MEDICAL COLLEGE OF GEORGIA

AUGUSTA, GEORGIA 30902

DEPARTMENT OF BIOCHEMISTRY

LABORATORY OF PROTEIN CHEMISTRY

April 1, 1970

Dr. P. K. Sukumaran
Cancer Research Institute
Parel Bombay 12, India

Dear Doctor Sukumaran:

Dr. Walter Schroeder (California Institute of Technology) and I have been engaged in a research project concerning the chemical heterogeneity of human fetal hemoglobins. The first results of these investigations were published in the Proceedings of the National Academy of Science; a copy of this paper is enclosed. There seems to be convincing evidence for a duplication of the γ chain structural gene, leading to the production of two fetal hemoglobins which differ at a minimum in one position of the γ chain. We have thus far examined over 40 cord blood samples and all showed this chemical heterogeneity; moreover the ratio between the two types of fetal hemoglobin was found to be rather constant, namely some 3 to 1. These cord blood samples were obtained mainly from Caucasian and Negro babies, whereas a few were from Japanese and Eskimo newborns. We are now anxious to extend this survey worldwide, and it is for this reason that I turn to you for help.

I would appreciate it if you could obtain for me cord blood samples from a few native newborns to be used in this study. The amount required is relatively small, 3 to 5 ml of blood from each baby is sufficient. If you are able to obtain material from newborns of other racial or ethnic origin, such samples would be of course most welcome. The samples should be mailed air mail special delivery and should be collected in tubes containing an anticoagulant, such as EDTA or heparin; it is also advisable to add penicillin and/or streptomycin as a preservative. I am enclosing several import permits which should be attached to the outside of the package to speed the customs process. Refrigeration of the samples are desirable, but not required.

I appreciate very much indeed your help in our project. With kind personal regards.

Yours sincerely,

Titus H. J. Huisman, Ph.D., D.Sc.
Regents' Professor of Protein Chemistry

THJH/ap
Encl.
CC: Dr. W. A. Schroeder

ED/2454/70 APRIL 14, 1970

Dear Professor Huisman:

Thank you very much for your letter dated April 1, 1970 and the enclosures.

I am glad to note that the study conducted by you alongwith Professor Schroeder on human fetal haemoglobin have shown some interesting results as to their chemical heterogeneity. I am sure further study on these lines is going to be very rewarding. As for your request for some cord blood samples from here, I shall be only glad to be of some help to you in your Project.

Perhaps in a day or two, I shall be in a position to send you few samples as a first consignment. This shall be sent by Air Mail Special Delivery with preservatives and anti-coagulants as per your instructions. After hearing from you of the condition of the samples on arrival at your end, I shall endevour to mail more samples.

The reprint you enclosed was very enlightening and I shall appreciate to receive the reprints of your other recent publications in order to keep myself informed of the interesting work carried out in your department.

With kind regards,

Yours sincerely,

P.K.S

P. K. Sukumaran
Scientific Officer

Prof. Titus H.J.Huisman, Ph.D.,D.Sc.,
Regents' Professor of Protein Chemistry,
Medical College of Georgia,
Department of Biochemistry,
AUGUSTA, Georgia 30902, U.S.A.

ED/2592/70 APRIL 18, 1970

Dear Professor Huisman:

This is in continuation of my letter No. ED/2454/70 dated April 14,1970.

I have despatched by Air Mail Post Special Delivery five samples of Cord Blood labelled Con. Nos. 2547, 48, 49, 51 and 53, with Penicillin and Streptomycin as preservative and EDTA (Pot. Salt) as anticoagulant. Hope this will reach in time and in satisfactory condition. After hearing from you, I shall arrange to send some more if need by by Air Cargo packed in a Thermos Flask.

Hope to hear from you soon,

With kind regards,

Yours sincerely,

P. K. Sukumaran
Scientific Officer

Prof. Titus H.J. Huisman, Ph.D.,D.Sc.
Regents' Professor of Protein Chemistry,
Medical College of Georgia,
Department of Biochemistry,
AUGUSTA, Georgia 30902,
U.S.A.

MEDICAL COLLEGE OF GEORGIA

AUGUSTA, GEORGIA 30902

DEPARTMENT OF BIOCHEMISTRY

LABORATORY OF PROTEIN CHEMISTRY

April 28, 1970

Dr. P. K. Sukumaran
Scientific Officer
Cancer Research Institute
Tata Memorial Center
Parel, Bombay 12
India

Dear Doctor Sukumaran:

The five cord blood samples arrived in excellent condition yesterday, Monday April 27. Our first analysis by starch gel electrophoresis has just beencompleted; all show the presence of Hb-A and Hb-F. I am most pleased with the material and will continue our analyses in the near future. I will keep you informed of our progress.

Please feel free to send any sample you might think would aid our fetal hemoglobin project. We are particularly interested in patients with thalassemia and their relatives.

With kind regards.

Yours sincerely,

Titus H. J. Huisman, Ph.D., D.Sc.
Regents' Professor of Protein Chemistry

THJH/app
cc: Dr. W. A. Schroeder

AIR MAIL

ED/3203 /70 May 14, 1970

Dear Prof. Huisman:

Thank you for your letter of April 28th.

I am glad to hear that the cord blood samples that I sent to you reached in very good condition and that you are proceeding with them. I hope to hear from you of the results in due course.

I shall soon send you some more samples. As regards Thalassemia cases, what you need, I presume, are from those prior to blood transfusion, but I am not very clear about the relatives of thalassemia cases. Does it imply that, even though they may not show raised foetal haemoglobin (thalassemia traits) you are interested in their bloods as well? At any rate I shall include some such cases as soon as I get hold of them.

Incidentally I wish to mention that Dr.L.D.Sanghvi, who is the Head of our Division, and whom perhaps you know, is now in the University of Wisconsin, Madison, as Visiting Prof. of Anthropology. I wonder whether you will have an opportunity to contact him. All the same I am sending a copy of this letter to him to inform him of our collaboration this interesting work of yours.

I wish to have your guidance on the following: A technique(in detail) you can suggest to detect human embryonic haemoglobin (Gower - ζ chain) and the other is the details of separating pure fractions of foetal haemoglobin by column chromatography and lastly method of detecting and estimating Bart's haemoglobin on random cord blood samples. I do hope that you will write to me about these at your leisure.

Thanking you and with kind regards,

Yours sincerely,

PKS

P.K.Sukumaran,
Scientific Officer.

Prof. T.H.J.Huisman,
Regents' Prof. of Protein Chemistry,
Dept. of Biochemistry,
Medical College of Georgia,
AUGUSTA, Georgia 30902
U.S.A.

MEDICAL COLLEGE OF GEORGIA

AUGUSTA, GEORGIA 30902

DEPARTMENT OF BIOCHEMISTRY

LABORATORY OF PROTEIN CHEMISTRY

June 1, 1970

Dr. P. K. Sukumaran
Cancer Research Institute
Tata Memorial Center
Parel, Bombay 12
India

Dear Doctor Sukamaran:

The second batch of samples (six) arrived Thursday May 27. The condition of the samples was poor and we had to discard three (our numbers 6 (or 3328); 7 (or 3329); and 9 (or 3345); all three were cord blood samples. Sample number 8 (or mother B) is an interesting case of which I would like to obtain some more information. We will study that case as soon as time permits. The other two cord bloods (our numbers 10 and 11) were in an acceptable condition. We recently obtained blood samples from Zambia which were some 10 days in transit. Their condition upon arrival was really very good, thanks to the addition of 0.2 g% streptomycin. May be such an addition can be considered in the future.

Our analyses of the five previous cord blood samples (our numbers 1 through 5) proceed nicely. None of the five showed any unusual component; the alkali resistant hemoglobin levels in the samples were 68.0; 64.4; 57.6; 67.7; and 59.6 percent respectively. I do not believe that it is necessary at the moment to increase the number of cord bloods; we are, however, most interested in studying unusual cases (as for instance mother B), and I hope you will continue to send us material from such patients when convenient.

As far as the technical details of some procedures is concerned, I am enclosing a reprint of a recent survey article which will supply you with most information needed. I have the following additional comments.

a. The Gower hemoglobins are likely best demonstrated by starch gel electrophoresis at pH 9.0.

b. There is no single column chromatographic procedure that will give pure fetal hemoglobin fractions. In our laboratory we use DEAE-Sephadex chromatography as an initial step followed by rechromatography of the Hb-F containing fraction on CM-cellulose (see pages 468-471 of the enclosed reprint).

c. Again there is no good method available for the quantitation of Hb-Bart's in cord blood samples or in blood samples from certain adults. Some investigators prefer starch block electrophoresis which is likely an excellent procedure for

for this purpose. We on occasion have used CM-Cellulose chromatography.

Hopefully this information is of interest to you; thanking you again for your help in our project, I remain,

Yours sincerely,

Titus H. J. Huisman, Ph.D., D.Sc.
Regents' Professor of Protein Chemistry

THJH/app
Encl.
cc: Dr. W. A. Schroeder
Dr. L. D. Sanghvi

BY AIR MAIL

ED/3948/70 June 12, 1970.

Dear Prof. Huisman,

This has reference to your letter dated June 1,1970. I thank you for the same as well as the enclosed reprint.

I am sorry to find that three out of six samples sent last, though took only eight days in transit, were spoilt. I must confess that this time we could not collect all the blood on the same day. In fact last time, all the five samples were fresh and though it took 10 days in transit the samples were useful. I find all the three samples you mentioned as "in poor condition" were collected a day earlier by somebody else and sent to us. We have added streptomycin (50 mgm%) and penicillin (25,000 units%) to each sample. Perhaps in future it wise to add more streptomycin as suggested by you.

The sample number 8 (mother B) is from the mother of a child having high foetal-thalassemia. Father is a B thalassemia trait and mother (mother B) showed normal Hb A_2 and increased HbF (24.0%). A-gar electrophoresis (pH 6.2) and cellulose acetate electrophoresis showed Hb A and F well separated. Foetal staining of mother's blood smear showed almost even distribution of HbF.

It may be mentioned that we have quite a few families on our record with hereditary persistence of foetal haemoglobin. The first case with thalassemia-HPF came to our notice in the early fifties but unfortunately could only make a mention of this case in 1959 as in the earlier years such an entity was not known.

I shall soon send you some more samples including, if available, thalassemia families as mentioned in your earlier letter. I do hope they would reach you in good condition.

I am grateful to you for all the technical details given in your letter.

Thanking you and with kind regards.

Yours sincerely,

P.K. Sukumaran,
Scientific Officer,

Prof. Titus H.J.Huisman,
Medical College of Georgia,
AUGUSTA, GEORGIA 30902.

/snn

MEDICAL COLLEGE OF GEORGIA

AUGUSTA, GEORGIA 30902

DEPARTMENT OF BIOCHEMISTRY

LABORATORY OF PROTEIN CHEMISTRY

June 17, 1969

Dr. P. K. Sukumaran
Scientific Officer
Cancer Research Institute
Tata Memorial Center
Parel, Bombay 12
India

Dear Doctor Sukumaran:

Thank you for your letter of June 12 which arrived after Doctor Huisman left for vacation. Upon his return (around July 1) Doctor Huisman will write. Should the samples mentioned in your letter come available, you may send them and work will begin as soon as possible.

Sincerely yours,

Ann P. Patch

/app

Mrs. Ann P. Patch
Administrative Secretary to
Titus H. J. Huisman, Ph.D., D.Sc.

MEDICAL COLLEGE OF GEORGIA

AUGUSTA, GEORGIA 30902

DEPARTMENT OF BIOCHEMISTRY

LABORATORY OF PROTEIN CHEMISTRY

July 2, 1970

Dr. P. K. Sukumaran
Scientific Officer
Cancer Research Institute
Tata Memorial Center
Parel, Bombay 12
India

Dear Doctor Sukumaran:

Enclosed I am sending a list of samples received from you which are at present under study.

a. There are seven cord blood samples; we have determined the gly/ala ratio thus far in one of these. The number of cord bloods is quite adequate, so that it is not necessary to mail additional samples.

b. The sample number 8 is a most interesting case; it would probably be most important to receive red cells from the father (from some 50 to 100 ml of blood) who is a β-thalassemia carrier, and from the child with the combination of HPFH and β-thal. It is also worthwhile to send us some information concerning the racial and/or ethnic background of these subjects.

In your letter you mention that you have additional HPFH subjects in your files. It would be most important if samples from such cases could be made available.

c. The samples 12, 13 and 14 represent a family with β-thalassemia. It seems that one of the characteristics of the β-thal. in India is the production of very low quantities of fetal hemoglobin in the heterozygote, whereas large amounts are found in homozygous patients. The material on case India 14 (your number 908) is rather adequate for our analyses; however, it is impossible to isolate a sufficient amount (15-20 mg) of Hb-F from the samples of the parents (India 12 and India 13). In any future sampling of β-thalassemia traits it seems then necessary to collect considerably more material (may be some 100 ml, if possible).

d. Cases 15 (father) and 16 (your number H-1072) are again most interesting. It is unfortunate that we have not yet completed our chromatographic analyses. The father produces large amounts of Hb-F and may be an HPFH carrier (the Hb-A_2 level seems not to be elevated in starch gel electrophoresis). However, he produces a considerably lower amount of Hb-F than observed in case 8 (mother B). His daughter (India 16) produces Hb-F+Hb-E without any noticeable Hb-A.

It might well be that the Hb-F abnormality in this family represents a special form of HPFH. Did you do any fetal hemoglobin staining experiments on blood from

these cases?

I am really most grateful to you for sending us material from these most interesting cases and I am looking forward to any future shipments.

With kind regards.

Yours sincerely,

THJH/app
cc: Dr. Walter A. Schroeder
Encl.

Titus H. J. Huisman, Ph.D., D.Sc.
Regents' Professor of Protein Chemistry

Case	Type of sample	Hb-A_2	Hb-$F_{A.D.}$	Notes
1	cord	-	68.0	gly/ala ratio 0.75/2.26
2	cord	-	64.4	
3	cord	-	57.6	
4	cord	-	67.7	
5	cord	-	59.6	
8	Mother-B	1.6	24.4	HPFH carrier
10	cord	-	63.3	
11	cord	-	59.6	
12	Thal.trait	n.d.*	0.5	Father of No. 14
13	Thal.trait	n.d.	1.1	Mother of No. 14
14	Thalassemia	n.d.	47.7	
15		n.d.	12.7	Father of No. 16
16		n.d.	31.1	Hb-E+Hb-F

* not yet determined