Agents Influencing the Production and Action of Erythropoietin in Man

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Current concepts of the physiology of erythropoietin have been reviewed by Gordon (1959), Stohlman (1962), and Boggs (1966). The emphasis of this paper will be on clinically encountered factors that could enhance or inhibit the production or action of erythropoietin. Reference to animal experiments will be limited to those clarifying physiological principles not yet demonstrated in man. A brief outline of the production and action of erythropoietin in man will identify the sites at which stimulants or inhibitors may act.

ERYTHROPOIETIN (ESF)

Mechanism of production. Reduction of oxygen tension within the kidney is the major stimulus to ESF production. Clinically, one of three mechanisms may be involved. The first, anaemic hypoxia, follows reduction of the red cell mass by haemolysis, haemorrhage, or reduced production of erythrocytes. The second, hypoxic hypoxia, can be induced by reduced atmospheric pressure (Reynafarje *et al.*, 1959; Siri *et al.*, 1966; Carmena *et al.*, 1967), reduced arterial saturation (Lertzman, 1962), and the presence of abnormal haemoglobins (Gerald, 1960; Adamson and Stamatoyannopoulos, 1967). The third, histotoxic hypoxia, is rare in man, but may follow excessive ingestion or impaired detoxification of cobalt (Morin and Daniel, 1967). Both hypoxic and histotoxic hypoxia may cause secondary polycythaemia.

Detection of ESF. Erythropoietic activity is detected in normal urine by concentrating specimens to small volumes which are then injected into polycythaemic mice (Finne, 1965; Van Dyke *et al.*, 1966; Alexanian, 1966; Adamson *et al.*, 1966). ESF is not detectable in the plasma of normal individuals, but is increased in both the plasma and urine of patients with a reduced red cell mass. The higher concentration of ESF in the urine of patients with hypoxia-induced secondary polycythaemia distinguishes them from those with polycythaemia vera (Adamson, 1966a); in the latter group of patients, erythropoiesis is autonomous and unresponsive to stimulation by ESF (Krantz, 1967). In patients with hypernephroma, or other tumours

associated with polycythaemia, the quantity of ESF excreted is variable and, in our hands, has not been helpful in diagnosis.

Site of Production. Production of ESF by the human kidney was suggested by the polycythaemia in patients with hypernephroma or other renal anomalies. More recently, the improved erythropoiesis following renal transplantation, the development of polycythaemia, and increased ESF in both urine and plasma of these patients support the concept that the human kidney produces ESF (Nies *et al.*, 1965; McMain *et al.*, 1965; Abbrecht and Greene, 1966; Manasc *et al.*, 1968).

Evidence that humans may produce extra-renal ESF or other erythropoietic factors has recently been presented. Anephric patients maintained

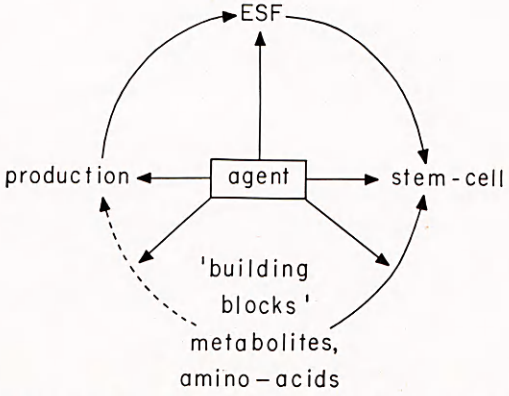

Fig. 1. Sites of action of agents which inhibit or enhance ESF.

adequate haemoglobin concentrations without detectable ESF in the plasma, despite evidence of haemolysis in some patients (Nathan *et al.*, 1964; Erslev *et al.*, 1967), and erythropoiesis could be enhanced in uraemic patients by the administration of cobalt despite the apparent absence of ESF (Gardner, 1962). Perhaps maintenance of baseline red cell production, independent of humoural regulation in such cases, is a result of the stimulation of erythropoiesis by products of haemoglobin degradation. This type of erythropoietic stimulation has been shown to occur in the absence of ESF (Brown *et al.*, 1963), and could be the primary control system upon which the action of ESF is superimposed. A comparable situation is seen during early foetal development where erythropoiesis proceeds in the absence of ESF (Jepson, 1968).

Site of Action. The major site of action of ESF is on stem cells which are

destined to differentiate into erythroid precursors. Some workers (Krantz and Goldwasser, 1965; Reissman and Ito, 1966; Keighley and Lowy, 1966; Hodgson, 1967) have suggested, more specifically, an effect at the level of messenger RNA synthesis, whereas others suggest that ESF stimulates DNA synthesis (Powsner and Berman, 1967).

It is apparent from the foregoing review that an agent may enhance or inhibit ESF directly or indirectly at its site of production, its site of action, or on the transfer or mobilisation of metabolites required for erythropoiesis (Fig. 1). A few examples of agents that affect one or more of these sites will be discussed.

INHIBITORY FACTORS

Immunological. The clinical course and laboratory findings in a number of cases of bone-marrow failure suggest that an immunological process could be involved. Definitive data to support this hypothesis are generally lacking. Attempts to demonstrate 'inhibitors' were unrewarding, and suggested that this mechanism was not a major factor in bone-marrow failure. On the other hand, the use of *in vivo* and *in vitro* techniques have provided evidence for antibody-like inhibitors in the plasma of patients with red-cell aplasia (Jepson and Lowenstein, 1966a; Krantz and Kao, 1967).

More recently, inhibitors that were detected in the plasma of two patients were shown to differ from each other in their biological behaviour. One, from the plasma of a patient with red-cell aplasia and thymoma, inhibited bone-marrow synthesis of nucleoprotein *in vitro*, but was not demonstrable *in vivo*. The second, found in the gamma globulin fraction of a patient with acute bone-marrow aplasia, behaved as an anti-erythropoietin (Jepson and Lowenstein, 1968a). Since the screening procedures employed are time-consuming and experimental, it is anticipated that simpler methods will be used on a routine basis for the detection of inhibitors, thus providing a more scientific approach to immuno-suppressant therapy for patients with demonstrable inhibitors.

Immuno-suppressants. Use of immuno-suppressants in the treatment of immunologically induced red-cell aplasia requires knowledge of their action on erythropoiesis. Induction of stem-cell differentiation by ESF in polycythaemic mice provides a model for studying the site of action of such drugs *in vivo*. Actinomycin D (Keighley and Lowy, 1966; Reissman and Ito, 1966) and vincristine (Morse and Stohlman, 1966) have been studied in a similar manner. More recently, azathioprine (Manasc and Jepson, 1968a) was tested because of its common use in patients with renal transplants. The doses employed were comparable to those used in patients. Results indicated a

suppression of erythropoiesis in normal mice and a specific inhibition of stem-cell differentiation induced by ESF. No effect on proliferation of erythroid precursors was observed, suggesting its primary effect was like Actinomycin D on RNA synthesis. Depression of erythropoiesis induced by azathioprine could be partially compensated by increasing the ESF titre in normal animals and appeared to be the case in man as well, since polycythaemia developed in patients with renal transplants despite continued administration of azathioprine (Manasc *et al.*, 1968). From the clinician's point of view, this suggests that the inhibiting effect of azathioprine on erythropoiesis in patients with red cell aplasia and inadequate endogenous ESF could be counteracted by the concurrent administration of ESF.

AGENTS ENHANCING ERYTHROPOIESIS

Androgens and anabolic steroids have been used frequently as erythropoietic stimulants in man; their use was reviewed by Gardner (1968). Their mechanism of action and that of anabolic protein hormones have only recently been explored, and will be reviewed briefly with emphasis on data obtained in humans.

Androgens

In experimental animals, testosterone synergistically enhances the effect of ESF (Naets and Wittek, 1964, 1966; Fried *et al.*, 1964; Jepson and Lowenstein, 1966b, 1967a). By itself, testosterone produces little effect when endogenous ESF is suppressed. This implies that the presence of ESF is required for its action to become apparent. It may act at several sites, enhancing the production of ESF (Fried *et al.*, 1964; Mirand *et al.*, 1965; Gurney and Fried, 1965), and potentiating the effect of ESF at the stem cell level in rodents (Naets and Wittek, 1964, 1966; Jepson and Lowenstein, 1967a, 1967b).

An effect on ESF production in man had been suggested by the greater erythropoietic activity of urine concentrates obtained from human males in comparison to that of females (Alexanian, 1966), and by the increased erythropoietic activity of urine concentrates of females given pharmacological doses of fluoxymesterone (Alexanian *et al.*, 1967). On the other hand, others have observed a decrease of erythropoietic activity in the urine of patients with bone-marrow failure treated with metho-androstanolone (Reynafarje and Faura, 1967), no change in the ESF content of specifically extracted plasma from a patient with siderocristic anaemia, collected at the same haematocrit both prior to and following injection of 600 mg of testosterone oenanthate weekly (Jepson, unpublished data), and no further increase in the amount of ESF excreted in the urine following addition of testosterone to the regimen of

human growth hormone (HGH) treated pituitary dwarfs (Jepson and McGarry, 1968b).

These findings suggest that ESF production was not stimulated by androgens, although it remains possible that the lack of stimulatory effect on the

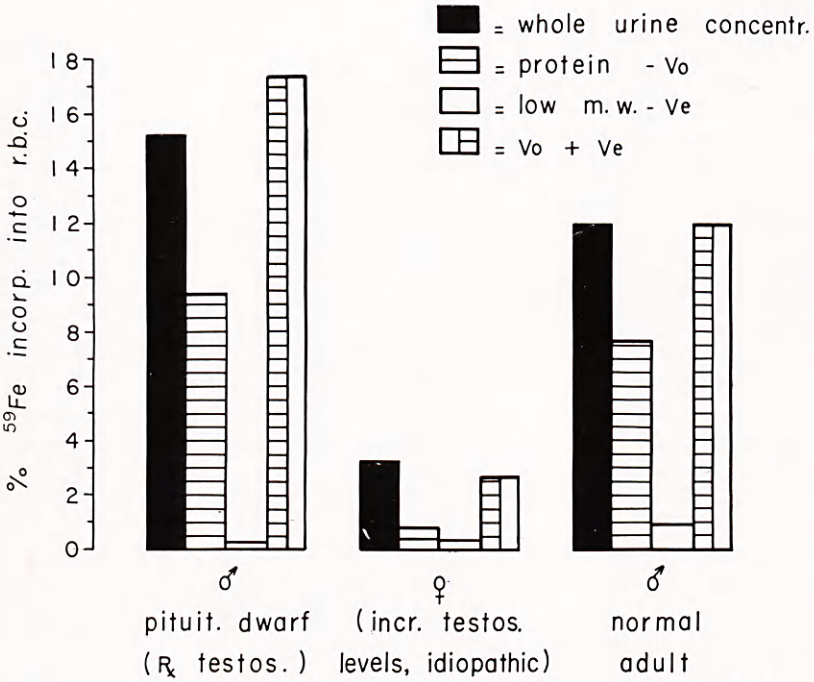

Fig. 2. Comparison of the erythropoietic effect of whole urine concentrate with that of the protein fraction (ESF) and small molecular weight fraction (<20,000) injected alone and when recombined.

production of ESF in patients with bone-marrow failure is due to the presence of a maximum ESF turnover rate present prior to addition of androgens. In the pituitary dwarfs, concurrent administration of HGH could have obscured the effect of testosterone. These observations would also be in accord with a common site of action of these hormones. Testosterone has been shown to aid transfer of metabolites, such as iron, from the reticulo-endothelial system to erythroid precursors (Haurani and Greene, 1967); to alter osmotic fragility and fatty acid composition of erythrocytes (March *et al.*, 1966), and, under some circumstances, to shorten the survival of erythrocytes in man (Reynafarje and Faura, 1967; Jepson and McGarry, 1968a).

When evaluating the ESF content of biological fluids, one must consider

the effect of hormone administration on renal clearance or tubular secretion of ESF. The renal haemodynamics of hypopituitary patients are improved by the administration of HGH and, presumably, by testosterone (Beck *et al.*, 1964). In addition, contamination of specimens with substances that could enhance or inhibit the effect of ESF must be considered when biological fluids are not specifically extracted for ESF; for example, when whole urine concentrates were fractionated on Sephadex G-25 into a protein fraction (ESF) and a small molecular weight fraction, the 'erythropoietic activity' of the ESF fraction was reduced, and that of the small molecular weight fraction abolished. The potentiating effect of the latter fraction was apparent when recombination of these fractions resulted in restoration of the originally observed erythropoietic activity (Fig. 2).

Androgens probably have a multi-site action on erythropoiesis in man, including a general anabolic effect when given to hypopituitary patients. Their action on bone marrow has been demonstrated *in vitro* (Reisner, 1967), and their effect at other sites, including transfer of metabolites from the reticulo-endothelial cells and alteration of cell membrane characteristics, indicate the complexity of their action. Specific stimulation of ESF production in man is suggested by some data, but definitive proof will rest on the demonstration of an increased turnover rate of ESF following their administration.

Anabolic Protein Hormones

Human growth hormone is of theoretical interest and illustrates the role of ancillary erythropoietic mechanisms. In hypophysectomised animals the effect of growth hormone on erythropoiesis was due to its anabolic action (Crafts and Meineke, 1959). In normal rabbits it produced a reticulocytosis (Halvorsen, 1966).

In prepubertal pituitary dwarfs, HGH increased the red cell mass, transferrin concentration, plasma iron turnover, and excretion of ESF (Jepson and McGarry, 1968b). Although the increased excretion of ESF suggests that HGH stimulates production of ESF, its general anabolic effect could result in an adjustment of both ESF production and erythropoiesis, with associated changes in other haematological parameters. In addition, the improved renal haemodynamics induced by HGH (Beck *et al.*, 1964) could produce increased levels of ESF in urine concentrates, and the small amount of HGH (30 μg) present, although devoid of an erythropoietic effect of its own, could potentiate the ESF in the specimen. No increase of erythropoietic activity was found in urine from an acromegalic patient and this finding could indicate that the effect observed in hypopituitary patients was due to the anabolic action of HGH. Prolactin, which is similar to HGH, stimulates

erythropoiesis in rodents when given in large doses (Jepson and Lowenstein, 1967a). When given in small doses and for a short period (three to six weeks) to patients with bone-marrow failure, there was no long-term beneficial

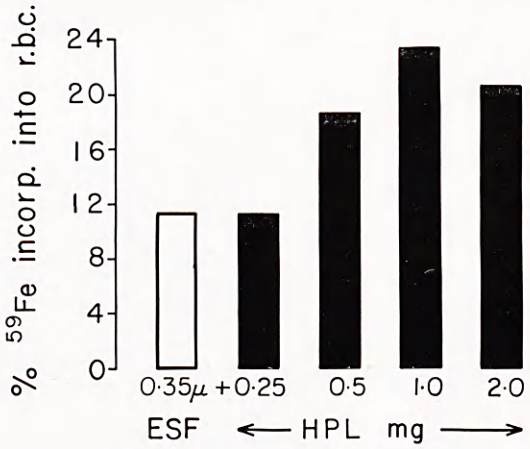

Fig. 3. Effect of the combined injection of increasing doses of HPL with a constant dose of 0·35 units ESF on the 48-hour per cent incorporation of ^{59}Fe into erythrocytes of polycythaemic mice.

effect, although increased and accelerated utilisation of ^{59}Fe by erythrocytes was evident (Jepson and Lowenstein, 1968d).

Human placental lactogen (HPL) found in high titres during human pregnancy may be an ancillary mechanism in enhancing the active erythropoiesis present, as well as increasing the plasma volume (Jepson and Lowenstein, 1968b, 1968c). Its action appears to be through enhancement of ESF rather than a direct effect on erythropoiesis. Maximum plasma 'erythropoietic activity' and excretion of ESF occur during the second trimester of human pregnancy, although increased plasma 'erythropoietic activity' occurred during all three trimesters (Manasc and Jepson, 1968b). Separation of erythropoietically-active retroplacental plasma into ESF and HPL fractions resulted in a decrease of the 'erythropoietic activity' of plasma (Jepson, 1968). The synergistic enhancement of ESF by HPL is demonstrated by injecting increasing doses of HPL in combination with a constant dose of ESF into groups of polycythaemic mice (Fig. 3). The same potentiation of ESF by HPL was demonstrated at increasing dose levels of ESF (Jepson and Friesen, 1967a, 1967b). The site of action of HPL appeared to be at the level of the stem cell, with little effect on late erythroid precursors (Fig. 4). These data

demonstrate the generally typical effect obtained with protein and steroid anabolic hormones (Jepson and Lowenstein, 1967a).

All of these protein hormones have marked anabolic effects. Their effect on

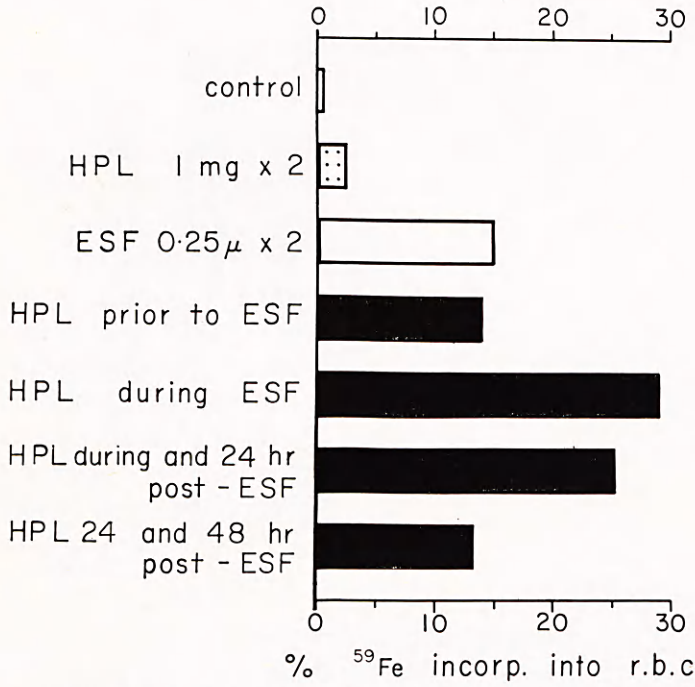

Fig. 4. Effect of HPL given at various intervals prior to, during, or following the initiation of stem-cell differentiation by ESF on the per cent incorporation of ^{59}Fe into erythrocytes of polycythaemic mice.

erythropoiesis could therefore be related to stimulation and mobilisation of protein with consequent improvement of erythropoiesis (Fig. 1). That more specific effects may be present is illustrated by the effect of HPL on stem-cell differentiation. By themselves they have little effect, requiring the presence of ESF for their action to become manifest. Thus they appear to serve an ancillary function.

ENDOCRINE RELATIONSHIPS OF ESF AND HORMONES RELATED TO THE KIDNEY

Patients with increased plasma or urine ESF titres, polycythaemia, and elevation of other hormones such as aldosterone, renin and angiotensin have recently been reported (Mann *et al.*, 1967; Jepson *et al.*, 1967). It is possible

that patients with polycythaemia and increased excretion of ESF following injection of vasopressin (Jepson and McGarry, 1968a), and patients with pheochromocytoma, polycythaemia, and increased plasma ESF (Sjoerdsma *et al.*, 1966) may fall into this category. The common denominator in these patients is the increased level of adrenal hormones or hormones related by their production in, or action on, the kidney. All, at appropriate doses, could directly or indirectly produce haemodynamic changes in the kidney and a consequent increased production of ESF. This hypothesis is somewhat supported by experiments carried out in animals.

Aldosterone and renin did not by themselves increase erythropoiesis (Mann *et al.*, 1967), but infusion of angiotensin II into rabbits reduced the renal blood flow and resulted in increased production of ESF (Nakao *et al.*, 1967). Increased production of ESF in rodents injected with vasopressin was also thought to be due to renal ischaemia secondary to its vasopressor effect (Jepson and Lowenstein, 1966b). Therefore, increased concentrations of these hormones with vasopressor activity could result, directly or indirectly, in an increased production of ESF.

The normotensive patient with hypertrophy of the juxtaglomerular apparatus and increased aldosterone, renin, angiotensin and ESF levels was of interest in view of the hypothesis that these cells produce ESF. Elevated angiotensin levels could produce ischaemic changes in the kidney like those demonstrated by Nakao *et al.* (1967), but such changes were not clinically demonstrable (Jepson *et al.*, 1967). Although these observations can be explained on the basis of renal ischaemia, the data could indicate a more direct effect of these 'renal hormones' on the production of ESF. Several authors (Kuratowska *et al.*, 1964; Contrera *et al.*, 1966; Gould *et al.*, 1968) suggest that a system of ESF production, release, and activation may parallel that of the aldosterone-renin-angiotensin system; a hypothesis recently reviewed by Gordon *et al.* (1967). The possible connexion between these two systems remains to be explored.

In summary, the effect of various types of inhibition and stimulation of the production or action of ESF has been reviewed. The importance of detecting the presence of plasma 'inhibitors' as a primary or secondary cause of erythropoietic depression is emphasised, since detection of such factors would form a basis for treatment with immuno-suppressants. The mechanism of action of various anabolic hormones which potentiate ESF is discussed, with the conclusion that many of these hormones, directly or indirectly, could enhance both its action and production. The curious association of increased levels of renal and adrenal hormones, polycythaemia and increased ESF production is discussed.

Acknowledgements

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To Make Snayle Water

Take a good peck of garden snayles in their shells and wash them in a great bowle of beere; then make your Chimney hearth very clean and pour out halfe a bushell of Charcoales and set them on fire, and when they are throughly kindled, then with a fire shovell make a great hole in the midst of them and pour the snayles and scatter some fire of yr quick coales amongst them, and soe let them rest till they have done makeing a noise . . . Then in a stone Mortar bruise them shells and all, take alsoe a quart of earth worms, and scoure them with water very well divers times over . . . Then take a clean Iron pot upon which sett yr Limbeck or Still, then take 2 handfulls of Sallondine and lay in the bottome of yr pott and 2 handfulls of Angelica must be laid upon that, then put in a quart or two of rosmary flowers alsoe boarsfoot Agrimony Reddock roots, Barke of Barbary trees, wood Sorrill, Bittony, of each of them two handfulls, halfe a handfull of Rue of Fenegrick and Tumricke of each an ounce, well beaten . . . Then pour in three galls of the strongest Ale . . . and lett it stand all night . . . In the morninge you must put in three ounces of Cloves beaten to powder, sixpeny waight of Saffron dry'd to powder Harts horne fyled six ounces . . . This water is by experience good for yellow Jaundise and for all griefes cominge from the obstructions of the Liver helps the Consumption.

(From Lady Sedley's Receipt Book. In Manuscript 1686. Original in College Library.)