HÆMATOLOGY

Use of Phenol in the Isolation of Erythropoietic Glycoprotein

THE erythropoietic activity in the plasma of rabbits made anæmic with phenylhydrazine can be isolated as an acid glycoprotein. A dialysed and lyophilized ethanol fraction from heat-coagulated plasma consistently gives us a potent stable highly water-soluble preparation. Six laboratories have found good correlation in a comparative assay of a batch prepared by us[1]. Further di-ethyl-amino-ethyl cellulose column fractionation removes 40–50 per cent of inert protein and results in the active glycoprotein previously described[2].

While the active peak could be re-chromatographed on di-ethyl-amino-ethyl cellulose without apparent change in electrophoretic and elution properties, there was usually a drop of 25–75 per cent in specific activity. Even more striking losses occurred in the fractionation of erythropoietin from human urines. Urine from patients with congenital hypoplastic anæmia was precipitated with 4 volumes of ethanol. The non-dialysable portion of the precipitate, a mixture consisting in the main of glycoproteins, contained essentially all the erythropoietic activity of the original urine. It could be further resolved on di-ethyl-amino-ethyl cellulose but with variable, sometimes complete, loss of activity.

One ethanol precipitate from human urine, after removal of the dialysable part, had this activity: in a fasted rat assay[1] 1 mgm. total dose per rat gave 26·8 per cent ^{59}Fe-uptake in the blood. When 100 mgm. portions of the precipitate were fractionated on di-ethyl-amino-ethyl cellulose we repeatedly lost 90–100 per cent of the activity although all the protein was recovered in successive eluates with 0·01 to 1·0 *M* sodium chloride.

We had observed before that plasma erythropoietin is not damaged by phenol and is extractable into a phenol upper layer like some other glycoproteins in the procedures of Morgan and Partridge[3], Westphal *et al.*[4] and Kirby[5]. By using as little as 0·1 per cent phenol in all equilibration and elution buffers in di-ethyl-amino-ethyl cellulose chromatography, as well as on both sides of the membrane during dialysis, we are now consistently recovering more than 50 per cent of the activity contained in the original urine.

The dialysed and lyophilized ethanol fraction from plasma, fractionated as previously described on di-ethyl-amino-ethyl cellulose[2], but in the presence of phenol, yields a peak with 2–3 times the specific activity of the original fraction, corresponding with the removal of inert material.

Evidence to date indicates that erythropoietic activity requires integrity of a glycoprotein since proteolytic enzymes as well as neuraminidase inactivate. Erythropoietin could be a glycoprotein associated with inert glycoprotein or even some other compound which becomes labile on disintegration of carrier glycoprotein. Enzymes like those mentioned, or other as yet undefined carbohydrases, either secreted with the urine or coming from other sources, could be responsible for large losses of urinary activity. The presence of phenol during isolation may prevent subtle changes through bacteriostasis, enzyme inactivation, anti-oxidant action, etc.

If it were not for measurement of the biological activity the protective effect of phenol would not have been noted. We hope that this preliminary report might be useful to those working with glycoproteins with or without biological activities.

This work was supported by a grant of the American Cancer Society.

PETER H. LOWY
GEOFFREY KEIGHLEY

Division of Biology,
Kerckhoff Laboratories of the
Biological Sciences,
California Institute of Technology,
Pasadena,
California.

[1] Keighley, G., Lowy, P. H., Borsook, H., Goldwasser, E., Gordon, A. S., Prentice, T. C., Rambach, W. A., Stohlman, jun., F., and Van Dyke, D. C., *Blood*, **16**, 1424 (1960).
[2] Lowy, P. H., Keighley, G., and Borsook, H., *Nature*, **185**, 102 (1960).
[3] Morgan, W. T. J., and Partridge, S. M., *Biochem. J.*, **35**, 1140 (1941).
[4] Westphal, O., Lüderitz, O., and Bister, F., *Zeitschr. f. Naturforsch.*, **7**, B, 148 (1952).
[5] Kirby, K. S., *Biochem. J.*, **64**, 405 (1956).

Thalassæmia in Melanesia

THALASSÆMIA has previously been regarded as non-existent in Melanesians[1]. In August 1960, a Papuan female from the Milne Bay area of New Guinea, aged 18–20 yr., was admitted to the General Hospital, Port Moresby, for re-investigation of a refractory anæmia first noticed during pregnancy in 1957. On that occasion she had been delivered of a full-term, apparently normal, infant, her hæmoglobin-level having been maintained by transfusions. Since discharge she had received no treatment and had been able to carry on normal village life, in spite of a hæmoglobin-level remaining fairly constant at 5–6 gm. per cent. Investigation in August 1960 showed typical bony changes, a hypochromic microcytic anæmia with target cells and normoblasts present in the peripheral blood, and the alkali denaturation test[2] showed 67 per cent undenatured hæmoglobin. The presence of hæmoglobin *F* as a major component was confirmed by hæmoglobin electrophoresis on cellulose acetate strips. Since then three other cases of thalassæmia major in Papuan children have been diagnosed, two of the children aged 5 yr. and 1·5 yr. came from the Kerema district, and one aged 3 yr. from the Oro Bay area in Papua.

All showed typical bony changes on radiological examination, a hypochromic microcytic anæmia with normoblasts and target cells in the peripheral blood and excess hæmoglobin *F*—32 per cent, 39 per cent and 46 per cent—by alkali denaturation, confirmed by hæmoglobin electrophoresis. All had been treated by transfusion on many occasions. In two of them diagnosis was delayed, presumably in one because of suppression of endogenous erythropoiesis by transfusion[3], and in the other by the presence of an aplastic crisis[4]. One of the children from Kerema has undergone splenectomy and his transfusion requirements have been greatly diminished since operation. Glucose-6-phosphate dehydrogenase estimation in this child on two occasions showed 46 and 56 units/100 ml. red blood cells respectively, suggesting that he has the negroid type of glucose-6-phosphate dehydrogenase deficiency in addition to thalassæmia.

In view of the fact that these cases have come from widely separated districts, it is considered that thalassæmia may be widespread in New Guinea.

Thanks are due to Dr. P. Brain of the Red Cross Transfusion Service, Perth, who carried out the hæmoglobin electrophoreses, Dr. D. Curnow and