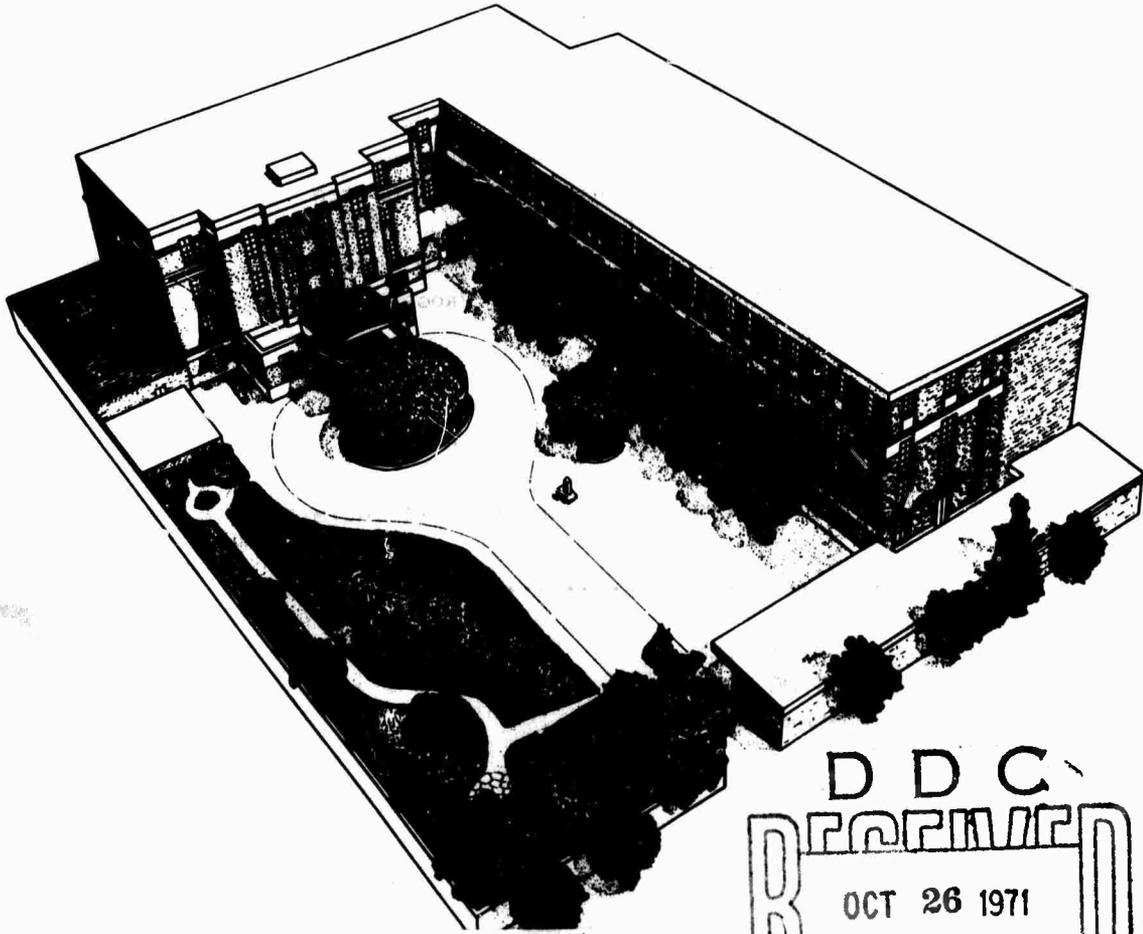


AD 781510

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

# NAMRU-2

HEREDITARY PERSISTENCE OF FOETAL HAEMOGLOBIN IN MEMBERS  
OF TWO CHINESE FAMILIES IN TAIWAN



Reproduced by  
**NATIONAL TECHNICAL  
INFORMATION SERVICE**  
Springfield, Va. 22151

DDC  
**RECEIVED**  
OCT 26 1971  
**RECEIVED**

NAMRU-2-TR-443  
1971

United States Naval  
Medical Research Unit No. Two  
Taipei, Taiwan

DISTRIBUTION STATEMENT A  
Approved for public release;  
Distribution Unlimited

8

CFOTI	WHITE SECTION <input checked="" type="checkbox"/>
DBC	BUFF SECTION <input type="checkbox"/>
UNANNOUNCED	<input type="checkbox"/>
JUSTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODES	
DIST.	AVAIL. and/or SPECIAL
A	20

BIOCHEMISTRY DEPARTMENT

R. QUENTIN BLACKWELL, PH.D., HEAD

ADMINISTRATIVE INFORMATION

THIS WORK WAS ACCOMPLISHED UNDER THE BUREAU OF MEDICINE AND SURGERY WORK UNIT MRO05.20.01-0099. THE STUDY WAS SUPPORTED IN PART BY THE BUREAU OF MEDICINE AND SURGERY, DEPARTMENT OF THE NAVY, WASHINGTON, D.C., AND IN PART BY THE ADVANCED RESEARCH PROJECTS AGENCY (PROJECT AGILE) WITH FUNDS MONITORED BY THE NUTRITION PROGRAM, NATIONAL CENTER FOR CHRONIC DISEASE CONTROL, U.S. PUBLIC HEALTH SERVICE, DHEW, UNDER ARPA ORDER NUMBER 580, PROGRAM PLAN NUMBER 298.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

R. H. WATTEN  
 CAPT MC USN  
 COMMANDING OFFICER



DOCUMENT CONTROL DATA - R & D

Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified

1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION	
U. S. NAVAL MEDICAL RESEARCH UNIT No. 2 Box 14, APO SAN FRANCISCO 96263		UNCLASSIFIED	
3. REPORT TITLE		2b. GROUP	
HEREDITARY PERSISTENCE OF FOETAL HAEMOGLOBIN IN MEMBERS OF TWO CHINESE FAMILIES IN TAIWAN			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)			
TECHNICAL REPORT			
5. AUTHOR(S) (First name, middle initial, last name)			
R. Q. BLACKWELL, C -S. LIU, C.-L. WANG, JEANETTE T.-H. HUANG, AND YEUH-O HUNG			
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS	
1971	4	48	
8a. CONTRACT OR GRANT NO.	8b. ORIGINATOR'S REPORT NUMBER(S)		
b. PROJECT NO.	NAMRU-2-TR-448		
c. MRO05.20.01-0099.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)		
d.			
10. DISTRIBUTION STATEMENT			
DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY	
PUBLISHED IN TROP. GEOGR. MED., 23: 145-148 1971		BUREAU OF MEDICINE AND SURGERY DEPARTMENT OF THE NAVY WASHINGTON, D. C. 20350	
13. ABSTRACT			
<p>HEREDITARY PERSISTENCE OF FOETAL HAEMOGLOBIN (HPFH) HAS BEEN FOUND IN CHINESE SUBJECT FROM TWO FAMILIES IN TAIWAN. THE PERCENTAGES OF FOETAL HAEMOGLOBIN AS DETERMINED BY AN ALKALI DENATURATION TEST IN THE THREE SUBJECTS FROM ONE FAMILY WERE 16, 17, AND 18. THE SINGLE MALE SUBJECT FROM THE SECOND FAMILY HAD 19 PER CENT FOETAL HAEMOGLOBIN. IN ADDITION TO ELEVATED FOETAL HAEMOGLOBIN THE SUBJECTS HAD THE FOETAL HAEMOGLOBIN DISTRIBUTED IN ALL ERYTHROCYTES IN A MANNER CHARACTERISTIC OF HPFH AND DISTINCTLY DIFFERENT FROM THE DISTRIBUTION SEEN IN THALASSAEMIA.</p> <p>ALL OF THE SUBJECTS WERE ASYMPTOMATIC AND REFUSED TO COOPERATE IN FURTHER HAEMATOLOGICAL STUDIES.</p> <p>THE FOETAL HAEMOGLOBINS FROM THE HPFH SUBJECTS HAD ELECTROPHORETIC MOBILITIES IDENTICAL TO THAT OF FOETAL HAEMOGLOBIN FROM CORD BLOOD WHEN EXAMINED BY STANDARD ELECTROPHORESIS PROCEDURES ON CELLULOSE ACETATE, AGAR, AND STARCH GEL. THE IDENTIFICATION WAS VERIFIED IN TWO UNRELATED SUBJECTS WITH HPFH BY PEPTIDE MAPPING OF THE TRYPTIC DIGEST OF THEIR FOETAL HAEMOGLOBIN FRACTIONS. THE MAPS SHOWED IDENTICAL PATTERNS FOR THE F HAEMOGLOBIN FROM THE SUBJECTS AND FROM CORD BLOOD HAEMOGLOBIN.</p> <p>FROM PRESENT INFORMATION THE INCIDENCE OF HETEROZYGOTES FOR HPFH AMONG CHINESE RESIDENTS OF TAIWAN IS ESTIMATED TO BE APPROXIMATELY ONE PER 40,000.</p>			

14.

KEY WORDS

LINK A

LINK B

LINK C

ROLE

WT

ROLE

WT

ROLE

WT

CHINESE SUBJECTS  
HEREDITARY PERSISTENCE OF FOETAL HAEMOGLOBIN

## HEREDITARY PERSISTENCE OF FOETAL HAEMOGLOBIN IN MEMBERS OF TWO CHINESE FAMILIES IN TAIWAN

R. QUENTIN BLACKWELL, C.-S. LIU, C.-L. WANG,  
JEANETTE T.-H. HUANG AND YEUH-O HUNG

*Department of Biochemistry, U.S. Naval Medical Research Unit No. 2,  
Taipei, Taiwan, Republic of China*

Received November 5th, 1970

### SUMMARY

Hereditary persistence of foetal haemoglobin (HPFH) has been found in Chinese subjects from two families in Taiwan. The percentages of foetal haemoglobin as determined by an alkali denaturation test in the three subjects from one family were 16, 17, and 18; the single male subject from the second family had 19 per cent foetal haemoglobin. In addition to elevated foetal haemoglobin the subjects had the foetal haemoglobin distributed in all erythrocytes in a manner characteristic of HPFH and distinctly different from the distribution seen in thalassaemia.

All of the subjects were asymptomatic and refused to cooperate in further haematological studies.

The foetal haemoglobins from the HPFH subjects had electrophoretic mobilities identical to that of foetal haemoglobin from cord blood when examined by standard electrophoresis procedures on cellulose acetate, agar, and starch gel. The identification was verified in two unrelated subjects with HPFH by peptide mapping of the tryptic digest of their foetal haemoglobin fractions; the maps showed identical patterns for the F haemoglobin from the subjects and from cord blood haemoglobin.

From present information the incidence of heterozygotes for HPFH among Chinese residents of Taiwan is estimated to be approximately one per 40,000.

### INTRODUCTION

Hereditary persistence of foetal haemoglobin is a condition in which otherwise haematologically normal individuals have relatively high percentages of Haemoglobin F. The early reports of the condition concerned Negro subjects in Ghana<sup>1</sup>, Liberia<sup>2,3</sup>, and Uganda<sup>4</sup>; likewise, many of the later reports have involved Negroes<sup>5-22</sup>. The condition has also been observed in Mediterranean groups, including Greeks<sup>23,24,25</sup>, Italians<sup>26,27</sup>, and Sicilians<sup>28,29</sup>, as well as in Portugese-Indians<sup>30</sup>, Indians<sup>31,32</sup>, and Puerto Ricans<sup>33</sup>. In addition, occasional reports have appeared of the condition in other Caucasian subjects<sup>34,35,36</sup>.

Only relatively recently has the condition been reported in South East Asian ethnic groups; WASI *et al.*<sup>37</sup> described the occurrence of HPFH in a Thai family and

WONG<sup>38</sup> reported it in a Malayan family. In the present report its occurrence in members of two Chinese families in Taiwan is described.

#### MATERIALS AND METHODS

The two initial cases were located during our continuing survey of Chinese school children and adults for haemoglobin variants. Both subjects were apparently healthy and normal males with no symptoms of anaemia; their ages were 15 and 19 years. Small blood samples were obtained from the father, mother, and a sister of one subject but no blood could be procured from members of the other family. (Both families refused to cooperate in further studies.)

Standard techniques were employed for the studies. The acid-elution technique of BETKE AND KLEIHAUER<sup>39</sup> was used to demonstrate the presence of foetal haemoglobin in the erythrocytes. The quantitative determinations of foetal haemoglobin were made by the two-minute alkali denaturation procedure of BETKE *et al.*<sup>40</sup>. In addition, the haemoglobin fractions were partially separated by column chromatography<sup>41</sup> on DEAE-Sephadex A-50-120 with a gradient of TRIS-HCl buffer ranging from pH 7.8 down to 6.9 followed by the final buffer of pH 6.5, and the relative amounts of the haemoglobin fractions determined spectrophotometrically. Tryptic digests were made of the purified foetal haemoglobin and peptide maps<sup>42,43</sup> were produced as described previously<sup>44</sup>. Standard procedures were used for electrophoretic examination of haemoglobin fractions with starch gel, cellulose acetate (cellogel), and agar.

#### RESULTS AND DISCUSSION

The slow haemoglobin component from both of the initial subjects was studied intensively and identified as Haemoglobin F by several methods. The electrophoretic mobilities of the slow haemoglobin from both subjects were compared with that of foetal haemoglobin from cord blood samples using agar gel at pH 6.2, cellogel at pH 8.9, and starch gel at pH 8.9. In addition, the mobility of the slow haemoglobin component in the DEAE-Sephadex column<sup>41</sup> was identical to that of foetal haemoglobin obtained from cord blood.

Finally, the peptide maps of the slow haemoglobin component from both subjects were found to be the same as that of purified Haemoglobin F from cord blood. Furthermore peptides  $\gamma T_1$ ,  $\gamma T_3$ ,  $\gamma T_{12}$  and  $\gamma T_{14}$  from the peptide maps of both subjects were eluted, analyzed, and found to have the expected amino acid compositions. These findings not only served to complete the identification of the slow haemoglobins from both subjects and eliminate the possibility of an adult haemoglobin variant but also indicated the likelihood of absence of changes from normal Haemoglobin F in their amino acid composition. Therefore it was concluded that the two individuals with HPPH apparently had normal foetal haemoglobin and not a variant of foetal haemoglobin. The same findings in Negro subjects with HPPH was reported previously by THOMPSON *et al.*<sup>13</sup>, SCHROEDER *et al.*<sup>45</sup>, and BAGLIONI<sup>46</sup>. More detailed structural analysis has not been carried out to investigate the possibility of electrophoretically silent mutations including any possible heterogeneity at position 136 in the gamma chain<sup>47</sup>.

Further substantiation that both index cases had hereditary persistence of foetal haemoglobin rather than another cause of elevated Haemoglobin F such as beta-thalassaemia was made by use of the acid-elution staining technique of BETKE AND KLEIHAUER<sup>39</sup> for visualization of foetal haemoglobin in the erythrocytes. Red cells from both index cases of HPPH were compared with those from a normal adult, as well as with cord blood, and with erythrocytes from an infant with thalassaemia. As described by MITCHENER *et al.*<sup>13</sup> and other previous workers<sup>13,19-22,24,30,32,34,37,38</sup>

the erythrocytes from our two index cases exhibited uniform staining for Haemoglobin F.

The relative amounts of foetal haemoglobin in the bloods of the two index cases, determined by alkali denaturation<sup>40</sup>, were 17 and 19 per cent. Apparently higher values were obtained by column chromatographic analysis where the ratios of Haemoglobin A<sub>0</sub>/F + A<sub>3</sub> in the two subjects were 73/27 and 75/25 respectively. However no further effort was made to separate the Haemoglobins A<sub>3</sub> and F from each other, therefore the actual amounts of Haemoglobin F determined by this procedure are uncertain. The percentages of Haemoglobin F in three subjects from the family of the index case whose foetal haemoglobin level was 17 per cent, also determined by alkali denaturation, were: father, 16; sister, 18; and mother 1.8 per cent.

The amounts of foetal haemoglobin present in the Chinese subjects are similar to those seen in the Greek subjects<sup>24</sup> and some Negro subjects<sup>22</sup> but definitely lower than those reported in most of the studies on Negroes<sup>1,3,7,16,17,19-21</sup>. The Thai subjects reported by WASI *et al.*<sup>37</sup> had slightly higher levels.

From our present information an estimate can be made of the incidence of heterozygotes for the HPFH condition among Chinese in Taiwan. Approximately 150,000 presumably normal Chinese subjects have been included in our haemoglobin screening survey thus far. Of that number nearly 70 were found to have haemoglobin variants with the singly-slow electrophoretic mobility of Haemoglobin G; Haemoglobin F would also be included in this group. Variants from approximately one-half of those 70 subjects have been studied up until the present time and two subjects (the index cases of the present study) were found to have Haemoglobin F as the slow variant. From this we may estimate that two more will be found when the remaining half of the 70 subjects are examined. Therefore approximately one in 40,000 can be taken as a conservative estimate of the incidence of heterozygotes for HPFH among Chinese residents of Taiwan. This is considerably lower than the estimated one heterozygote per thousand for certain Negro populations in the U.S.<sup>5,9</sup> and the one per 500 estimated for a Greek population<sup>24</sup>.

#### ACKNOWLEDGEMENTS

We thank Misses Ruth Jean, Linda Ting, and Maggie Huang for their help in procuring blood samples required for this work. Dr. Kuo-Sin Lin, Department of Paediatrics, National Taiwan University College of Medicine, kindly made available to us, for comparison purposes, blood samples from an infant with  $\beta$ -thalassaemia.

This work was accomplished under the Bureau of Medicine and Surgery Work Unit MR005.20.01-0099. The study was supported in part by the Bureau of Medicine and Surgery, Department of the Navy, Washington, D.C., and in part by the Advanced Research Projects Agency (Project AGILE) with funds monitored by the Nutrition Program, National Center for Chronic Disease Control, U.S. Public Health Service, DHEW, under ARPA Order Number 580, Program Plan Number 298.

## REFERENCES

- <sup>1</sup> G. M. EDINGTON AND H. LEHMANN, *Brit. med. J.*, i (1955) 1308; ii (1955) 1328.
- <sup>2</sup> J. V. NEEL, J. HIERNAX, J. LINHARD, A. R. ROBINSON, W. W. ZUELZER AND F. B. LIVINGSTONE, *Amer. J. hum. Genet.*, 8 (1956) 138.
- <sup>3</sup> E. B. OLESON, K. OLESON, F. B. LIVINGSTONE, F. COHEN, W. W. ZUELZER, A. R. ROBINSON AND J. V. NEEL, *Brit. med. J.*, i (1959) 1385.
- <sup>4</sup> G. F. JACOB AND A. B. RAPER, *Brit. J. Haemat.*, 4 (1958) 138.
- <sup>5</sup> L. N. WENT AND J. E. MACIVER, *Blood*, 13 (1958) 559.
- <sup>6</sup> T. B. BRADLEY, JR. AND C. L. CONLEY, *Trans. Assoc. Amer. Physicians*, 73 (1960) 72.
- <sup>7</sup> E. C. HERMAN, JR. AND C. L. CONLEY, *Am. J. Med.*, 29 (1960) 9.
- <sup>8</sup> J. OLIVIA AND R. M. MYERSON, *Amer. J. med. Sci.*, 241 (1961) 215.
- <sup>9</sup> T. B. BRADLEY, JR., J. N. BRAWNER, III, AND C. L. CONLEY, *Bull. Johns Hopkins Hosp.*, 108 (1961) 242.
- <sup>10</sup> J. T. WHEELER AND J. R. KREVANS, *Bull. Johns Hopkins Hosp.*, 109 (1961) 217.
- <sup>11</sup> A. P. KRAUS, B. KOCH AND L. BURCKETT, *Brit. med. J.*, i (1961) 1434.
- <sup>12</sup> J. E. MACIVER, L. N. WENT AND R. A. IRVINE, *Brit. J. Haemat.*, 7 (1961) 373.
- <sup>13</sup> R. B. THOMPSON, J. W. MITCHENER AND T. H. J. HUISMAN, *Blood*, 18 (1961) 267.
- <sup>14</sup> L. C. BRUMPT, P. M. DE TRAVERSE AND M. L. COQUELET, *Compt. rend. Soc. Biol. (Paris)*, 155 (1961) 246.
- <sup>15</sup> R. G. SCHNEIDER, W. C. LEVIN AND C. EVERETT, *New Eng. J. Med.*, 265 (1961) 1278.
- <sup>16</sup> G. R. THOMPSON AND H. LEHMANN, *Brit. med. J.*, i (1962) 1521.
- <sup>17</sup> C. L. CONLEY, D. J. WEATHERALL, S. N. RICHARDSON, M. K. SHEPARD AND S. CHARACHE, *Blood*, 21 (1963) 261.
- <sup>18</sup> T. JENKINS, *Cent. Afr. med. J.*, 8 (1963) 212.
- <sup>19</sup> R. B. THOMPSON, R. WARRINGTON, J. ODOM AND W. N. BELL, *Acta genet. (Basel)*, 15 (1965) 190.
- <sup>20</sup> G. A. BECKER AND E. C. ROSSI, *Ann. intern. Med.*, 65 (1966) 1071.
- <sup>21</sup> B. RINGELHANN, F. I. D. KONOTEY-AHULU, H. LEHMANN AND P. A. LORKIN, *Acta haemat.*, 43 (1970) 100.
- <sup>22</sup> T. JENKINS AND K. STEVENS, *S. Afr. med. J.*, 44 (1970) 111.
- <sup>23</sup> P. FESSAS, in: *Haemoglobin-Colloquium*, eds: H. Lehmann and K. Betke, Georg Thieme Verlag, Stuttgart, (1962) 82.
- <sup>24</sup> P. FESSAS AND G. STAMATOYANNOPOULOS, *Blood*, 24 (1964) 223.
- <sup>25</sup> LIE-INJO LUAN ENG AND R. TARAIL, *Nature*, 211 (1966) 47.
- <sup>26</sup> G. MANGANELLI, G. DALFINO AND N. TANNOIA, *Haematologica*, 47 (1962) 353.
- <sup>27</sup> P. POLOSA, L. MOTTA, G. CALCAGNO AND M. LUNETTA, *Haemat. lat.*, 7 (1964) 43.
- <sup>28</sup> L. MOTTA AND P. POLOSA, (abstract) 11th Cong. intern. Soc. Haemat., Sydney, (1966) 267.
- <sup>29</sup> L. MOTTA AND P. POLOSA, *Haemat. lat.*, 9 (1966) 173.
- <sup>30</sup> P. BARKHAN AND M. ADINOLFI, *J. clin. Path.*, 15 (1962) 350.
- <sup>31</sup> P. K. SUKUMARAN, H. P. RANDELIA, L. D. SANGHVI AND S. M. MERCHANT, *J. Assoc. Physicians India*, 9 (1961) 477.
- <sup>32</sup> G. W. G. BIRD, M. I. HASAN, O. P. MALHOTRA AND H. LEHMANN, *J. med. Genet.*, 1 (1964) 24.
- <sup>33</sup> A. DISTENFELD, *N.Y. State J. Med.*, 66 (1966) 981.
- <sup>34</sup> W. H. HITZIG, H. R. MARTI AND I. ROY, *Schweiz Med. Wschr.*, 95 (1965) 1459.
- <sup>35</sup> F. VELLA AND T. A. CUNNINGHAM, *Canad. med. Assoc. J.*, 96 (1967) 398-401.
- <sup>36</sup> A. L. PAWLAK AND F. KOZLOWSKA, *Acta haemat.*, 43 (1970) 184.
- <sup>37</sup> P. WASI, S. POOTRAKUL AND S. NA-NAKORN, *Brit. J. Haemat.*, 14 (1968) 501.
- <sup>38</sup> H. B. WONG, *Med. J. Malaya*, 23 (1968) 61.
- <sup>39</sup> K. BETKE AND E. KLEIHauer, *Blut*, 4 (1958) 241.
- <sup>40</sup> K. BETKE, H. R. MARTI AND I. SCHLICHT, *Nature*, 184 (1959) 1877.
- <sup>41</sup> T. H. J. HUISMAN AND A. M. DOZY, *J. Chromatog.*, 19 (1965) 160.
- <sup>42</sup> V. M. INGRAM, *Biochim. Biophys. Acta*, 28 (1958) 539.
- <sup>43</sup> C. BAGLIONI, *Biochim. Biophys. Acta*, 48 (1961) 392.
- <sup>44</sup> R. Q. BLACKWELL, H.-J. YANG AND C.-C. WANG, *Biochim. Biophys. Acta*, 175 (1969) 237.
- <sup>45</sup> W. A. SCHROEDER, P. STURGEON AND W. R. BERGREN, *Nature*, 193 (1962) 1161.
- <sup>46</sup> C. BAGLIONI, *Nature*, 198 (1963) 1177.
- <sup>47</sup> T. H. J. HUISMAN, W. A. SCHROEDER, A. M. DOZY, J. R. SHELTON, J. B. SHELTON, E. M. BOYD AND G. APELL, *Annals N.Y. Acad. Sci.*, 165 (1969) 320.
- <sup>48</sup> J. W. MITCHENER, R. B. THOMPSON AND T. H. J. HUISMAN, *Lancet*, 1 (1961) 1169.