IAEA ICSH Panel on Diagnostic Applications
of Radioisotopes in Haematology

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Summary of discussion on paper by P.A. McIntyre: Techniques of Ferrokinetics

＝國文抄錄＝

이 論文은 1969年 9月 부터 시작된 IAEA, Internal Society of Hematology 後援인 ICSH (International Committee for Standavdization of Haematology 주최로 열린 제5차 ICSH Panel에서 거론된 Ferrokinetics에 관한 몇 가지 문제점을 초록한 것이다.

1973년 11월 19일 IAEA/ICSH 는 Ferrokinetics 의 手技上의 문제점에 관한 토론이 있었다.

즉 血淸鐵이나 總鐵結合能을 검사용 혈액 채취시마다 매번 측정해야 되는지에 관한 문제, 患者의 總鐵結合能이 낮을 때 ^{59}Fe 를 注射하기전 직업적 供血者의 血漿과 미리 incubation 한 후 주어야 하는지의 문제, 검사용 혈액채취시 그 혈액양, 주입하는 방사성동위원소의 量, 抗凝固劑로 Heparin 대신 ACD 를 사용할 수 있는지 여부, 通常의 注射器를 사용할 때 그 오차를 줄이는 방법에 관한 문제 放射能 計測時 血漿대신 全血을 사용해도 좋은가에 관한 문제점등이 토론되었다.

여기서 대체로 의견이 일치된 점으로는 注入하는 ^{59}Fe 는 比放射能이 $5\mu Ci/\mu g$ 이상일 것, 이의 量은 體重當 얼마로 표시할 것, 放射能計測에 사용되는 血液은 반드시 血漿으로 통일할 것. 血淸鐵의 測定에는 첫번째 및 네번째의 검사용 血液을 사용할 것, 血液채취후 즉시 血漿을 分離하여 보관할 것, 等에 의견을 모았으며 앞으로 이에 관한 詳報를 마련하기로 하였다.

Najean considered that the Panel had three options, first not to attempt any recommendations relating to ferrokintic studies, second to recommend a procedure for simple routine studies, or third to recommed a procedure for detailed studies. *Belcher* expressed the hope that the last two of these might be combined and that a procedure for simple routine studies might be elaborated that could also provide a basis for detailed studies. *Szur* (chairman) suggested that discussions should proceed along the latter lines.

Szur questioned the need to measure the patient's serum unsaturated iron binding capacity on a blood sample taken at the same time of day as ferrokinetic studies are begun, pointing out that the measured value is not used subsequent calculations. Discussion then centred on the need to incubate the ^{59}Fe with plasma from a professional blood donor before injection if the patient's own unsaturated iron binding capacity was low. *Najean* reported that professional donors were not so used in France and that he used AB blood bank plasma stored frozen 10 ml lots for the purpose. This he could store up to 3—4 months. *Eernisse* considered that to use laboratory personnel as a source of plasma for such purposes, as was often done, was dangerous. *Najean* considered the use of professional donors no less so. *Eernisse* believed that the need to use a donor arose quite infrequently. *Najean* said that on the contrary it arose often, for example in both haemochromatosis and aplastic anaemia. *Najean* further questioned the need to use heparinized blood, stating that his blood bank plasma contained ACD. He believed that 5—10 ml of plasma was sufficient but that the amount of radioactivity had to be adapted both to the patient and to the procedure to be carried out.

He used 0.1 μCi/kg for routine studies and 0.2 μCi/kg for detailed studies. *Ganzoni* recalled that Finch used 0.25 μCi/kg. *Najean* stressed the importance of checking female patients for pregnancy before commencing ferrokinetic studies. *Belcher* asked whether the use of ACD delayed the binding of ^{59}Fe by transferrin. *Najean* had no evidence of this. *Ganzoni* stated that there was some evidence of competition for iron between citrate and transferrin, for which reason he preferred to use ^{59}Fe in the form of ferric chloride. Finally *Eernisse* mentioned that if plasma was to be stored frozen, it should not be heparinized.

Najean referred to the need or otherwise for red cell volume determinations in ferrokinetic studies. He considered that for routine studies, it might be sufficient to express the incorporation of ^{59}Fe in the red cells per 100 ml of whole blood, in which case no estimate of red cell volume was needed. For detailed studies, on the other hand, it was essential and should then be based on a determination with ^{51}Cr-labelled cells. He pointed out that it was usual when ferrokinetic studies were performed also to perform red cell survival studies with ^{51}Cr-labelled cells. When this was done, an estimate of red cell volume was in any case available. *Belcher* then questioned the need for a separate determination of plasma volume. *Najean* considered that this was not required. He pointed out that the distribution volume of ^{59}Fe-transferrin as determined from the plasma ^{59}Fe disappearance curve extrapolated back to zero time was some 10% greater than that of ^{131}I-albumin. He believed that the former was the correct value to use in the calculation of plasma iron turnover rate. Some discussion ensued as to whether this discrepancy represented a real difference in distribution volume or was due to mixing phenomena.

Ganzoni thought that it might be due to a rapid initial loss of ^{59}Fe from the plasma. *Belcher* recalled that certain workers had reported a similar difference in distribution volume between radioiodine-labelled transferrin and albumin. He suggested that the point could readily be cleared up by a limited comparison between ^{59}Fe-transferrin and ^{113m}In-transferrin. *Najean* agreed to undertake such a comparison. ACTION: NAJEAN.

Najean reiterated the need to adapt the amount of radioactivity injected both to the patient and to the procedure to be carried out. Discussion then centred on the need to incubate the ^{59}Fe with plasma before injection. *Szur* recalled that studies he had carried out in dogs showed identical disappearance curves for ^{59}Fe injected as citrate and injected as transferrin complex after incubation with plasma. *Belcher* reported similar findings in rats. *Ganzoni* considered that it was necessary not only to compare plasma disappearance but also red cell incorporation curves, since direct uptake of ^{59}Fe by reticulocytes might depend on the form in which it was injected. *Najean* did not, however, regard this as being of practical importance. He stated that he used ^{59}Fe in the form of ferric citrate, but incubated with plasma before injection. Even in the presence of citrate, binding to transferrin was practically 100%. *Ganzoni* reported that Finch followed a similar procedure. *Najean* referred to relevant studies by Monti, Glynn and Dern (1963). *Szur* suggested that the point at issue could be cleared up by a limited double tracer study with ^{52}Fe and ^{59}Fe in human subjects. He agreed to carry out such a study. ACTION: SZUR. *Najean* questioned the specific activity of the ^{59}Fe, considering that a value of $>10\mu$Ci/μg should be given. *Szur* suggested $>5\mu$Ci/μg and after some discussion this was accepted. Finally *Eernisse* reiterated that the amount of radioactivity injected should be expressed on a

body weight basis.

Lewis questioned the use of a glass syringe, referring to reports of significant absorption of ^{59}Fe on glassware by Fielding and by Smith. *Najean* emphasized that he injected ^{59}Fe in plasma, not in saline. *Lewis* believed that absorption occurred even with ^{59}Fe in plasma, but agreed to check this point. ACTION; LEWIS.

Eernisse pointed out that if absorption of ^{59}Fe on glassware took place it was not acceptable to measure the injected dose by weighing. He also questioned the procedure described for preparation of the standard, which he considered wasteful. *Ganzoni* stated that he filled the syringe, weighed it, ejected 1 ml of the contents for preparation of the standard, weighed again, injected the bulk of the contents into the patient and weighed a third time. *Najean* recommended that instructions on this question should refer to the Panel's recommended method for the measurement of red cell and plasma volume.

Discussion first centred on the acceptability of using whole blood rather than plasma for the initial plasma radioactivity measurements. *Najean* pointed out that plasma was essential for plasma radioactivity measurements extending over more than an hour or two. *Ganzoni* pointed out that even during the first hour direct uptake of ^{59}Fe by reticulocytes could be considerable in patient reticulocytosis. There was general agreement by the Panel that the additional labour involved in the separation of plasma was trivial and that plasma samples were essential. *Najean* considered that 2 ml plasma samples were adequate. Four samples were adequate. Four samples were needed to identify the first component of the plasma ^{59}Fe disappearance curve in routine studies. For detailed studies he continued sampling over 15 hours, taking 6—8 samples during this period. *Ganzoni* preferred to take 6 blood

samples within the initial period. For the earliest samples 1 ml of plasma was sufficient, though more might be needed for later ones. *Szur* pointed out that the times of sampling had to be adapted to the patient. *Najean* agreed. For example, in cases of aplastic anaemia he normally only performed routine studies, but sampling had to be extended over 5 or more hours. He instructed his technicians appropriately in each individual case. *Murphy* pointed out that the spacing of the samples would influence errors in subsequent calculations. *Belcher* raised the question of serum iron concentration measurements. *Eernisse* stated that he measured serum iron concentration on the first sample only. *Lewis* indicated that he normally did the same. *Najean* measured serum iron concentration on the first and fourth of fifth blood sample and used the mean value for subsequent calculations. A small change in serum iron concentration during the initial period could significantly influence the ^{59}Fe disappearance curve. *Ganzoni* followed a similar procedure. The Panel agreed to recommend duplicate measurements on the first and fourth blood samples, which should be increased appropriately in volume and taken under appropriate conditions.

Najean questioned the spacing of the blood samples for red cell radioactivity measurements. For routine studies he took samples at 24 hours, 48 hours and thereafter 3 times per week up to 2 weeks. Haematocrit determinations were carried out on each occasion, these serving the dual puspose of demonstrating the existence or otherwise of a steady state and giving warning of any urgent need for transfusion of the patient. Radioactivity measurements were made on 2 ml whole blood samples. Since the red cell radioactivity at 24 hours was usually low, it was. necessary low, it was necessary to apply a correction for plasma radioactivity in this sample.

This was based on a separate measurement on a plasma sample, for which additional blood was needed. For detailed studies he took an additional sample at 32 hours, measuring both whole blood and plasma radioactivity on this. For such studies he also measured plasma radioactivity on the 48 hour sample. The later plasma radioactivity measurements were then used not only to correct the corresponding whole blood measurements for plasma radioactivity but also to determine the terminal portion of the ^{59}Fe disappearance curve. These procedures met with the general approval of the Panel. *Lewis* emphasized the need for advice on the control of the patient, for example as regards transfusion, during ferrokinetic studies.

Najean considered that the paragraphs on sample preparation should appear earlier, since it is essential in ferrokinetic studies to separate the plasma as soon as possible after blood sampling. *Belcher* asked about the need to haemolyze whole blood samples before radioactivity measurement. *Najean* stated that he did not always do this, but accepted its desirability. The Panel agreed to recommend it.

Belcher questioned the need for photo-peak counting and pointed out that for many samples it would be virtually impossible to achieve a counting error of 1% or less. The Panel agreed to use its other recommendations as models in formulating the relevant paragraphs.

Discussion first centred on the determination of the plasma ^{59}Fe half-disappearence time in simple routine studies. *Najean* considered this best done by fitting a straight line through the first 4 points on the ^{59}Fe disappearance curve plotted on semilog paper. *Eernisse* considered

that more points should be used but believed that results might be erroneous if points earlier in time than 10 min were included, becuse of an initial rapid loss of ^{59}Fe from the plasma. *Ganzoni* suggested that it might be better not to take the first sample earlier than 10 min. *Szur* pointed out that the plasma ^{59}Fe half-disappearance time could itself be less than 10 min. *Mollison* considered that a 5 min sample should be taken, but the corresponding point omitted from the analysis if this should seem desirable. *Belcher* considered that if it were inclued, the resulting error was unlikely to be very significant.

Najean pointed out that Finch's expression of the plasma iron turnover rate per 100 ml blood avoided the need to use a value for the plasma volume. He believed this to be asuitable basis the analysis of the results in routine ferrokinetic studies. *Belcher* emphasized that whatever the mode expression, the calculation of plasma iron turnover rate from the plasma ^{59}Fe half-disappearance time or the initial slope of the plasma ^{59}Fe disappearance curve i.e. on the assumption the assumption of an open single-compartment model for iron metabolism-gave results that were over-estimated by some 20% in normal subjects and as much as 100% in some patients. He considered the only adequate basis for a calculation of plasma iron turnover rate to be the complete disappearance curve. After some further discussion of these issues *Szur* suggested that Belcher and Najean should prepare a protocol for detailed ferrokinetic studies, including the determination of plasma iron turnover rate. This agreed. ACTION; BELCHER, NAJEAN.