Journal of Supramolecular Structure
and Cellular Biochemistry 15:73–81 (1981)
Control of Cellular Division and Development: Part B: 301–309

Effect of Heme on Globin Messenger RNA Synthesis in Spleen Erythroid Cells

O. Fuchs, P. Ponka, J. Borova, J. Neuwirt, and M. Travnicek

Department of Pathophysiology, Charles University, Prague, Czechoslovakia (O.F., J.B., J.N.) and Czechoslovak Academy of Sciences, Prague, Czechoslovakia (M.T.)

Synthesis of globin mRNA in erythroid spleen cells from anemic mice was measured after in vitro incubation under conditions in which the level of intracellular heme was manipulated. This newly synthesized globin mRNA was isolated by hybridization with globin cDNA covalently bound to cellulose. Isonicotinic acid hydrazide (INH) and penicillamine were used as specific inhibitors of heme synthesis. It has been found that a 120-min incubation of spleen erythroid cells with 5mM INH or 5mM penicillamine reduced [^{3}H] uridine incorporation into globin mRNA by 24% or 36%, respectively. The addition of heme to INH- or penicillamine-treated cells almost completely restored [^{3}H] uridine incorporation into globin mRNA. These results indicate that heme stimulates transcription or processing of globin mRNA.

Key words: erythroid cells, globin messenger RNA, heme, isonicotinic acid hydrazide, penicillamine, transcription

The synthesis of all components of hemoglobin is very closely coordinated in both reticulocytes [1, 2] and nucleated erythroid cells [3, 4]. The basic principles of the regulation of hemoglobin synthesis are the dependence of globin synthesis on the presence of heme and the feedback inhibition of heme synthesis by heme.

Globin synthesis in rabbit reticulocytes [5] and their cell-free lysates [6] is controlled by the availability of heme. Rabinovitz and co-workers [7, 8] showed that heme deficiency in the reticulocyte lysates is associated with the formation of an inhibitor of polypeptide chain initiation, the so called hemin-controlled repressor (HCR). It is of interest that a similar translation inhibitor is present in other eukaryotic cells [9–12]. There is also other evidence that heme may have other regulatory properties [13].

We previously reported that a specific inhibitor of heme synthesis, isonicotinic acid hydrazide (INH), reduced [^{3}H] uridine incorporation into 9S RNA fraction of mouse spleen erythroid cells incubated in vitro [14]. Although this result indicates the involvement of hemin in RNA synthesis, it does not allow any final conclusion concerning its effect on globin mRNA synthesis. On the other hand, Ross and Sautner [15] and Dabney and Beaudet [16] demonstrated that heme is required for globin mRNA accumulation in Friend erythroid cells. However, neither of these groups of investigators provided any direct evidence for the effect of heme on globin mRNA synthesis rather than for example, on stability of exisiting globin mRNA.

Dr. P. Ponka is now at Department of Physiology, McGill University, 3655 Drummond Street, Montreal, P.Q., Canada H3G 1Y6.

Received May 5, 1980; revised and accepted October 15, 1980.

0275-3723/81/1501-0073$03.00

In the work described in this paper newly synthesized globin mRNA was isolated by hybridization with globin cDNA covalently bound to a solid matrix. This technique was used to estimate the synthesis of globin mRNA in erythroid spleen cells from anaemic mice after incubation in vitro under conditions in which the level of intracellular heme was manipulated.

MATERIALS AND METHODS

Chemicals

Amino acids for cell culture medium were obtained from Koch-Light Laboratories Ltd., guanosine, cytosine, unlabelled deoxynucleoside triphosphates, and dithiothreitol from Calbiochem and adenosine from Lachema (Czechoslovakia). Poly(rA)-oligo$(dt)_{10}$ and oligo$(dT)_{12-18}$ were from P-L Biochemicals. Oligo(dT)-cellulose type 7 (P-L Biochemicals) was a generous gift of Dr. Gerard Marbaix (Brussels). DL-penicillamine and hemin were purchased from Calbiochem, 4-deoxypyridoxine and levulinic acid from Koch-Light, 3-amino-1,2,4-triazole from Fluka and 2,2-bipyridine and INH from Lachema.

[^{3}H] dGTP (30 Ci/mM), [^{3}H] dTTP (15 Ci/mM), and [4,5-^{3}H] L-leucine (57 Ci/mM) were obtained from Radiochemical Centre Amersham, [5-^{3}H] Uridine (26.7 Ci/mM) from the Institute for Production, Research and Use of Radioisotopes, Prague, ^{59}Fe-citrate (about 10 mCi/mg Fe) and [2-^{14}C] glycine (5.4 mCi/mM) from the Zentral Institut für Kernforschung, Dresden.

Animals and Cell Preparation

ICR mice weighing approximately 23 gm were given 0.1 ml 0.8% neutralized phenylhydrazine ip for four days. Their spleens were removed on the sixth day and minced with fine scissors in medium, gently dispersed by passage through a plastic syringe, and filtered through nylon net. The spleen cells were collected by centrifugation at 600g for 10 min and washed twice with culture medium and then resuspended in medium to give a final cell concentration of 6×10^7 cells per ml. All manipulations of cells were performed using siliconized glassware.

Incubation Procedures

All incubations were performed in Dulbecco modified Eagle medium (MEM-D) [17] containing 10% fetal calf serum and 15% mouse serum at 37°C in a humified atmosphere containing 10% CO_2.

Protein synthesis in spleen cells. The spleen cells (6×10^7 cells per ml) were preincubated in culture medium for 45 min either alone or with various additions as indicated in the figures. After preincubation [^{3}H] L-leucine (10 μCi/ml) was added and the incubation continued for various time intervals as indicated in the Figures. After incubation, cells were washed three times with cold phosphate-buffered saline (PBS) (137 mM NaCl, 27 mM KCl, 8 mM Na_2HPO_4, 15 mM KH_2PO_4) containing 12% fetal calf serum. The spleen cells were lysed in redistilled water by repeated freezing and thawing. Protein was precipitated by acetone-HCl [18] and washed several times until a white protein precipitate was obtained. The protein was dissolved in 1 N NaOH and reprecipitated with trichloroacetic acid (TCA) according to Mans and Novelli [19]. TCA-precipitated protein was dissolved in 85% formic acid, aliquots were applied to Whatman 3 MM filters, dried, and the radioactivity determined in a liquid scintillating counter Mark II.

Heme synthesis in spleen cells. The spleen cells (6×10^7 cells per ml) were preincubated for 45 min with or without various additions before adding 1 μCi of ^{59}Fe bound to transferrin in mouse serum per ml of final incubation mixture. At the indicated time intervals (see Figures), the incubation was stopped and cells were washed as above. The incorporation of ^{59}Fe heme was determined according to Borová et al [20] .

In the experiment with 2,2-bipyridine, [2-^{14}C] glycine was used as a labelled substrate for heme synthesis. All procedures were similar to the ^{59}Fe-transferrin experiments except that 10 μCi of [2-^{14}C] glycine were added per ml of culture mixture (which contained unlabelled mouse serum). After washing and lysis of the cells a known amount of unlabelled hemoglobin carrier was added, then hemin was extracted according to Labbe and Nishida [21] and its specific activity determined. Total ^{14}C-heme radioactivity per cell sample was calculated from specific activity of heme in the hemoglobin carrier.

Incorporation of [^{3}H] uridine into RNA. The spleen cells (6×10^7 cells per ml) were preincubated for 45 min in culture medium under similar conditions as described above except that the medium was supplemented with 10^{-5} M each of guanosine, adenosine, and cytidine. Ten μCi of [^{3}H] uridine were added per ml of the incubation mixture, the incubation continued for 75 min, and then the cells were washed three times in the medium before total RNA was extracted [22, 23] . The washed cells were resuspended in 0.9% NaCl to give a final concentration of approximately 6×10^7 per ml. An equal volume of extraction buffer (0.1 M NaCl, 1 mM EDTA, 1% SDS, 0.1 M Tris-HCl, pH 9.0) was added followed by two volumes of phenol-chloroform (11:1). The mixture was stirred for 15 min at room temperature and the aqueous phase recovered by centrifugation at 18,000g for 10 min at room temperature. The phenol phase was then re-extracted with the extraction buffer. Total cellular RNA from the pooled aqueous phases was precipitated at -20°C overnight by the addition of 2.5 volumes of precooled ethanol and 2% potassium acetate. RNA was collected by low-speed centrifugation (3,000g, 30 min) at -20°C, then suspended in 2 M LiCl at 4°C, stirred for 1 h and then collected by centrifugation (3,000g, 30 min) at 4°C. The supernatant containing the majority of DNA residual proteins, and low molecular weight RNA (< 5S) was discarded [24] . The RNA pellet was resuspended in 2 M LiCl and the procedure was repeated. The purified RNA was then dissolved in 0.24 M ammonium acetate and precipitated by two volumes of precooled ethanol and finally dissolved in double-distilled water. Aliquots were applied to Whatman 3 MM filters and counted in a liquid scintillation counter Mark II. The concentration of isolated RNA was determined from its absorbance at 260 nm.

RNA-directed DNA polymerase from avian myeloblastosis virus. BAI strain A avian myeloblastosis virus (AMV) was purified from blood plasma of leukemic chicks (Leghorn white) exsanquinated in terminal stages of myeloblastic leukaemia, as previously described [25] . RNA-directed DNA polymerase (reverse transcriptase) was isolated from 50 mg of purified AMV essentially by the method of Kacian and Spiegelman [26] , including DEAE-cellulose and phosphocellulose chromatography. The enzyme eluate from the phosphocellulose column was used for the synthesis of mouse globin cDNA both free and bound to oligo(dT)-cellulose. The activity of the enzyme was 40 units/ml. One unit of enzyme activity is defined as the amount of AMV reverse transcriptase catalyzing the incorporation of 1 nmol of dTTP into an acid insoluble product at 37°C in 10 min in the assay mixture containing: 50 mM Tris-HCl pH 8.3, 6 mM $MgCl_2$, 60 mM KCl, 4mM dithiothreitol, 200 μM dATP, dCTP and dGTP, 30 μM [^{3}H] dTTP (final specific activity 125 cmp/pmol) and 0.1 A_{260} units/ml of poly(rA)-oligo$(dT)_{10}$.

Synthesis of Mouse Globin cDNA-Cellulose and Mouse Globin cDNA

The mouse globin mRNA used as template was prepared from total RNA isolated [27] from mouse reticulocyte polyribosomes by affinity chromatography on a poly(U)-Sepharose column [28]. A 9S fraction of this poly(A) containing RNA, which directed globin chain synthesis in the wheat embryo cell-free system [29], was used for cDNA synthesis.

cDNA-cellulose was synthesized as described by Wood and Lingrel [30] and Friedman and Rosbash [31] in 10 ml final reaction micture containing 50 mM Tris-HCl pH 8.3, 120 mM potassium phosphate, 6 mM magnesium acetate, 20 mM dithiothreitol, 1 mM dATP, dGTP, dCTP, and dTTP, 100 μg/ml actinomycin D, 20 μg/ml mouse globin mRNA, 30 mg/ml oligo(dT)-cellulose, and 8 units/ml of RNA-dependent DNA polymerase. The mixture was gently rocked at 37°C for 18 hr to keep the cellulose in suspension. After incubation, the cellulose was transferred to a small glass column and washed at room temperature with 10 volumes of 0.1 M NaOH followed by 25 volumes of double-distilled water. This procedure removed the mRNA while the globin cDNA remains covalently attached to the cellulose matrix through the oligo(dT)-primer.

Soluble, labelled cDNA was synthesized in 200 μl control sample run in parallel under the same condition with the following changes: oligo(dT)-cellulose was omitted and 0.02 A_{260} units of oligo(dT) and 160 μM [^{3}H] dGTP (final specific activity 500 cpm/pmol) were added. Twenty μl aliquots of reaction mixture were precipitated with TCA, collected on cellulose nitrate filters (Synpor, Czechoslovakia), dried and counted in a liquid scintillation counter Mark II.

Hybridization of mRNA to Globin cDNA-Cellulose

To detect the amount of cDNA covalently bound to the cellulose column, it was hybridized to an excess of labelled globin mRNA. [^{3}H]-labelled mouse globin mRNA was prepared by the same procedure as unlabelled mRNA (see previous sections) except that phenylhydrazine-treated mice were injected with [^{3}H] uridine (280 μCi per mouse) 14 h before sacrifice.

All analytical determinations of de novo synthesized labelled mRNA were performed using an excess of cDNA.

Indicated amounts (see Tables I and II) of labelled RNA from spleen cells were hybridized with 6.8 μg globin cDNA linked to cellulose (packed in a water-jacketed column) at 65°C for 2 h in hybridization buffer (10 mM Tris-HCl, pH 7.5, 0.1 M NaCl, 0.1 mM EDTA, and 0.2% SDS). The cDNA-cellulose was washed with 20 column volumes of hybridization buffer at 65° to remove non-hybridized sequences. The hybridized RNA was then eluted with double-distilled water at the same temperature. The globin cDNA-cellulose is relatively stable: 8% of globin cDNA was lost from the cDNA-cellulose after 10 hybridization experiments.

RESULTS AND DISCUSSION

Mouse Globin cDNA-Cellulose

Figure 1 shows the kinetics of synthesis of labelled mouse globin cDNA in solution. The reaction was run in parallel with the synthesis of mouse globin cDNA cellulose under basically same conditions with two exceptions: labelled [^{3}H] dGTP and oligo(dT) instead

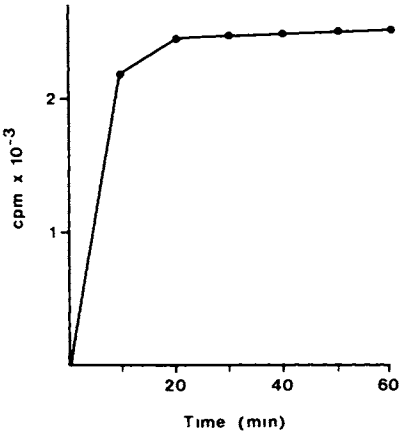

Fig. 1. Kinetics of cDNA synthesis in solution. The reaction conditions were described in Materials and Methods. The incubation was carried out in 200 μl reaction mixture, 20 μl aliquots were removed at indicated time intervals and assayed for TCA insoluble radioactivity.

of oligo(dT)-cellulose were used. The quantity of synthesized soluble cDNA was calculated assuming that the mRNA for globin contains 27.4% guanosine [32]. The cDNA yield in solution was 10% of the mass of the input mouse globin mRNA.

The quantity of unlabelled cDNA bound to the oligo(dT)-cellulose was determined by hybridization with ^{3}H-labelled mouse globin mRNA (see Materials and Methods). Our cDNA-cellulose preparation contained 0.023 μg of mouse globin cDNA per mg of cellulose, which is an amount comparable to the value of 0.025 μg to 0.08 μg of bound mouse cDNA obtained by Levy and Aviv [33]. The yield of the cDNA in suspension was approximately 66% less than cDNA yield in solution, presumably immobilized oligo(dT) was a less effective primer.

To establish the specificity of the globin cDNA-cellulose, nonglobin poly(A)-containing RNA (^{3}H-labelled 60S poly(A)-containing high molecular weight RNA from AMV) was examined for its ability to hybridize to the immobilized globin cDNA. Nonglobin (0.5 μg) RNA containing poly(A) was hybridized using an excess of the globin cDNA (6.8 μg cDNA linked to cellulose). Only 8.5 ng ^{3}H-labelled 60S poly(A)-containing RNA from AMV was bound to the globin cDNA-cellulose. This amount is about 750 times less than amount of globin mRNA bound to the globin cDNA under the same conditions.

Heme and Globin Synthesis in Spleen Erythroid Cells

In order to manipulate the level of intracellular nonhemoglobin heme in spleen erythroid cells, we tested several inhibitors of heme synthesis for their ability to inhibit ^{59}Fe or [2-^{14}C] glycine incorporation into heme. In addition, we tested the effect of these inhibitors on the incorporation of labelled leucine into protein. Figure 2 shows that penicillamine, INH, 2,2-bipyridine and deoxypyridoxine are potent inhibitors of heme synthesis. However, aminotriazole (AT) inhibited ^{59}Fe incorporation into heme only slightly and levulinic acid (15 mM, not shown in the Figure) was ineffective. All the effective inhibitors of heme synthesis also decreased the incorporation of labelled leucine into pro-

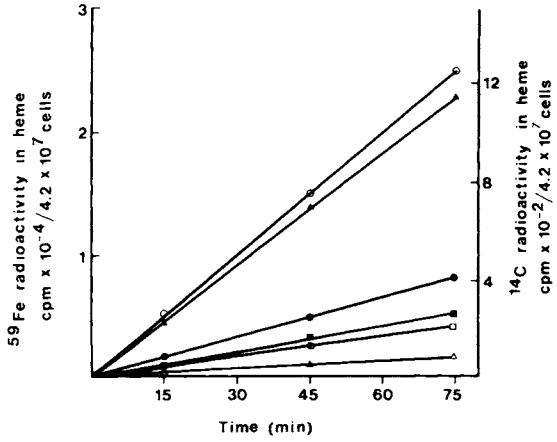

Fig. 2. ^{59}Fe or [2-^{14}C] glycine incorporation into heme. The spleen cells (6 X 10^7 cells per ml) were preincubated in MEM-D, containing fetal calf serum (10%), with or without various inhibitors of heme synthesis for 45 min. After preincubation ^{59}Fe bound to transferrin in mouse serum (final concentration 15%) was added to all samples except those with bipyridine and the incubation was continued. Fractions were removed at 15,45, and 75 min and incorporation of ^{59}Fe into heme was determined according to Borová et al [20]. (○ — ○) control; (▲ — ▲) 15 mM AT; (□ — □) 5 mM INH; (△— △) 5 mM penicillamine; (● —●) 15 mM deoxypyridoxine. To estimate the effect of bipyridine [2-^{14}C] glycine was added after 45 min preincubation. The incubation mixture contained unlabelled mouse serum (15%) and fetal calf serum (10%). ^{14}C-heme radioactivity was determined as described in Materials and Methods. (■ — ■) bipyridine 5 mM.

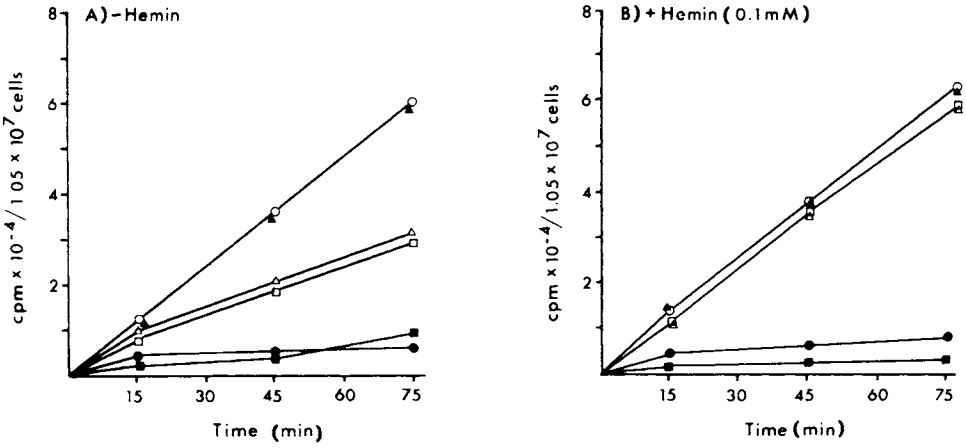

Fig. 3. [^{14}C] leucine incorporation into TCA-precipitated protein. The spleen cells (6 X 10^7 cells per ml) were preincubated in MEM-D containing fetal calf serum (10%) and mouse serum (15%) for 45 min. (A) spleen cells with or without various inhibitors of heme synthesis; (B) as in (A) with addition of hemin (0.1 mM) to all groups. The symbols are the same as in Fig. 2. After preincubation [^{3}H] leucine was added and the incubation continued. Fractions were removed at 15, 45, and 75 min and treated as described in Materials and Methods.

tein in spleen erythroid cells (Fig. 3). Heme completely restored protein synthesis to the control level in INH- and penicillamine-treated spleen erythroid cells. However, added heme was not capable of preventing inhibition of protein synthesis by deoxypyridoxine or 2,2-bipyridine. This result suggested that neither deoxypyridoxine nor 2,2-bipyridine were specific inhibitors of heme synthesis but also inhibited protein synthesis independently of any effect of the decrease in heme pool.

Both INH and penicillamine interfere with pyridoxal-5′-phosphate, which is a necessary cofactor in δ-aminolevulinic acid synthesis [see ref. 13]. These two inhibitors were therefore selected for further experiments since they appear to inhibit heme synthesis specifically.

Globin mRNA Synthesis in Spleen Erythroid Cells After Heme Depletion

Table I shows that a 120-min incubation of spleen erythroid cells with 5 mM INH induces [^{3}H] uridine incorporation into globin mRNA by 24%. The addition of heme to INH-treated cells almost completely restores globin mRNA synthesis. Similar results were obtained with a different heme synthesis inhibitor – 5 mM penicillamine (Table II). It may be of interest that heme alone slightly increases the incorporation of [^{3}H] uridine into globin mRNA. These results seem to indicate that globin mRNA formation requires the availability of heme. Although INH or penicillamine inhibit heme synthesis to 10–20% of control, the inhibition of [^{3}H] uridine incorporation into globin mRNA does not exceed 36%. This discrepancy can be explained by the fact that in nucleated erythroid cells there is a rather large nonhemoglobin heme pool that persists for about 60 min when heme synthesis is inhibited by INH [4].

Certain objections may be raised that the inhibitors used can influence RNA synthesis in a more direct way. This possibility has to be taken into consideration especially in the case of INH, which has been reported to react with the CMP moiety of a polynucleotide and to inhibit transcription [34]. However, INH concentrations that were used to inhibit transcription [34] were 132 times higher than those used in our experiments. Moreover, a different inhibitor of heme synthesis, penicillamine, also inhibited [^{3}H] uridine incorporation into globin mRNA. Moreover, in addition heme reversed the effects of both these inhibitors. Finally, [^{3}H] uridine incorporation into total RNA was only slightly affected by the addition of either compound. Therefore, it can be concluded that changes in [^{3}H] uridine incorporation into globin mRNA are due to changes in intracellular heme pool level.

From the two possibilities suggested in previous reports [15, 16] ie, that heme either stimulates synthesis or inhibits degradation of globin mRNA, our results favour the former one. However, the present experiments do not solve the question whether heme affects transcription or processing of globin mRNA precursors. Since the half-life of mouse globin mRNA is about 17 h [35], whereas in our experiments the spleen cells were incubated with the labelled uridine only for 75 min, then changes in globin mRNA degradation would hardly affect the synthesis of globin mRNA observed in our experiments.

It may be argued that INH and penicillamine can decrease the stability of globin mRNA by reducing intracellular heme pool. Heme-deficient erythroblasts with decreased rate of globin chain initiation [6–8] will probably contain more ribosome-free globin mRNA that may be more susceptible to inactivation by a ribonuclease as suggested by Del Monte and Kazazian [36]. On the other hand, Marks et al [37] used NaF to inhibit chain initiation and dissociate reticulocyte polysomes for various periods up to 120 min and found that protein synthesis could be completely restored after removal of the NaF,

Table I. Effect of INH, Hemin, or Combination of Both on the Incorporation of [^{3}H] Uridine Into Total RNA or Globin Messenger RNA in Spleen Cells*

	Radioactivity of total spleen RNA		Radioactivity of globin mRNA	
	cpm/mg	%	cpm[a]	%[b]
Control	2.64×10^6	100	843	100
Hemin (0.1 mM)	2.73×10^6	103	900	107 (104–107)
INH (5 mM)	2.46×10^6	93	636	75 (72–76)
Hemin (0.1 mM) + INH (5mM)	2.61×10^6	99	792	94 (90–94)

*77.8% erythroblasts.
[a]15 μg of total spleen RNA was applied to the column of globin cDNA-cellulose and radioactivity was determined in hybrid.
[b]Figures in this table are from one representative experiment; the figures in parentheses represent the range of the values obtained in three experiments.

TABLE II. Effect of Penicillamine, Hemin, or Combination of Both on the Incorporation of [^{3}H] Uridine Into Total RNA or Globin Messenger RNA in Spleen Cells* Incubation In Vitro

	Radioactivity of total spleen RNA		Radioactivity of globin mRNA	
	cpm/mg	%	cpm[a]	%[b]
Control	1.83×10^6	100	822	100
Hemin (0.1 mM)	1.86×10^6	102	855	104 (104–107)
Penicillamine (5 mM)	1.66×10^6	92	522	64 (61–65)
Hemin (0.1 mM) + Penicliiamine (5 mM)	1.77×10^6	97	741	90 (88–92)

*61.2% erythroblasts.
[a]19 μg of total spleen RNA was applied to the column of globin cDNA-cellulose and radioactivity was determined in hybrid.
[b]Figures in this table are from one representative experiment; the figures in parentheses represent the range of the values obtained in three experiments.

indicating that non translated globin mRNA remained stable. Nevertheless, further experiments are planned to determine whether in INH- or penicillamine-treated erythroid cells the half-life of globin mRNA is reduced and if so whether this can contribute to a decrease in [^{3}H] uridine incorporation into globin mRNA.

A recent report of Loeb and Creuzet [38] has indicated that heme inhibits phosphorylation of the nonhistone proteins of rat liver chromatin. This process has been implicated as a mechanism for gene activation [39]. However, there is some evidence against a necessary

relationship between increased protein kinase activity and specific gene activation [40]. Further experiments are needed to elucidate the mechanism of heme action on transcription.

ACKNOWLEDGMENTS

We are especially grateful to Dr. Gerard Marbaix for oligo(dT)-cellulose and Dr. Sulová from the Institute of Molecular Genetics, Prague for purified ^{3}H-labelled poly(A)-containing high molecular weight 60S RNA from acian myeloblastosis virus. We also are indebted to Dr. Paul R. Harrison from the Beatson Institute for Cancer Research, Glasgow for reading and correcting the manuscript.

REFERENCES

1. Kruh J, Borsook H: J Biol Chem 220:905, 1956.
2. London IM, Bruns GP, Karibian B: Medicine 43:789, 1964.
3. Tavill AS, Vanderhoff G, London IM: J Biol Chem 247:326, 1972.
4. Glass J, Yannoni CZ. Robinson SH: Blood Cells 1:557, 1975.
5. Ponka P, Neuwirt J, Sperl M, Brezik Z: Biochem Ciophys Res Commun 38:817, 1970.
6. Zucker WV, Schulman HM: Proc Natl Acad Sci US 59:582, 1968.
7. Maxwell CR, Rabinovitz M: Biochem Biophys Res Commun 35:79, 1969.
8. Gross M, Rabinovitz M: Biochem Biophys Res Commun 54:315, 1973.
9. Clemens MJ, Pain VM, Henshaw EC, London IM: Biochem Biophys Res Commun 72:768, 1976.
10. Delaunay J, Ranu RS, Levin DH, Ernst V, London IM: Proc Natl Acad Sci US 74:2264, 1977.
11. Pinphanichakarn P, Kramer G, Hardesty B: J Biol Chem 252:2106, 1977.
12. Sierra JM, De Haro C, Datta A, Ochoa S: Proc Natl Acad Sci US 74:4356, 1977.
13. Neuwirt J, Ponka P: "Regulation of Haemoglobin Synthesis," The Hague: Martinus Nijhof, 1977.
14. Ponka P, Fuchs O, Borova J, Neuwirt J, Necas E: Acta Biol Med Germ 36:353, 1977.
15. Ross J, Sautner D: Cell 8:513, 1976.
16. Dabney BJ, Beaudet AL: Arch Biochem Biophys 179:106, 1977.
17. Morton HJ: Minutes of the Seventh Meeting of the Committee on Cell Cultures, In: Hayflick L, Perkins FT (eds), Geneva, pp 114, 1971.
18. Anson ML, Mirsky AE: J Gen Physiol 13:469, 1930.
19. Mans RJ, Novelli GD: Arch Biochem Biophys 94:48, 1961.
20. Borova J, Ponka P, Neuwirt J: Biochim Biophys Acta 26:437, 1957.
21. Labbe R, Nishida G: Biochim Biophys Acta 26:437, 1957.
22. Cheng TCh, Kazazian HH: Proc Natl Acad Sci US 73:1811, 1976.
23. Merkel ChG, Kwan S-P, Lingrel JB: J Biol Chem 250:3725, 1975.
24. Nokin P, Gautier F: Mol Biol Rep 1:47, 1973.
25. Travnicek M, Riman J: Nature New Biol 241:60, 1973.
26. Kacian DL, Spiegelman S: In Grossman L, Moldave K (eds): "Methods in Enzymology." New York: Academic Press, 29(E):150, 1974..
27. Perry RP, La Torre J, Kelley DE, Greenberg JR: Biochim Biophys Acta 262:220, 1972.
28. Lindberg U, Persson T: Eur J Biochem 31:246, 1972.
29. Borova J, Fuchs O, Ponka P, Neuwirt J: Coll Czech Chem Commun 43:900, 1978.
30. Wood TG, Lingrel JB: J Biol Chem 252:457, 1977.
31. Friedman EY, Rosbash M: Nucl Acids Res 4:3455, 1977.
32. Burney A, Marbaix G: Biochim Biophys Acta 103:409, 1965.
33. Levy S, Aviv H: Biochemistry 15:1844, 1976.
34. Klamerth OL: Mutation Res 35:53, 1976.
35. Bastos RN, Volloch Z, Aviv H: J Mol Biol 110:191, 1977.
36. Del Monte MA, Kazazian MM: J Mol Biol 56:429, 1971.
37. Marks PA, Burka ER, Conconi FM, Perl W, Rifkind RA: Proc Nat Acad Sci Wash 54:1437, 1965.
38. Loeb JE, Creuzet C: FEBS Lett 81:423, 1977.
39. Stein GS, Spelsberg TC, Kleinsmith LJ: Science 183:817, 1974 .
40. Man N-T, Morris GE, Cole RJ: Develop Biol 47:81, 1975.