Engl. J. Med. **283**, 1370, 1441, 1498 (1970).
18. R. O. Brady, *Annu. Rev. Med.* **21**, 317 (1970).
19. S. Pell and C. A. D'Alonzo, *J. Amer. Med. Ass.* **214**, 1833 (1970).
20. R. D. Hotchkiss and M. Gabor, *Annu. Rev. Genet.* **4**, 193 (1970).
21. A. S. Fox, S. B. Yoon, W. M. Gelbart, *Proc. Nat. Acad. Sci. U.S.* **68**, 342 (1971).
22. S. A. Aaronson and G. J. Todaro, *Science* **166**, 390 (1969).
23. H. J. P. Ryser, *ibid.* **159**, 390 (1968).
24. J. T. Dingle and H. B. Fell, *Frontiers Biol.* **14A**, 220 (1969).
25. R. Dulbecco, *Science* **166**, 962 (1969); M. Green, *Annu. Rev. Biochem.* **39**, 701 (1970).
26. J. Sambrook, H. Westphal, P. R. Srinivasan, R. Dulbecco, *Proc. Nat. Acad. Sci. U.S.* **60**, 1288 (1968).
27. M. Fried, *Virology* 40, 605 (1970).
28. E. H. Szybalska and W. Szybalski, *Proc. Nat. Acad. Sci. U.S.* **48**, 2026 (1962); M. Fox, B. W. Fox, S. R. Ayad, *Nature* **222**, 1086 (1969); R. A. Roosa and E. Bailey, *J. Cell. Physiol.* **75**, 137 (1970); L. M. Kraus, *Nature* **192**, 1055 (1961); D. Roth, M. Manjon, M. London, *Exp. Cell Res.* **53**, 101 (1968); J. L. Glick and C. Sahler, *Cancer Res.* **27**, 2342 (1967); E. Ottolenghi-Nightingale, *Proc. Nat. Acad. Sci. U.S.* **64**, 184 (1969).
29. T. Friedmann, J. H. Subak-Sharpe, W. Fujimoto, J. E. Seegmiller, paper presented at Society of Human Genetics Meeting, San Francisco, Oct. 1969.
30. Calculated with the assumption that (i) the DNA contents of a diploid human cell is 4×10^{12} daltons; (ii) representative gene codes for a protein containing 200 amino acids equivalent to 4×10^{5} daltons of DNA; and (iii) there are two copies of each gene per cell. This would represent a minimum estimate if the redundant DNA segments in human cells include genes which specify enzymes.
31. H. M. Temin and S. Mizutani, *Nature* **226**, 1211 (1970); D. Baltimore, *ibid.*, p. 1209.
32. P. Berg, and D. M. Jackson, personal communication.
33. J. V. Osterman, A. Waddell, H. V. Aposhian, *Proc. Nat. Acad. Sci. U.S.* **67**, 37 (1970).
34. L. Grady, D. Axelrod, D. Trilling, *ibid.*, p. 1886.
35. B. Hirt, seminar at Brandeis University, Oct. 1970.
36. H. Fraenkel-Conrat, *Annu. Rev. Microbiol.* **24**, 463 (1970).
37. H. G. Terheggen, A. Schwenk, A. Lowenthal, M. Van Sande, J. P. Colombo, *Lancet* **II-1969**, 748 (1969); *Z. Kinderheilk* **107**, 298 and 313 (1970).
38. S. Rogers, *Nature* **183**, 1815 (1959).
39. ——— and M. Moore, *J. Exp. Med.* **117**, 521 (1963).
40. P. S. Satoh, T. O. Yoshida, Y. Ito, *Virology* **33**, 354 (1967); G. Orth, F. Vielle, J. B. Changeux, *ibid.* **31**, 729 (1967).
41. This objection would be straightforward if only one type of arginase were present in human cells. Even though there seem to be two arginase isozymes in both human liver and erythrocytes, the objection is still cogent since one isozyme contributes 90 to 95 percent of the total arginase activity, and the two isozymes cross-react immunologically. See J. Cabello, V. Prajoux, M. Plaza, *Biochim. Biophys. Acta* **105**, 583 (1965).
42. R. N. Feinstein, M. E. Seaholm, J. B. Howard, W. L. Russel, *Proc. Nat. Acad. Sci. U.S.* **52**, 661 (1964).
43. H. Harris, *Frontiers Biol.* **19**, 167 (1970).
44. W. Munyon, E. Kraiselburd, D. Davis, J. Mann, *J. Virol.* **7**, 813 (1971).
45. C. R. Merril, M. R. Geier, J. C. Petricciani, *Nature* **233**, 398 (1971).

Protein Synthesis: Its Control in Erythropoiesis

Cellular differentiation is explored in the erythroid cell system of the mouse.

Paul A. Marks and Richard A. Rifkind

The regulation of mammalian cell differentiation remains one of the major unresolved problems in biology. Operationally, the definition of the differentiated state generally involves the capacity for synthesis of specialized proteins. To elucidate the mechanisms controlling the initiation of synthesis of differentiated proteins one must study the precursor of the differentiated cell. In almost all mammalian cell populations there is heterogeneity with regard to the stage of differentiation and with regard to the type of differentiated cell. To identify the initiating event in the onset of synthesis of differentiated protein of a given cell line, the precursor cell must be isolated for quantitative, analytical studies. In the total cell population of most differentiating mammalian cell systems such cells are present in a very low proportion.

In erythroid cell systems a substantial beginning has been made in the analysis of aspects of the regulation of the biologic activity of the precursor cell (*1, 2*). Erythroid cells synthesize a predominant type of protein, the hemoglobins. There is a considerable body of knowledge with respect to the genetic, chemical, physiological, and cytological aspects of erythroid cells. The hormone, erythropoietin, has a role in inducing erythropoiesis and hemoglobin synthesis. While no single mammalian cell system is ideal for exploring the full range of problems that are critical to the understanding of the regulation of protein synthesis in differentiating cells, the erythroid cell system has provided considerable insight into a number of these, such as: (i) the cellular basis for changing patterns of hemoglobin synthesis during fetal and postfetal development; (ii) the relationship between primitive and definitive erythroid cell lines; (iii) the mitotic activity of erythroblast and the relationship to hemoglobin synthesis; (iv) the stability of messenger RNA in relation to the synthesis of different classes of proteins during erythroid cell differentiation; and (v) the nature of erythropoietin action on cell differentiation and hemoglobin synthesis.

Patterns of Hemoglobin Synthesis

Changes in the types of hemoglobins synthesized during fetal and postfetal development have been described for the mouse, man, tadpole, chick, and a variety of other species (*3*). Studies in the mouse have provided perhaps the most direct evidence on the relationship between alterations in the cell line active in erythropoiesis and the type of hemoglobin formed (*4*). In the mouse, whose gestation period is 21 days, the first morphologically identifiable site of erythropoiesis occurs in the blood islands of the yolk sac at approximately the eighth day of gestation (*1*). Proliferation of erythroid cell precursors is active in these sites from about the eighth to the tenth days (*1, 5*). These cells enter the fetal circulation as nucleated erythroblasts by the ninth day of gestation. From the tenth day onward, further division and differentiation of these cells proceeds in the circulation. Mitosis may be observed through day 13 of gestation. Ribosomes and polyribosomes, abun-

Dr. Marks is dean of the Faculty of Medicine, College of Physicians and Surgeons, Columbia University, New York 10032, and he and Dr. Rifkind are members of the Departments of Human Genetics and Development and of Medicine at the university.

Table 1. Patterns of hemoglobin synthesis compared with development. This table is based on data reported in (*3, 4, 7*).

Species	Cell lines			
	Embryonic		Definitive	
	Site	Hemoglobin	Site	Hemoglobin
Mouse	Yolk sac	E_I (xy) E_{II} (αy) E_{III} (αz)	Liver and bone marrow	(Fast A?) and A($\alpha\beta$)
Man	Yolk sac (?)	Gower ($\alpha\epsilon$)	Liver and bone marrow	F ($\alpha\gamma$), A($\alpha\beta$), and A_2($\alpha\delta$)
Chicken	Yolk sac	E (α?) P	Yolk sac and bone marrow	A (α?), D (α?)
Frog	Liver	Type I Type II t	Liver and bone marrow	Frog I and frog II

dant in early stages of these cells, progressively decrease in concentration as the cells accumulate hemoglobin. This process is accompanied by progressive condensation of the nuclear chromatin, shrinkage and disappearance of the nucleolus, and overall nuclear pycnosis. The mature circulating erythrocyte derived from the yolk sac is nucleated but devoid of cytoplasmic mitochondria and ribosomes. Three hemoglobins are formed in erythroid cells of the yolk sacs of strain C57Bl/6J mice. These have been characterized as embryonic hemoglobin, E_I, composed of x and y globin chains, embryonic hemoglobin E_{II}, composed of α and y globin chains, and embryonic hemoglobin E_{III}, composed of α and z globin chains (*4*). There is no detectable synthesis of β globin and, hence, no adult hemoglobin ($\alpha_2\beta_2$) in these cells. During the tenth day of gestation, a second site of erythropoiesis, the liver, becomes morphologically detectable. Erythroid cell presursors, apparently derived from mesenchymal cells adjacent to cords of hepatic epithelial cells of endodermal origin (*6*), give rise to hemocytoblasts and, in turn, to proerythroblasts which differentiate through a series of developmental stages, to nonnucleated reticulocytes. Terminal differentiation of these erythroid cells is characterized by nuclear expulsion prior to loss of the cytoplasmic ribosomes and mitochondria. Liver erythroid cells synthesize a single type of hemoglobin with a globin composition indistinguishable from that of hemoglobin present in the adult of this species, designated α and β globin (*4*).

These data establish that there are two distinct populations of erythroid cells appearing at different times during fetal development of the mouse. The change in pattern of hemoglobin synthesis from the embryonic to the adult type of hemoglobin is associated with the substitution of liver erythropoiesis for yolk sac erythropoiesis. This cellular basis for changing patterns of hemoglobin synthesis appears to be a more general phenomenon in animal development (Table 1). Evidence indicates that an analogous shift from a primitive to a definitive erythroid cell line is associated with a change in types of hemoglobin synthesized in the chick (*3*), man (*1*), and the tadpole (*7*). In addition to this pattern of cellular changes which determines changes in hemoglobin formation, a further suggestion of pattern may be discerned in the structural alterations in the hemoglobins formed in primitive and definitive cell lines. In the mouse, man, and chicken, conversion from embryonic to adult hemoglobin includes the substitution of one globin chain (*3, 4*). In the mouse and in man this substitution is in the beta-like chain. The alpha chain is constant in structure in the embryonic and adult hemoglobins, presumably reflecting constant genetic activity for the alpha chain and altered genetic activity for the beta-type chain. In the chicken, likewise, one globin chain appears to be constant between the embryonic and adult hemoglobin, although this is not the case with the tadpole-frog system which has no common peptide chains (*7*).

Primitive and Definitive Cell Lines

These observations raise questions as to the relationship between the primitive and definitive erythroid cell lines. At what stage of embryogenesis does determination occur in a progenitor cell toward the yolk sac erythroid cell line and, subsequently, toward the hepatic erythroid cell line? Do the blood island cells of the yolk sac seed the liver as direct precursors of liver erythropoiesis?

There is no definitive evidence in any species to indicate that the primitive erythroid cells are direct precursors of definitive erythroid cells. Mouse yolk sacs contain pluripotential cells capable of producing granulocytic, megakaryocytic, and erythroid spleen colonies in vivo, and of producing granulocytic and mononuclear macrophage colonies in vitro, on agar (*8*). There is no detectable megakaryocytic or granulocytic cell formation in mouse yolk sac cells in situ. These findings suggest that in the yolk sac environment differentiation is restricted to the erythropoietic pathway and the synthesis of embryonic hemoglobins. Neither in vivo nor in vitro is there substantive evidence that yolk sac cells seed fetal liver erythropoiesis (*8–10*). No evidence for embryonic hemoglobin synthesis in liver cells has been detected (*5*). These negative results do not exclude the possibility that the yolk sac contains cells which are the direct precursors of fetal liver erythropoiesis. An alternative possibility is that the primitive or yolk sac cells and the definitive or liver cells derive from a common precursor at a point considerably earlier than the development of yolk sac cells. There is considerable evidence that expression of a differentiated lineage may reflect the interaction of a programmed cell and environmental factors (*11*).

Mitosis and Hemoglobin Synthesis

Erythroid cells in the yolk sac differentiate as a relatively homogeneous population in vivo (*12*). In these cells, hemoglobin formation proceeds in cells synthesizing DNA and dividing. This was indicated by studies in which yolk sac erythroid cells derived from 11-day fetuses were incubated with [^{3}H]thymidine and examined by autoradiographic techniques to determine which cells synthesize DNA (*12*). At 11 days, approximately 80 percent of the cells were labeled with thymidine within 4 hours of incubation. At this stage, at least 45 percent of all the hemoglobin that would be synthesized by these cells had been formed. All of the dividing erythroid cells contained hemoglobin, as evaluated by histochemical techniques. Yolk sac erythroid cells differentiate between days 10 and 14 of gestation, and the cells, on the average, undergo two to three mitoses. With each succeeding division, the increment in hemoglobin content per cell decreased (Table 2). Between day 10 and

day 11, there was approximately a sixfold greater increase in the content of hemoglobin than occurred in cells between days 12 and 13. These data indicate that the rate of hemoglobin synthesis per cell decreased with each succeeding cell division between days 10 and 17. There was continued hemoglobin synthesis following the terminal mitosis, but at a relatively low rate. Cessation of hemoglobin synthesis occurred by day 15.

The nucleus of mature erythroid cells, following the terminal division, is essentially inactive in nucleic acid synthesis, but may be capable of reactivation under special circumstances. Hen nuclei, freed of cytoplasm by treatment of the mature nucleated erythrocytes with Sendai virus, and introduced into fibroblasts of a variety of species including man and mouse, resume nucleic acid synthesis and direct the synthesis of certain species-specific proteins but not hemoglobins (*13*). Since the fibroblast does not synthesize hemoglobin despite the fact that its nucleus contains the necessary genes, the failure to reactivate hemoglobin formation in these experimental circumstances may be due to the fact that fibroblast cytoplasm is not an appropriate environment for hemoglobin messenger RNA transcription or translation.

It has been suggested that synthesis of specialized protein does not occur in cells which are actively proliferating (*14, 15*). This is not the case for differentiation of erythroid cells in the yolk sac. Similarly, DNA synthesis has been demonstrated to occur in differentiating cells of the galea of the silk moth, in fibroblasts capable of collagen synthesis, and in cells producing antibodies (*16*). Holtzer (*15*) suggested that erythroid cell differentiation is a variation on the theme of obligatory separation of mitotic activity and synthesis of differentiated proteins, which does appear valid for differentiation of skeletal muscle. In differentiation there is a critical mitosis, at which point the definitive commitment to the synthesis of differentiated proteins occurs. This definitive, or "quantal" (*14*), mitosis need not be the terminal division of a cell lineage. It differs from divisions for self-renewal of the precursor cells by virtue of initiation of expression of the program of differentiation. This program determines the transition from precursor cell to erythroblast, including the number of divisions and the amount of hemoglobin to be synthesized by the cell and its progeny (*12, 15*). This definitive mitosis results in a process which, at least in vivo, is irreversible. The findings in the fetal mouse cited above, and those reported by Holtzer and co-workers in studies with primitive chick erythropoiesis (*12, 15, 17*), suggest that the number of cell divisions and the amount of hemoglobin synthesis of these cells is programmed and independent of specific external influences.

Table. 2. Number of yolk sac erythroid cells, content of embryonic hemoglobins, and mitotic index on days 10 to 14 of gestation. These data are derived from (*12*).

Day	No. of cells ($\times 10^6$/embryo)	Content of embryonic hemoglobins*		Increment in hemoglobin content per cell per day (μg/10^6 cells)	Mitotic Index†
		μg/embryo	μg/10^6 cells		
10	0.31	3.5	11.3		3.2
				42.3	
11	0.70	37.5	53.6		3.8
				10.4	
12	1.22	78.0	64.0		3.4
				7.3	
13	1.41	102.0	72.3		0.4
				7.3	
14	1.50	121.0	80.6		0.0

* These values represent the average of at least three separate experiments on each day. The values for micrograms of hemoglobin per 10^6 cells were calculated as the quotient of the average value for micrograms of hemoglobin per embryo divided by the average number of cells. † Based on counts of 2000 cells from blood pooled from at least 100 fetuses on each day.

In mouse yolk sac erythroid cells, there is evidence that mitotic history and hemoglobin synthesis are not inextricably linked and selective interference with one process will not necessarily affect the other. Fantoni *et al.* (*18*) have shown that by exposing fetal mice to a suitable dose of x-irradiation, the number of mitoses which the yolk sac erythroid cells undergo can be decreased, on the average, by one, while the content of hemoglobin per mature cell in irradiated fetuses is twice normal. This suggests that yolk sac erythroid cells are programmed to synthesize a specific amount of hemoglobin at a relatively early stage in differentiation and that this program is independent of that which determines the number of cell divisions that the erythroblasts will undergo.

Stability of Messenger RNA

As yolk sac erythroid cells differentiate between days 11 and 15 of gestation, there is a progressive decrease in the content of RNA (*12*). This decrease in RNA content per cell correlates with the disappearance of cytoplasmic ribosomes observed in electron microscopic studies of these cells. The capacity for RNA synthesis falls sharply between days 11 and 13 of gestation. Paralleling this decrease in the rate of RNA synthesis is a decrease in the formation of nonhemoglobin protein, while the capacity for hemoglobin synthesis remains relatively unchanged during this period. Over 90 percent of the nonhemoglobin proteins synthesized in yolk sac erythroid cells are nuclear proteins (*19*). Of this nuclear protein, approximately 50 percent is insoluble in acid.

In yolk sac erythroid cells the formation of nonhemoglobin protein appears to proceed predominantly on relatively short-lived molecules of messenger RNA. On the other hand, differentiated protein synthesis, that is, hemoglobin formation, is directed by relatively stable messenger RNA formed at some time prior to day 10 of gestation. Thus, incubation of 11-day yolk sac erythroid cells with actinomycin D has little or no effect on the rate of synthesis of hemoglobins, but inhibits nuclear protein synthesis by at least 90 percent (*12, 19*). The synthesis of differentiated protein on relatively stable messenger RNA templates is characteristic of several differential cell lines studied to date, including the lens, fibroblasts, pancreas, and others (*20*).

At 11 days, only one-third of the total protein formed in mouse yolk sac erythroid cells are hemoglobins, but by day 13 essentially all of the proteins synthesized in the cells are hemoglobins. The formation of nuclear and other nonhemoglobin proteins decreases to nil between days 11 and 13.

These alterations in the pattern of proteins formed are associated with changes in the types of RNA synthesized at different stages of differentiation. In the RNA purified from polyribosomes of day 11 erythroid cells, there is a major peak of rapidly syn-

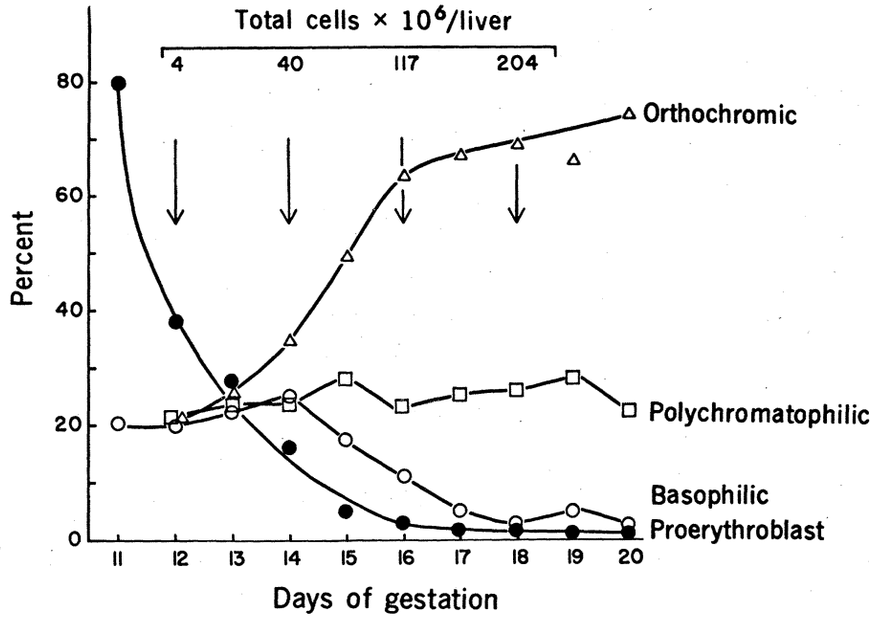

Fig. 1. Alterations in the erythroid cell population of the liver in terms of proportion of the cells morphologically classified as proerythroblasts (solid circles), basophilic erythroblasts (open circles), polychromatophilic erythroblasts (squares) and orthochromic erythroblasts (triangles) on days 11 through 18 of gestation of fetal mice. The numbers at the top of the figure are from studies by Barker *et al.* (*10*) for the total erythroid cells per liver. The data on the different stages of differentiation of erythroid cells are from Djaldetti and Rifkind (unpublished results).

thesized RNA which migrates on agarose acrylamide gel in a position corresponding to 9*S* (*19*). This 9*S* RNA is not recovered from 80*S* ribosomes and is not a degradation product of ribosomal RNA. Between days 11 and 13 of gestation, while there is a marked decrease in the overall rate of synthesis of RNA, the rate of synthesis of 9*S* RNA falls almost to nil, in parallel with the decrease in nonhemoglobin protein formation. It was further shown that the 9*S* RNA synthesized in 11-day cells is not identical, by the criteria of electrophoresis in agarose acrylamide gel with the messenger RNA for hemoglobin isolated from polyribosomes of adult mouse reticulocytes (*19, 21*). Because mouse yolk sac erythroid cells synthesize three types of embryonic hemoglobins, two of which contain alpha chains indistinguishable from the alpha chains of adult hemoglobin, one would anticipate that if the 9*S* RNA synthesized in these cells is messenger RNA for globin, it should be identical to the messenger RNA for globin found in adult mouse reticulocyte polyribosomes. It has also been found that agents such as actinomycin D or hydroxyurea, which block the synthesis of 9*S* RNA in yolk sac erythroid cells, inhibit nuclear protein formation but not hemoglobin synthesis (*19*). The characteristics of this 9*S* RNA, namely, that it turns over rapidly, that it is electrophoretically distinct from adult reticulocyte messenger RNA for globin, and that it is not synthesized in the presence of actinomycin D or hydroxyurea, agents which inhibit the synthesis of nuclear proteins but not hemoglobins, suggest it may be messenger RNA for one or more of the nuclear proteins. The messenger RNA for globin in yolk sac erythroid cells appears to be synthesized prior to the tenth day of gestation and remains stable while these erythroblasts proliferate fourfold. The stable messenger RNA's for hemoglobins may be distributed to daughter cells through, on the average, two to three cell divisions.

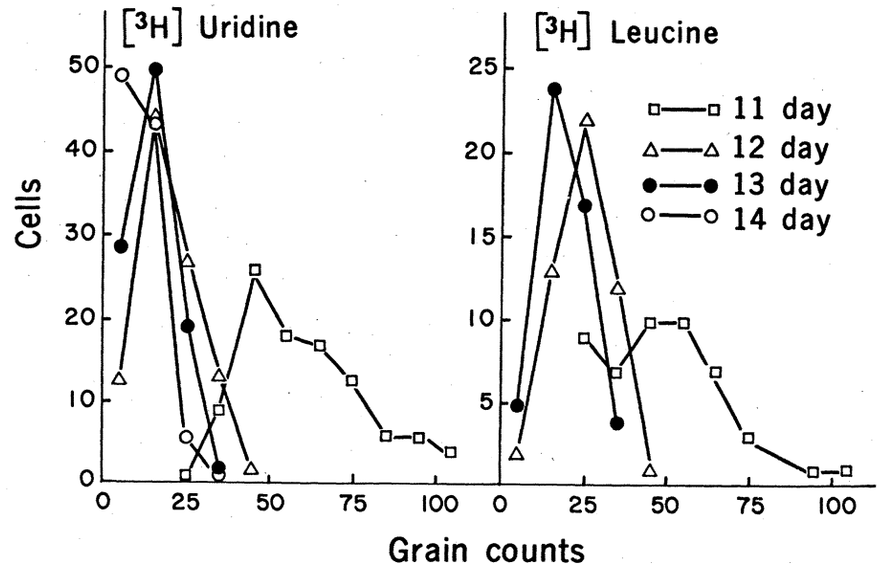

Fig. 2. Uptake of [³H]uridine and [³H]leucine by proerythroblasts of the livers of 11-, 12-, 13-, and 14-day fetuses. The cells were incubated with [³H]uridine or with [³H]leucine for 15 minutes and isotope incorporation was determined by quantitative radioautography. Grain counts for these cell populations with major differences in rate of incorporation were calculated by extrapolating observed counts to an arbitrary exposure time, after establishing the linear relationship between duration of exposure and grain count.

In contrast with yolk sac erythropoiesis, it has been possible to demonstrate in liver erythroid cells a transition from hemoglobin synthesis which is sensitive to inhibition by actinomycin D on days 11 and 12 to one which is insensitive to the effects of this antibiotic by day 13 of gestation (*12*). This development of resistance to actinomycin D appears to reflect an alteration in the environment in which erythropoiesis is proceeding. On both days 12 and 15, actinomycin D inhibits RNA synthesis by more than 90 percent at all stages of differentiation between proerythroblasts and orthochromic erythroblasts (*22*). On day 12, the antibiotic inhibits the uptake of labeled iron or leucine, employed as precursors of hemoglobin synthesis, at all stages of erythroblasts. By day 15, the antibiotic has little or no effect on the uptake of these isotopes into polychromatophilic and orthochromic erythroblasts which synthesize hemoglobin. These observations suggest that the stabilization of the hemoglobin synthetic capacity in liver erythroid cells occurs with fetal development.

Erythropoietin Action

Central to our understanding of the regulation of erythroid cell differentiation and hemoglobin synthesis is identification of the cell in which the synthesis of these proteins is initiated. From day 11 of gestation, the fetal liver is a site of erythropoiesis and the population of liver erythroid cells becomes increasingly more differentiated (*6, 23, 24*). On day 11, approximately 80 percent of the erythroid cells present in the liver are at a very immature stage, but by day 14, the proportion of these immature cells decreases sharply, to less than 5 percent, and there is a concomitant increase in the proportion of orthochromic erythroblasts which have synthesized hemoglobin (Fig. 1) (*25*). During this period, there is a greater than tenfold increase in the total number of erythroid cells in the liver (*9, 10*). Erythropoiesis in the fetal liver proceeds as a heterogeneous population with regard to cell stage, in contrast to yolk sac erythroid cells which differentiate in a relatively homogeneous fashion. Thus, yolk sac erythroid cells appear to develop from precursor cells as a cohort, while liver erythropoiesis, at least for a short period, depends on a self-perpetuating precursor cell which yields differentiating erythroblasts over a period of time. Erythropoiesis in the liver, however, is a transitional process; by birth, the liver is no longer an active site of erythropoiesis (*26*).

In the livers of 11-day fetuses, there is a population of cells morphologically classified as proerythroblasts, which show a very active incorporation of uridine into RNA and leucine into protein. This is indicated by a rate of uptake of [^{3}H]uridine and of [^{3}H]leucine by these cells which is three to five times greater than that in morphologically comparable cells on subsequent days of gestation (Fig. 2) (*25*). In addition to morphological criteria, as will be indicated below, evidence that these cells are erythroid cell precursors includes their selective response to the physiological regulator of erythropoiesis, the hormone erythropoietin (*27*). Its disappearance and the consequent loss of the capacity for sustained erythropoiesis may account for the transient nature of erythropoiesis in the liver of the fetal mouse.

There is considerable evidence that the precursor cell responsive to erythropoietin is distinct from the pluripotential hematopoietic stem cell. Thus, Stephenson and Axelrad (*28*), by means of velocity sedimentation of mouse fetal liver cells, separated erythropoietin-responsive cells from hematopoietic spleen colony-forming cells. Earlier biological studies provided evidence that pluripotential spleen colony-forming cells are distinct from (and probably include the progenitor of) the erythropoietin-sensitive cell (*29*).

The nature of the erythropoietin-responsive cell, the immediate precursor of the hemoglobin-forming cell, has been examined further at the cellular level. Goldwasser and co-workers (*30*), studying bone marrow of the adult rat, and Paul and co-workers (*23, 31*), studying liver erythroid cells of the fetal mouse, demonstrated that erythropoietin induces increased hemoglobin formation. In fetal liver erythroid cells, the hormone-induced increase in hemoglobin formation does not result from a direct effect on the rate of hemoglobin synthesis per cell (*24*). This is indicated by the observation that erythropoietin does not increase the uptake of [^{3}H]-leucine per polychromatophic or per orthochromatic erythroblast in cultures in which the hormone causes approximately a twofold increase in hemoglobin formation. The erythropoietin-stimulated increase in hemoglobin synthesis is caused by an increase in the number of cells synthesizing hemoglobin. Incubation of erythroid cells derived from fetal liver with erythropoietin for 24 hours results in an approximately twofold increase in the number of erythroblasts compared with cultures without erythropoietin. The hormone acts to maintain the number of immature proerythroblasts and basophilic erythroblasts in the population, and to increase the total number of hemoglobin-forming cells. Erythropoietin is required for renewal of the immature population of erythroid cell precursors under these conditions in vitro. Yolk sac erythropoiesis, in which immature precursors do not persist, is not demonstrably responsive to erythropoietin (*23, 25, 32*). These observations are consistent with studies in several laboratories (*27*) which suggest that the continuous presence of erythropoietin is not necessary for erythroblast maturation but does stimulate erythropoiesis.

In erythroid cells of fetal liver, the first detectable effect of erythropoietin on macromolecular synthesis is a stimulation of the rate of RNA formation (*24, 33*). Radioautographic studies demonstrated that this effect of the hormone is selective, in that it is restricted in culture to a stimulation of

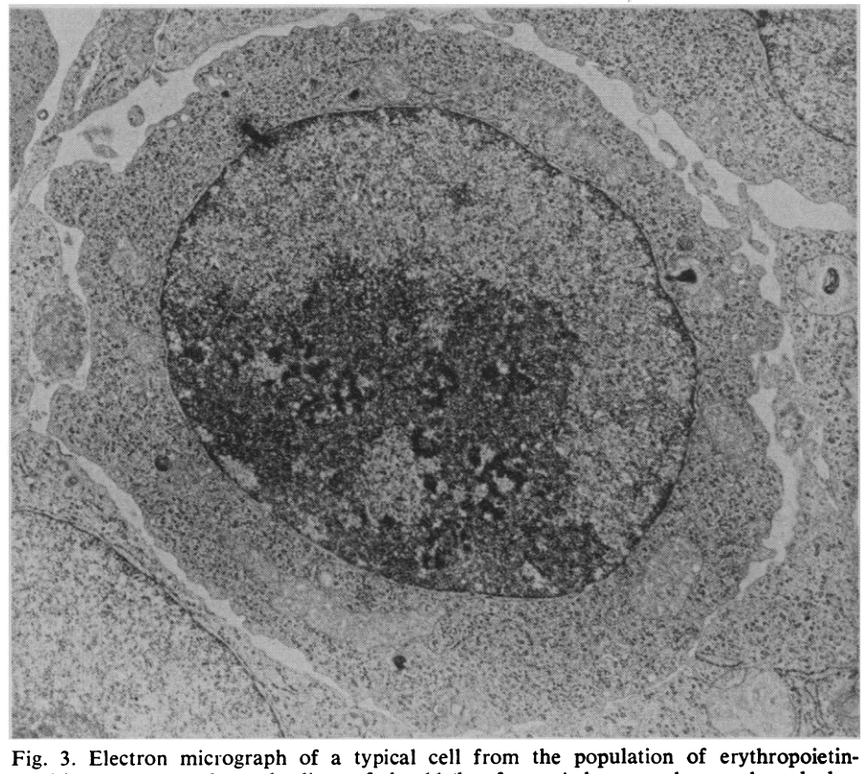

Fig. 3. Electron micrograph of a typical cell from the population of erythropoietin-sensitive precursors from the liver of the 11-day fetus. A large nucleus and nucleolus, with almost exclusively extended chromatin in the nucleoplasm, are characteristic. The cytoplasm contains free polyribosomes, mitochondria, and relatively few cisternae of endoplasmic reticulum (×9000).

the rate of RNA synthesis in the most immature erythroid cells, the proerythroblasts (*24*). The hormone had no effect on RNA formation in more differentiated erythroid cells or in nonerythroid cells.

These erythropoietin-responsive cells are included in a class of precursor cells designated proerythroblasts on the basis of cytological criteria (*34*). The cells in this population are large and, by light and electron microscopy, display a high ratio of nuclear to cytoplasmic material and an extended chromatin pattern within the large nucleus (Fig. 3). The cytoplasm contains abundant polyribosomes, mitochondria, and sparse elements of the endoplasmic reticulum.

In these cells, erythropoietin stimulation of RNA synthesis is not dependent on any hormone-mediated effect on DNA synthesis (*35*). Inhibition of DNA synthesis by hydroxyurea or cytosine arabinoside does not prevent the erythropoietin-stimulated RNA synthesis in proerythroblasts. Comparable results were reported for rat marrow (*35*). Erythropoietin-stimulated synthesis of RNA in fetal liver proerythroblasts in culture precedes detectable increase in cell number and in hemoglobin synthesis (*24, 35*).

The nature of the erythropoietin-stimulated RNA in fetal liver erythroblasts has not yet been determined. In rat marrow, Gross and Goldwasser (*33*) report that erythropoietin causes stimulation of a variety of RNA species including 150*S*, 55 to 65*S*, 45*S*, 28*S*, 18*S*, 9*S*, 6*S*, and 4*S*. This is in contrast to an earlier report from the same laboratory which concluded that erythropoietin selectively stimulated 10 to 12*S* RNA and suggested that the hormone might be acting primarily by increasing the synthesis of messenger RNA for globin (*33*).

In considering the mechanisms of action of erythropoietin, it is interesting to note that a number of substances which affect, selectively, cell growth and differentiation, such as nerve growth factor (*36*), phytohemagglutinin (*37*), insulin (*38*), and others (*39*), stimulate the synthesis of RNA as the initial effect on macromolecular formation. It has been suggested that this requires a constant set of biochemical reactions responding coordinately to the environmental stimulus, possibly via a common mediator substance (*38*).

On the basis of the data presently available, we suggest the following hypothesis: erythropoietin acts on a specific cell, a proerythroblast, which is differentiated from a progenitor pluripotential stem cell. This erythropoietin-responsive cell is programmed to differentiate to erythroid cells capable of synthesizing hemoglobin. The erythropoietin-responsive cell is differentiated to have a receptor site for erythropoietin, possibly on the cell membrane. The hormone is necessary to induce the transition from the erythropoietin-responsive cell to the erythroblast stages capable of hemoglobin synthesis. The primary effect of the hormone on macromolecular synthesis is to stimulate the formation of a variety of species of RNA in the erythropoietin-responsive cell. The hormone-induced RNA synthesis in turn leads to cell replication, possibly mediated by yet unidentified factors whose synthesis is determined by the RNA. This response may be representative of a more general phenomenon, characterizing hormone-dependent cell differentiation.

Summary

Erythropoiesis in the fetal mouse provides a model to study several important aspects of the regulation of cell differentiation and differentiated protein synthesis. Changes in the patterns of hemoglobins formed during fetal and postfetal development are shown to be associated with the substitution of the liver erythroid cell line. In the course of differentiation of yolk sac erythroid cells there are at least two classes of proteins distinguishable with respect to dependence on continued RNA formation. The bulk of nuclear proteins, "nondifferentiated" proteins, appear to be dependent on relatively short-lived messenger RNA while synthesis of differentiated proteins, the hemoglobins, proceeds on relatively stable molecules of messenger RNA. Hemoglobin formation occurs in those cells which are actively synthesizing DNA and dividing. On the average, two to three cell divisions may occur after the formation and stabilization of the messenger RNA for globin. Yolk sac erythropoiesis, at least from day 10 of gestation, is unresponsive to erythropoietin. By comparison, in fetal liver erythropoiesis, the hormone, erythropoietin, acts selectively on the most immature erythroid cell precursor to induce differentiation, cell replication, and hemoglobin formation. The erythropoietin responsive cell in the liver is apparently differentiated from the progenitor, pluripotential stem cell and committed to erythroblast formation and hemoglobin synthesis on exposure to the hormone. The initial effects of erythropoietin on macromolecular synthesis are to stimulate RNA synthesis, which temporally is followed by cell replication and the increase in hemoglobin formation. During liver erythropoiesis, there appears to be a transition from hemoglobin synthesis dependent on RNA formation to hemoglobin synthesis directed by relatively stable messenger RNA.

References and Notes

1. A. Bank, R. A. Rifkind, P. A. Marks, in *Regulation of Hematopoiesis*, A. S. Gordon, Ed. (Appleton-Century-Crofts, New York, 1970), vol. 1, p. 701.
2. W. F. Loomis, *Papers on Regulation of Gene Activity During Development* (Harper & Row, New York, 1970), p. 323.
3. J. S. Kovach, P. A. Marks, E. S. Russell, H. J. Epler, *J. Mol. Biol.* **25**, 131 (1967); V. M. Ingram, *The Hemoglobins in Genetics and Evolution* (Columbia Univ. Press, New York, 1963); C. Baglioni and C. E. Sparks, *Develop. Biol.* **8**, 272 (1963); L. M. Kraus, A. Rasad, Y. Ohba, M. T. Patterson, *Fed. Proc.* **30**, 1128 (1971); F. H. Wilt, *Advan. Morphog.* **6**, 89 (1967).
4. A. Fantoni, A. Bank, P. A. Marks, *Science* **157**, 1327 (1967); J. G. Gilman and O. Smithies, *ibid.* **160**, 885 (1968).
5. M. Attfield, *J. Genet.* **50**, 250 (1951).
6. R. A. Rifkind, D. H. K. Chui, H. Epler, *J. Cell Biol.* **40**, 343 (1969).
7. B. Moss and V. M. Ingram, *J. Mol. Biol.* **32**, 493 (1968); G. M. Maniatis and V. M. Ingram, *J. Cell Biol.* **49**, 373 (1971); J. L. Brown, G. P. Bruns, V. M. Ingram, unpublished observations; V. M. Ingram, *Nature*, in press.
8. M. A. S. Moore and D. Metcalf, *Brit. J. Haematol.* **18**, 279 (1970).
9. J. E. Barker, M. A. Keenan, L. Raphals, *J. Cell. Physiol.* **74**, 51 (1969).
10. G. T. Matioli, H. Niewisch, H. Vogel, *Fed. Proc.* **27**, 672 (1968).
11. J. J. Trentin, in *Regulation of Hematopoiesis*, A. S. Gordon, Ed. (Appleton-Century-Crofts, New York, 1970), vol. 1, p. 159; E. A. McCulloch, *ibid.*, p. 133; E. S. Russell, *ibid.*, p. 649.
12. A. Fantoni, A. de la Chapelle, P. A. Marks, *J. Mol. Biol.* **33**, 79 (1968); A. de la Chapelle, A. Fantoni, P. A. Marks, *Proc. Nat. Acad. Sci. U.S.* **63**, 812 (1969).
13. H. Harris, *Cell Fusion* (Clarendon, Oxford, 1970), p. 52.
14. J. D. Ebert and M. E. Kaighn, in *Major Problems in Developmental Biology*, M. Locke, Ed. (Academic Press, New York, 1966), p. 29.
15. H. Holtzer, *Symp. Int. Soc. Cell Biol.* **9**, 69 (1970).
16. F. C. Kafatos and N. Feder, *Science* **161**, 470 (1968); L. M. Davies, J. H. Priest, R. E. Priest, *ibid.* **159**, 91 (1968).
17. G. L. Campbell, H. Weintraub, H. Holtzer, *J. Cell Biol.*, in press; H. Weintraub, G. LeM. Campbell, H. Holtzer, *ibid.*, in press.
18. A. Fantoni, L. Chiara, L. V. Potzi, *J. Mol. Biol.*, in press.
19. M. Terada, A. Fried, J. Banks, P. A. Marks, *Fed. Proc.* **29**, 730 (1970); M. Terada, J. Banks, P. A. Marks, *J. Mol. Biol.* **62**, 347 (1971).
20. J. A. Stewart and J. Papaconstantinou, *J. Mol. Biol.* **29**, 357 (1967); N. K. Wessells, *J. Cell Biol.* **20**, 415 (1964).
21. R. E. Lockhard and J. B. Lingrel, *Biochem. Biophys. Res. Commun.* **37**, 204 (1969); R. Williamson, G. Lanyon, J. Paul, *Nature* **223**, 628 (1969).
22. M. Djaldetti, D. Chui, P. A. Marks, R. A. Rifkind, *J. Mol. Biol.* **50**, 345 (1970).
23. R. J. Cole and J. Paul, *J. Embryol. Exp. Morphol.* **15**, 245 (1966); J. Paul and J. A.

Hunter, *J. Mol. Biol.* **42**, 31 (1969); J. Paul, D. Conkie, R. I. Freshney, *Cell Tissue Kinet.* **2**, 283 (1969).
24. R. A. Rifkind, D. Chui, M. Djaldetti, P. A. Marks, *Trans. Amer. Ass. Phys.* **82**, 380 (1969); D. Chui, M. Djaldetti, P. A. Marks, R. A. Rifkind, *J. Cell Biol.* **51**, 585 (1971).
25. M. Djaldetti, D. Chui, R. A. Rifkind, unpublished observations.
26. E. S. Russell and S. E. Bernstein, in *Biology of the Laboratory Mouse*, E. L. Green, Ed. (McGraw-Hill, New York, 1966), p. 351.
27. S. B. Krantz and L. O. Jacobson, *Erythropoietin and Regulation of Erythropoiesis* (Univ. of Chicago Press, Chicago, 1970), p. 118.
28. J. R. Stephenson and A. A. Axelrad, *Blood* **37**, 417 (1971).
29. E. A. McCulloch, in *Regulation of Hematopoiesis*, A. S. Gordon, Ed. (Appleton-Century-Crofts, New York, 1970), vol. 1, p. 132.
30. E. Goldwasser, in *Current Topics in Developmental Biology*, A. Monroy and A. A. Moscona, Eds. (Academic Press, New York, 1966), p. 73.
31. J. A. Hunter and J. Paul, *J. Embryol. Exp. Morphol.* **21**, 361 (1969); J. Paul and J. A. Hunter, *Nature* **219**, 1362 (1968).
32. It should be noted that the responsiveness of yolk sac erythroid cells was examined only from day 10. It is conceivable that at an earlier stage a precursor of these cells exists which is responsive to erythropoietin. This possibility is raised by the finding, summarized later in this review, that in the liver, only the most immature erythroid cell is responsive to erythropoietin. Yolk sac erythroid cells, unlike liver erythroid cells, differentiate as a cohort, and a comparable precursor cell may not be present after day 8 of gestation.
33. M. Gross and E. Goldwasser, *Biochemistry* **8**, 1795 (1969), S. B. Krantz and E. Goldwasser, *Biochim. Biophys. Acta* **103**, 325 (1965); M. Gross and E. Goldwasser, *J. Biol. Chem.* **246**, 2480 (1971).
34. The most immature erythroid cells in the fetal mouse liver are nominally designated proerythroblasts. It is recognized that cytological criteria are inadequate to distinguish the erythropoietin-responsive cells from cells of the first erythroblast stage already committed to hemoglobin synthesis and differentiation.
35. M. Gross and E. Goldwasser, *J. Biol. Chem.* **245**, 1632 (1970); M. Djaldetti, H. Preisler, P. A. Marks, R. A. Rifkind, *ibid.*, in press.
36. P. U. Angeletti, R. Levi-Montalcini, P. Calissano, *Advan. Enzymol.* **31**, 51 (1968).
37. J. E. Kay and S. D. Handmaker, *Exp. Cell Res.* **63**, 411 (1970).
38. A. Hershko, P. Mamont, R. Shields, G. M. Tomkins, *Nature* **232**, 206 (1971).
39. R. W. Turkington, in *Developmental Aspects of the Cell Cycle*, I. L. Cameron, G. M. Padilla, A. M. Zimmerman, Eds. (Academic Press, New York, 1971), p. 315.
40. Supported in part by NIH grant GM-14552 and NSF grant GB4631. R.A.R. is a career investigator of the Health Research Council of New York City.

Information for a Changing Society

For society's purposes, technical information must be credible, interpreted, and appropriately packaged.

Edward L. Brady and Lewis M. Branscomb

The wise society, like the wise individual, does not act without attempting to determine what the consequences of its actions might be. Surely then, few societies can be called completely wise. On all sides we see concrete evidence of our own society's failure to be wise. Some of those failures are literally embodied in concrete—the concrete of highways that have uprooted populations and changed the character of towns and major cities, the concrete of dams that have now turned into ecological disasters. Others are found in the pollution of our streams, in urban ghettos, in blackouts and brownouts, in ill-fed and poorly-educated children.

Our national administration and our state and local administrations have dedicated themselves to repairing the damage of past imprudence and to ensuring that future decisions are wise ones. This is not a partisan political goal, but a goal of our entire society, one that surely is endorsed by every citizen.

We do not want to appear to line up on the side of those who say that technology has been disastrous and has led to all the evils of our society. Quite the contrary. We believe that technology has been responsible for the material virtues of society, making it possible for much of the world's population to achieve a high standard of living, and pointing the way to peace and material abundance for all. While the misuse of technology has contributed to some of today's problems, the future can be improved only through the better application of new and existing technology.

What is needed to ensure that changing societies make wise decisions?

First, information. Information is needed to tell us what our society is like now, how rapidly and in what ways it is changing, and what scientific and technological alternatives to present practices exist or can be found.

Second, social analysis. Analysis is needed to determine what relations exist between current actions and future effects, to weigh the merits of alternative priority systems, to derive practical, achievable goals for society, and to determine how best to allocate our finite resources to attain those goals.

Third, well-informed decision makers. Society requires mechanisms to ensure that decision makers, including the public, have access to the information they need, have available the results of the analyses carried out, and have alternative courses of action formulated for their consideration.

Fourth, appropriate institutions. Institutions are needed to ensure that decisions can be put into practice. They take many forms—political institutions, financial institutions, legal institutions, and educational institutions.

A report prepared under the auspices of the Organization for Economic Cooperation and Development (OECD) has addressed the first need listed above —that for information. The report, entitled *Information for a Changing Society* (*1*), is an outgrowth of OECD concern with national and international policy relating to information systems in science and technology; its content and its implications are the subject of this article.

In 1969 OECD Secretary General Emile Van Lennep appointed the Ad Hoc Group on Scientific and Technical Information (*2*) to examine the information program of his organization and to advise OECD member states on information policy. The ad hoc group decided in its earliest deliberations that policy for information in science and technology could not be considered separately from policy for science and technology, which in turn could not be separated from policy for economic growth and other social goals. The task of the group thus became an examination of the information needs of the

Edward L. Brady is associate director for information programs, and Lewis M. Branscomb is director, National Bureau of Standards, Department of Commerce, Washington, D.C. 20234.