791.
Meldolesi, G., Siedel, W., and Möller, H. (1939). *Hoppe-Seyler's Z. physiol. Chem.*, 259, 137.
Robinson, S., Vanier, T., Desforges, J. F., and Schmid, R. (1962). *New Engl. J. Med.*, 267, 523.
Sansone, G., and Pik, C. (1965). *Brit. J. Haemat.*, 11, 511.
Schmid, R., Brecher, G., and Clemens, T. (1959). *Blood*, 14, 991.
Siedel, W., and Möller, H. (1939). *Hoppe-Seyler's Z. physiol. Chem.*, 259, 113.

TABLE I.—Elemental Analysis of Esterified Dipyrrolic Pigments Derived from Various Sources

Reference	Diagnosis	Source	Nitrogen (%)	Carbon (%)	Hydrogen (%)
1. Siedel and Möller (1939)		Theoretical values	8.80	64.11	6.97
		Bilirubin	8.63	63.98	6.97
Siedel and Möller (1939)		Mesobilirubinogen	8.43	64.26	6.74
		Neoxanthobilirubinic acid	—	62.71	6.51
2. Meldolesi <i>et al.</i> (1939)	Muscular dystrophy	Faeces	5.48	65.71	7.55
	Normal		8.81-8.96	—	—
3. Gilbertsen <i>et al.</i> (1959)	Haemolytic anaemia	Faeces	2.22-6.95	—	—
	Hypernephroma		6.43-6.78	—	—
	Reticuloendotheliosis		4.99	—	—
4. Schmid <i>et al.</i> (1959)	Congenital Heinz-body anaemia	Urine	6.60	59.78	6.56
5. Robinson <i>et al.</i> (1962)	Thalassaemia minor	Faeces	7.95	—	—
Present study	Thalassaemia intermedia	Urine	5.95	61.23	7.08