Minutes of ICSH Panel Meeting Held in the Palace Hotel, Noordwijk, Netherlands from 18th-23rd November, 1973

PRESENT: **E.H. Belcher,** IAEA
J.G. Eernisse, Netherlands
H.I. Glass, U.K.
H. Heimpel, W. Germany
S.M. Lewis, U.K.
P. Mollison, U.K.
A.E. Murphy, U.S.A.
Y. Naiean, France
L. Szur, U.K.
I. Dormer, W. Germany(by invitation)
A. Ganzoni, Switzerland(by invitation)
J. de Koning, Netherland(by invitation)
K.E. Scheer, WHO(by invitation)

=國文抄錄=

이 論文은 1973年 ICSH주최로 열린 panel에서 血小板壽命 測定法의 標準化에 關한 討論結果를 技錄한 것이다.

이 prnel에서는 主로 血小板壽命測定의 技術的인 面과 分析的인 面을 討論하고 있다. 血小板에 放射性 物質의 標識法으로는 "Cohort"法과 "Random"標識法이 있으며 前者는 分析的인 面에서는 "Random" 標識法 보다 좋으나 현재로서는 標識法으로서 만족하지는 않다.

"Random"標識法으로는 ^{14}C-serotonime, DEP, ^{32}P, ^{3}H와 ^{51}Cr等이 利用되고 있다. DEP는 현재 널리 사용되고 있으나 DEP는 注入後 2週까지 放射能이 처음의 10~15%程度가 계속 남아있고 또한 血小板以外에도 白血球, 赤血球等에도 상당히 많이 標識되므로 血小板만 따로 分離해야 되는 단점이 있어서 이 panel에서는 사용하지 않는 것이 좋다고 하였다. 반면 ^{51}Cr은 技術的인 問題가 이미 많이 해결되어 있어 추천하고 있다.

여기서는 血小板壽命 測定法에서 血小板分離 및 標識, 採血 및 計測, data分析法等에 關하여 論하고 있다.

(1). Apologies for Absence

N.I. Berlin, U.S.A., L. Garby, Denmark, L. Halberg, Sweden, M. Lee, Korea, P. McInlyre, U.S.A.

(2). Introductory Remarks

The panel was welcomed by Dr. J.G. Eernisse and Dr. Szur replied on behalf of the panel. Dr. Belcher expressed his pleasure on behalf of the IAEA on having been able to provide support for the meeting, and he was asked by Dr Szur to convey to the IAEA the thanks of the panel members. Dr. Szur also welcomed Dr. Scheer representing WHO and Dr. Dormer, who was an observer on behalf of the West German Ministry of Science. The panel agreed to write to Dr. McIntyre to wish her a speedy

recovery following her recent illness. Dr. Scheer expressed the strong interest of WHO in the work of the panel and pointed out that one of the tasks of WHO was to collect data on health throughout the world and to compare the information via the regional officers. An accurate comparison of the data made standardization very necessary. WHO also had a strong interest in reducing radiation dosage to as low a level as possible.

Dr. Heimpel pointed out that he had been asked to lead a discussion on a subject which did not accord with the item on the agenda. It was agreed that the task of preparing a draft section on Iron Turnover and Incorporation Studies should be undertaken by Drs. Heimpel, Belcher, Ganzoni and Najean.

(3). Minutes of Previous Meeting

The Minutes of the previous meeting were adopted. It was pointed out that Professor Fliedner's name had been mis-spelt.

(4). Matters Arising

The following two papers were presented which were concerned with the Red Cell and Plasma Volume Document.

1. Use of $^{99}Tc^{m}$ for red cell volume and $^{131}In^{m}$ for Plasma Volume Document. P. Mollison.
2. Elution Table for ACD in red cell survival studies. S.M. Lewis.

Professor Mollison concluded that the use of $^{99}Tc^{m}$ for red cell volume cannot yet be recommended and it was proposed that Dr. Heimpel, Dr. Lewis and Professor Mollison should provide a document on the method which should be used by members of the panel. The results obtained using this method would be reported to Professor Mollison, and then the problem would be reconsidered by the panel at the next meeting.

Following Dr. Lewis' presentation, it was agreed that the same elution table could be used for both CPD and ACD methods. It was proposed that should a revision of the red cell survival document be undertaken, an amendment to include this new information would be made.

(5). Report on Panel Publications

(i) Dr. Lewis pointed out that it was essential that the national ICSH representative should be shown the panel documents in the first instance, and that arrangements should be made through this representative for the document to be published in different national languages.

(ii) Dr. Scheer and Dr. Belcher agreed to inquire from WHO and IAEA whether they would support the cost of publishing a pamphlet which would include the documents on Red Cell Survival, Blood Volume Measurements and if possible, Surface Counting, and make these available free of charge. Dr. Belcher pointed out the advantage of publishing the recommendations as individual papers rather than pamphlets, as papers could be published in many languages whilst pamphlets are likely to be published in one language only, possibly English.

(iii) Dr. Heimpel urged that the documents should be published in Nuclear Medicine as well as Haematology journals to ensure that they should be available to all workers interested in the subject. He also felt that the document suffered from a lack of references. It was agreed however, that the present style would be maintained.

(iv) Dr. Lewis, Dr. Szur and Professor Mollison agreed to raise the necessary money to pay for the reprints of the Blood Volume Document. The question of the financing of further documents was deferred until a reply was rece-

ived from Dr. Scheer and Dr. Belcher.

(6). Investigations on Surface Counting

Dr. Glass reported that no information had been received on the inter-comparison invesigations. However, Dr Najean reported his findings directly to the meeting and his results were supported by the findings of Dr. Eernisse.

Dr. Glass reported the results obtained using a dual detector system and also using multihole and single hole collimators.

A draft document on "The Determination of Sites of Red Cell Destruction" was presented by Dr. Lewis and a sub-cemmittee was formed to consider the points raised in discussion and to prepare a further version of this document for reconsideration by the panel. This was discussed and further revisions and comments were made. It was agreed to circulate the draft to members for further consideration with a veiw to publication.

(7). Platelet Survival Studies

The following two papers were presented.

1. The Value of surface counting in Platelet Survival Studies. J. de Koning.

2. Draft document on Platelet Survival Studies. A.E. Murphy.

Following Dr. Murphy's presentation three sub-committees were formed to redraft the Platelet Document for further discussion. It was agreed that a second draft of the document would be circulated after consultation with experts on some practical points which were raised in discussion.

(8). Ferrokinetics

The following papers were presented.

1. The value of ferrokinetic studies in clinical medicine. A. Ganzoni(based on a submission of N.I. Berlin).

2. Purified transferrin and native serum in Ferrokinetics. A. Ganzoni.

3. Technical aspects pf ferrokinetic studies. E.H. Belcher.

4a. Studies of Iron Turnover. A. Ganzoni.

b. The interpretation of iron incorporation studies. H. Heimpel.

5. The technique and interpretation of surface counting using Iron 59. Y. Najean.

6. Proposal for draft documentation on Ferrokinetic Investigations. E. Belcher.

Dr. Belcher agreed to circulate to the panel members a report on the discussion of the ferrokinetics with special reference to methodology.

(9). Availability Computer Programmes

Dr. Glass reported numerous requests for copies of the computer programme which could be used for working out the red cell survival times using the methods recommended by the panel. Since the platelet document proposed by the panel depended considerably on the computer programme used by Dr. Murphy, he agreed to approach N.I.H. to enquire whether or not they would be prepared to finance the distribution of such programmes on request. It was pointed out that N.I.H. already have a mechanism for providing this type of assistance.

(10). Future meeting

It was felt that a subject which might be seriously considered at future meetings is the subject of labelled fibrinogen to indicate thrombosis. Dr. Murphy reported that he and Dr. McIntyre had made a grant application to the National Cancer Institutes for support to enable the nexthmeeting to be held in Baltimore, U.S.A.

The results of this application would not be known until July, 1974.

(11). Concluding Remaks

Dr. Szur expressed the thanks of the panel to Dr. and Mrs. Eernissie, to Dr. Spander and Dr. Coster of ICSH for providing generous hospitality and to the secretaries who helped throughout the meeting, and once again to the IAEA for their support.

Radio sotope Platelet Survival Studies

Recommendations by the Panel on Diagnostic Applications on Radioisotopes in Haematology of the ICSH.

The expert panel on diagnostic applications of radioisotopes in haematology at its recent meetings in Ulm(1972) and Leiden(1973) discussed recommendations for the standardization of platelet survival measurements. The panel comprises E.H. Belcher(I.A.E.A.), N.I. Berlin (U.S.A), J.G. Eernisse(Netherlands), L. Garby (Sweden), H.I. Glass(U.K., Secretary), H. Heimpel(Federal Republic of Germany), M. Lee(Republic of Korea), S.M. Lewis(U.K.), P. MacIntyre(U.S.A.), P.L. Mollison(U.K.), E.A. Murphy(U.S.A.), Y. Najean(France) and L. Szur(U.K., Chairman). In addition, the panel had the help of W. Adam(F.D.R.), J. de Koning(Netherlands) and L. Harker(U.S.A.) as consultants. The following document was prepared at these meetings and deals with the technical and analytical aspects of platelet survival determinations. The study of platelet survival has proved of value in the investigations of:

a). The mechanisms of thrombocytopneia and the quantitation of the factors contributing to it.

b). The effects of various diseases and environmental factors on platelet turnover.

c). The effect of immunological factors on the survival of allogenic platelets.

Both "cohort" and "random" labelling methods have been employed tn study platelet turnover. The former are preferable from the point of view of analysis; however, at the present time there does not exist any satisfactory cohort label for platelets. At the present time attempts at cohort labelling with ^{35}S or 75-Se-Methonine have not proved satisfactory largely because the biological availability is long compared to the platelet lifetime. 14C-serotonine, labelled with DFP, ^{32}P, ^{3}H and sodium-^{51}Cr chromate have been employed as random labels. 14C-serotonine have proved unsatisfactory and is no longer used. DEP, which has been widely used, has provided relevant information about platelet turnover and is still to be regarded as a valuable aid in clinical investigations. It has the advantage of being an in-vivo label, thus avoiding any possible damage during the procedures associated with labelling. However, the panel makes no recommendations on the use of DEP for the following reasons.

The DEP curve does not reach zero, but shows a plateau of residual radioactivity which amounts to 10~15% of the initial value and which persists for 2 weeks. It is not clear whether this is due to partial labelling of megakaryocytes or to reutilisation or to both. Furthermore, when DFP is injected, red cells, neutrophils and

monocytes take up the label to a greater extent that do platelets. Thus, this isolation of the platelets from the blood samples becomes critical; this is extremely difficult to achieve by current cell separation techniques. In view of the above shortcomings the recommendations have been restricted to the labelling oplatelets in vitro with ^{51}Cr. Since ^{51}Cr was first used as a platelet label a number of technical improvements have been introduced, which reduce damage to the cells, and increase the efficiency of labelling and make it possible to apply the method with autologous platelets in states of severe thrombocytopenia. The technique described below includes these improvements and makes it possible to carry out survival studies of autologous platelets, when the counts are as low as 20, 000. The method can be applied to label either autologous or homologous platelets and can be used to study the distribution of the radionuclide throughout the body by external counting.

D. Expression of the results

1. The corrected platelet-bound radioactivity $N(t_i)$ at each time of sampling is calculated with the following formula:

$$N(t_i)=C\times E$$

where C=radioactivity c/s in the platelet button.

$$E=\frac{\text{(number of platelets in the blood/ml)}}{\text{(number of platelets in the PRP)}\times\text{(volume of PRP) ml}}$$

=PRP: Platelet enriched plasma.

If the recovery is to be calculated, and the results are expressed as the relative radioactivity at particular times after infusion of the labelled material then the following formula should be used:

$$N(t_i)=\frac{C\times E\times B}{R}$$

where B=Blood volume estimated from suitable tables of height and weight(ml), or measured directly

R=Total radioactivity originally injected (c/s)

Technique

A. Materials.

1. All solutions must be sterile and pyrogen free. The containers in which the blood is drawn should be of high quality plastic bags, made from a suitable material. However, in certain laboratories as a matter of convenience siliconized glass bottles are used but no recommendations are made concerning the use of these bottles.

2. A centrifuge large enough to allow bags containing up to half a liter of blood to be centrifuged at 1, 500 G and with facilities for ensuring that the temperature does not rise above 25°C, should be used. A smooth braking action is essential.

3. A plasma exlractor, is required.

4. Sterile ACD solution(2, 2g trisodium citrate dihydrate; 0. 8 g citric acid; 2. 5 g dextrose; water to 100 ml.) in batches of 10 to 20 ml freshly made, should be used and kept refrigerated. Suitable containers include PL 130, PL 146(manufactured in the USA) and Biotest (manufactured in Frankfort).

5. Sodium ^{51}Chromate buffered in sterile saline (pH 6. 5) with specific activity more than 10 μCi per ug, is used for labelling.

B. Platelet isolation and labelling.

All operations should be carried out with sterile techniques.

1. The platelets own platelets are used except where the expected yield of platelets is expected to be inadequate. Survival studies should be conducted with the patient's own platelets. About 200 to 500 ml of blood, according to the size of the patient and the platelet count in the peripheral blood, is drawn from the subject into a sterile transfusion bag containing

acid citrate dextrose: 15 ml per 100 ml of blood. The tube containing the blood is isolated and sealed off. To avoid the risk of iso-immunization and of transferring infectious disease. Otherwise, donor blood collected not more than two hours previously can be maintained at room temperature. The donor should be of the same AB or RH group.

2. The entire bag containing 500 ml is then centrifuged at 300 G for 15 minutes, including acceleration but not deceleration time. When there is less blood the speed must be adjusted accordingly dependent on the characteristics of the centrifuge.

3. The platelet rich plasma(PRP) which constitutes the supernatant is transferred with the help of a plasma extractor into a satellite bag. The remaining packed red cells may be reinfused.

4. Approximately 5 ml of the ACD solution per 100 ml of PRP is added so that the pH of the PRP may be brought to 6.5 ± 0.2 with ACD.

5. The platelets are now sedimented into a button by centrifugation at $1,500 \times g$ for minutes.

6. All but 5 ml of the supernatant platelet poor plasma(PPP) is transferred without disturbing the button into a second dry satelite bag, in which the button is resuspended by the very gentlest of mixing.

7. Add 100~300 μCi of $Na_2\ ^{51}Cr\ O_4$ to insure an adequate platelet labelling. In the cases in which the platelet button is small, this dose may be increased up to 1,000 μCi.

8. The mixture of chromate and the resuspended platelets is now incubated for 30 minutes at room temperature. Do not shake during incubation.

9. All but 40 ml of the remaining PPP is now returned to the incubated mixture.

10. This bag is now centrifuged at 1,500 G for 15 minutes to form a second platelet button. The PPP is then carefully decanted, the platelet button being left undisturbed. If approximately 200 ml of filtered air is introduced into the bag, a good air-plasma interface is produced which may facilitate the complete removal of the supernatant.

11. 20 ml of non-radioactive PPP is then gently layered over the platelet button, and then discarded in such a way as not to disturb the button. The object of this step is to remove as much as possible of unbound radio-chromium.

12. The labelled platelets are then gently resuspended in 20 ml of non-radioactive PPP. If red cells are present they may be removed by contrifuging the suspension, (divided if necessary, into two sterile glass tubes) at 200 G for 5 minutes. The supernatant PPP is then removed.

13. A standards prepared in duplicate by adding 0.5 or 1.0 ml of the platelet suspension to each of two plastic tubes containing 2 ml of 1% ammonium oxalate solution and then centrifuging at 2,000 G for 30 minutes. The supernatant is removed without disturbing the platelet button. The two tubes are then sealed and saved for counting. According to the radio-activity and the apparatus available, it may be convenient to dilute these standards appropriately.

14. The remaining quantity in an accurately measured amount, (assayed by the methods described in The Blood Volume Document; about 15 ml; in about 15~25 μCi) of the ^{51}Cr platelet suspension is injected intravenously using a calibrated syringe and the time noted.

C. Sampling procedure and countings.

The number and timing of the samples will be decided by the purpoess of the study and the value which the mean survival may be expected to take. Early termination of the study is not recommended when it is evident that the platelet

bound radio-activity has completely disappeared. Gratuitous variations in the timing of the studies are to be avoided to obviate bias in the interpretation of the data.

Within any one patient, daily sampling should wherever possible be obtained at the same time of day.

It is recommended that samples be taken at 30 minutes and 3hrs. after injection, and daily thereafter; where survival is expected to be short, additional samples in the first day are needed.

1. For each measurement 5 to 20 ml of blood are taken into a syringe containing 0.2 ml of 10% dipotassium ethylene diamine tetracetic acid (EDTA) per ml of blood. The platelet count is determined in an aliquot of the blood.

2. A precise volume of blood is put in a plastic test tube and diluted with an equal volume of saline, and mixed.

3. The mixture is centrifuged at 300 G for 10 minutes and the PRP removed to a counting tube.

4. The procedure is repeated and the two harvests of PRP pooled, the volume precisely measured, and the platelet count determined in an aliquot.

5. The PRP is now centrifuged at 2,000 G for 30 minutes and the supernatant removed without disturbing the button. Where there is reason to suspect a continuation with labelled red cells, 2 ml of 1% ammonium oxalate may be added before the centrifugation. The tube is then capped and saved for counting.

6. The radioactivity is then measured with a gamma spectrometer, at the same time for the standards and the samples. The buttons are made up in equal volumes of distilled water, to insure strictly comparable geometry. Care should be taken to take account of the possibility that the button may sink and therefore sensitivity of the apparatus will be changed.

Analysis of Data

Two methods of analysing the data are recommended. One of these (Method I) is based on a multi-hit model and requires advanced computer facilities. A second simpler procedure (Method II), suitable for use on a desk calculator, is also proposed. This latter procedure has been shown to have an acceptable correlation with the more complex procedure and to yield similar mean values. In both methods it is essential that formal least squares fitting procedures be used and the error in the estimated mean survival time be calculated from the variance of the data.

Method I:

The values of radioactivity $N(t_i)$ at various times ti are fitted successively for increasing values of n to the function.

$N(t_i)=c.a^n.e^{-at_i}.t_i^{n-1}/(n-1)!$

a=mean waiting time between hits

n=no. of hits before platelet is extinct

c=estimated initial label or y-intercept

until no further improvement in fit is obtained for higher value of n. The value of (n/a) thus obtained is the mean survival time.

Method II:

The values of radioactivity at time t_i, $N(t_i)$, are fitted to the linear decay function

$N(t_i)=c-kt_i$

and the value, A, of t_i when $N(t_i)=o$ is estimated.

where c=y-intercept

k= slope of the line

The values are also fitted to the function

$Ln(N(t_i))=-mt_i$

and the value, B, of $1/m$ is calculated.

where m=slope of the transformed line.

A weighted mean of A and B is no.w obtained and this is taken to be the mean survival time.

Let A be the linear estimate of mean survival

B be the logarithmic estimate of mean survival

Let S_A be the sum of the squares of the deviations of the data points from the fitted straight line.

Let S_B be the sum of the squares of the deviations of the data points from the fitted exponential curve (the deviations are the discrepancies between the fitted line and the data and *not* of the logarithms of the data values)

Then the weighted mean estimate is:

$$\frac{AS_B+BS_A}{S_A+S_B}$$

Method of Calculating Weighted Mean Survival

Let the times at which samples are taken be denoted by t_i where $i=1, 2, 3\cdots\cdots$, k_i and the relative radioactivities be N_i with $y_i=\log_e N_i=2.3016 \log_{10} N_i$

Compute the following quantities:

(1) $\Sigma t_j(k=\bar{t})$

(2) $\Sigma t_i^2-(\Sigma t_i)^2/k=\Sigma(t_i-\bar{t})^2=F$

(3) $\Sigma N_i/k=\overline{N}$

(4) $\Sigma N_i^2-(\Sigma N_i^2)/k=\Sigma)N_i-\overline{N})^2=G$

(5) $\Sigma N_i t_i-(\Sigma N_i)(\Sigma t_i)/k=\Sigma(t_i-\bar{t})(N_i-\overline{N})$
$=H$

(6) $\Sigma y_i/k=\bar{y}$

(7) $\Sigma yi^2-(\Sigma y_i)^2/k=\Sigma(y_i-\bar{y})^2=J$

(8) $\Sigma y_i t_i-(\Sigma y_i)(\Sigma t_i)/k=\Sigma(y_i-\bar{y})(t_i-\bar{t})=M$

Then

$$A=\bar{t}-\frac{\overline{N}F}{H}$$

$$B=\frac{F}{M}$$

The residual sum 'of squares S_A associated with estimate A is given by:

$$S_A=G-\frac{M^2}{F}$$

and that S_B associated with estimate B is obtained by first computing

$C=\bar{y}-b\bar{t}$, and then

$S_B=\Sigma(e^{c-t_i'/B}-N_i)^2$

The weighted average estimate of mean survival, W, is given by

$$W=\frac{AS_B+BS_A}{S_B+S_A}$$