THE USE OF COMPARTMENT MODELS IN FERROKINETIC ANALYSIS.

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Abstract. In this paper we consider the study of a set of data from a normal population on which radioactive iron studies have been done. We used three tools: a well known compartment model, the conversational version of the SAAM language and a set of existing data. We investigate here the recovery of the parameters which have a clinical interest. Indeed just a few studies on normal subjects have so far appeared in the literature.

Keywords. Compartment model; SAAM language.

1.INTRODUCTION.

Metabolic and kinetic transfer rates of many specific substances in biological systems are often represented by compartmental models. These can be analyzed by using either mathematical tools, as outlined e.g. in Robertson (1983a) or Rescigno (1983), or with the aid of computer codes (Berman, 1978). The latter usually attempt to fit the data relative to a model formulated by means of a set of linear or nonlinear equations. This leads to the same kind of problem which arises in nonlinear optimization, namely that existing algorithms seek local minima which may or may not be the absolute minimum for the objective function.

In the present study we have undertaken the analysis of ferrokinetic data from several normal human subjects. Kinetic models are essential tools needed for radiation dose calculations (Robertson, 1983b). In addition, system analysis plays an important role in the description of physiological and metabolic processes. Studies of iron metabolism have been advanced greatly by the use of radioactive traces of iron (Bothwell, 1979). Iron-59 has been used extensively to trace the internal iron exchange (ferrokinetics). From time to time, various compartmental models have been used to describe ferrokinetics in human studies (Huff, 1951; Pollycove, 1961; Ricketts, 1975; Wasserman, 1964). Because of the central role of iron in oxygen transport in the blood (as an essential part of the hemoglobin molecule), many investigators have studied the metabolism, transport and dosimetry of radioiron in human and animal subjects. Nevertheless, few comprehensive studies have been reported and analyzed for normal human subjects. Data in 6 normal subjects are presented and analyzed here.

In order to pursue our investigation we used three tools. A well known and commonly accepted model was our starting point. The conversational version of the SAAM language, CONSAM (Foster, 1983), has proven to be extremely powerful. Its versatility can be seen in the possibility of quickly calculating the best fits for each compartment and obtaining the plots for the data and the interpolating functions. The data in this study were previously reported (Hosain, 1967), but are analyzed here using different models in order to compare the results to those that currently are used for accepted radiation dose estimates (Robertson, 1983b).

The paper is organized as follows. In section 2 we briefly outline how the data were collected. In section 3 we extensively examine the methodology we used to arrive at the final formulation of the model. Section 4 contains the results of our investigation. Tables with the average values of the transfer rates are provided, and comparisons between the various models studied are presented to indicate the best one. Finally a brief discussion of these results and a few open questions conclude the paper.

2. DATA ACQUISITION.

Data on 6 normal volunteers and several patients were obtained from previous work carried out by Hosain, Marsaglia and Finch (1967) in Seattle, the details of which were previously reported. Tracer doses of Fe-59 (approximately .5 μ Ci per Kg bound to plasma transferrin) were injected intravenuously. Heparinized blood samples were obtained frequently during the first 12 hours, and then daily or at alternate days for about 2 weeks. The samples consisted of whole blood, and separated plasma (free of hemolysis or corrected for any hemoglobin contamination), and packed red cells. In vivo activity counting with external probe detectors was also carried out at the same times when the blood samples were taken. The different sites examined were: liver, spleen, sacrum and sternum. We note here that there is a basic difference between these data and the ones in the MIRD report, which will become of great

importance in the next section. The model which we are going to describe has to incorporate the following feature, namely it has to interpolate data which are taken since early times (starting within 5-10 minutes after injection) and usually up to 15 days. The first consequence this remark entails is the deletion of any possible feedback from RBC's to rapidly equilibrating tissue, since the average life span of a red blood cell in normal individuals is about 120 days. The second consequence that can be drawn from the available data concerns the blood compartments. It is evident that since we have three sets of data on the blood, it will be simulated by as many compartments, one given by plasma, the second one by the RBC's, the last one by the "sum" of the two, which we call simply blood. This is necessary due to the fact that the data are contaminated by statistical errors and sometimes the above mentioned "sum" is not a sum at all. Indeed it is well known that counts for RBC's at early times after injection, usually before 1 day, are very difficult and thus these early values are not well established.

3. METHODS OF SIMULATION.

We would like at first to make a few remarks on how the computer code CONSAM proceeds to find the best fitting curves for the various compartments. The values of the transfer rates between the various compartments are adjusted manually until a reasonably good agreement between the calculated solutions and the observed values is obtained. Then the code can be used to iterate and seek to minimize the least squares deviations in order to approximate the final solution. Even though the number of compartments is moderately large, up to 13 in some of our analyses, the computer time to set up the model on a Vax 11/780 is found to be about 20 seconds and each iteration then requires about 60 to 90 seconds.

The starting point for the investigation has been the model proposed by Price et al. (1974) with the modification on the "total blood" compartment described above. The model itself is schematically represented in figure 1.

We tested it on the normal patient on which most data were availble. The solution plots obtained from this model revealed a systematic error at early times for the various "summer" compartments, i.e. the spleen, liver, sternum and sacrum. The modification we made to compensate for this misbehavior was to introduce the plasma in those organs as a summand. Another possible approach would have been to consider "total blood" instead of plasma and the RBC's separately as summands for each organ. However here we face one of the limitations of the program, namely that a compartment which is a summer cannot at the same time also be a summand.

The most difficult data to interpolate have been those pertaining to the red cell compartment. Some discrepancies between observed and calculated data were also observed in the "total blood" compartment. In order to overcome the difficulty, we introduced a delay element, which we assumed to correspond to the marrow compartment itself. The mean residence time was initially assumed to be about 39 hours, as indicated in (Robertson 1983b) for radioiron in normal subjects. Later we allowed it to be a free variable. At an early stage, we tried the data excluding the RBC's (compartment 6). Subsequently however, due to the bad results we obtained, we reincorporated them into the model. The RBC compartment was still not well fit by the model. At that point we introduced what appears to be the major difference with earlier published compartment models. It consists of a parallel link between the plasma compartment and the RBC's, which avoids the delay line through the marrow. The justification for this is given by the possible presence of reticulocytes (immature RBC's), which can incorporate Fe-59 from plasma, or due to artefacts from incomplete separation of plasma from RBC's. The results in the fittings we obtained were encouraging. We tried also to vary the number of elements in the delay line and found that a larger number seems to lead to better accuracy. We finally fixed it to the value 100. The final model constructed from the above trials is illustrated in figure 2.

One basic limitation of the SAAM language consists in the number of compartments and of adjustable parameters which can be present in the model. Indeed it seeks the least squares solution by an iterative procedure which is possible only under such restrictions. While the former did not constitute a problem, for the latter we ought to fix some of the parameters, let the other ones vary in order to make the program iterate and then interchange the situation. In order to reduce the degrees of freedom of the system, and since plasma would be seen from the organs as 100%, we restricted to the value 1 the summation factors (briefly sigmas in the SAAM terminology) originating from plasma. Even though in (Robertson 1983b) some of the transfer rate constants were fixed, namely the ones from plasma into extracellular fluid and vice versa, we performed the study letting these parameters be adjustable. With this modification the model gave reasonable answers and its results are reported in the next section under the labels D and E.

A different idea has at this point been taken into account, in an attempt to improve the model. It consists essentially of looking at the set of data in a different perspective. We should remark here that in putting into the code the whole set of data we assigned the same fractional standard deviation (FSD) to all of them. However not all of them should be considered of the same importance. Changing the FSD did not lead to a significant change and moreover if pursued it would have been arbitrary. We noted novertheless that it is not meaningful to obtain excellent fittings for the organs, while the sum of the squares of the deviations for the better defined blood compartments is perhaps one order of magnitude larger, or even more. Indeed the in vivo countings are technically obtained by measuring the radioactivity at the sites and are affected by the repositioning accuracy of the detector and by the fact that in measuring the activity say of the liver, also some marrow is present in the overlying ribs. This is also the reason for which the organs are summers of the blood, tissue and bone marrow, i.e. it explains the presence of the sigmas (S) in the model. The ratio of the sigmas relative to one organ to their sum gives in a

sense the proportion of each of those three different components which are present in the measurement, taken from outside the body, of the activity in that organ. On the other hand the counting of blood samples provided measures of activity which is not measured from outside the body, and thus is less subject to error. Note, however, that the separation of RBC's from plasma, to count the RBC's radioactivity is not an easy task. Even if these steps are performed carefully, misreadings are expected when the amount of activity in RBC's is small. It therefore seems to be more adequate to proceed as follows: try first to obtain the best fitting for the blood compartments and then fit the remaining ones. This amounts to considering the plasma activity data as the only true system data.

We then proceeded in the following way. By using a feature of SAAM, we fit the compartment 1 data by a sum of 5 decaying exponentials. This number is in a sense arbitrary, but necessarily larger than one and less than the total number of samplings. In just a few iterations the fittings were obtained. This function is then incorporated into the model as a forcing function on the plasma compartment. At this point all the other data are taken into account and the calculations which were previously performed were repeated also in this situation. This led to the results labeled A, B, C in the next section.

As a final check on the model, we wanted to see what changes would occur as the model was simplified. The basic reason for attempting such an operation is the fact that many parameters are present and some of their final values have a large variance.

In summary, some of the modifications we performed at this stage consisted in fixing the two transfer rates between the plasma and the extracellular fluid to the values of Robertson (1983b). Another alternative has been the deletion of compartment 4, i.e. we considered only one pool compartment for tissue. Also we studied the effect of a T-interrupt on the parameter L(6,1), on the direct link between plasma and RBC's. It was set to the value 0 after one day. However since the plasma clears quite rapidly, it is reasonable to expect that this modification does not lead to results too much different from the model not containing the T-interrupt, as indeed was the case. In all these circumstances however we observed a worse behavior in the fittings. We decided then to return to the model described in figure 1, and performed the remaining studies, both on normal subjects and patients, using this version of the compartment system.

4. RESULTS.

The purpose of the study was twofold. We were interested in testing the various options on the model, which have been extensively outlined in the previous section, and which are summarized below. Secondly, we wanted to recover values consistent with clinical knowledge of the system. For the normal subjects and two patients (aplastic anemia) we compared the five following different situations:

A) S(J,1) = 1 for J=7,8,9,10,11
presence of L(6,1)
presence of the forcing function

B) all S(J,1) adjustable parameters
presence of L(6,1)
presence of the forcing function

C) S(J,1) = 1 for J=7,8,9,10,11
absence of L(6,1)
presence of the forcing function

D) S(J,1) = 1 for J=7,8,9,10,11
presence of L(6,1)
absence of the forcing function

E) S(J,1) = 1 for J=7,8,9,10,11
absence of L(6,1)
absence of the forcing function

The criterion to decide which model is the best one has been the overall sum of the squares of the deviations between observed and calculated data. It appears that the comparison between the presence and absence of the direct link from plasma to RBC's is always in favor of the former, both in presence and absence of the forcing function, i.e. among all the studies, cases C and E are the worst ones. Comparing the models A and D we obtain information on the fittings in the case when the forcing function is incorporated in the system and when it is not. It is apparent that its presence yields better behavior for the calculated solutions. The comparison between cases A and B suggests that letting all the sigmas be adjustable parameters leads to no sensible variation in the solution. However this includes more degrees of freedom and converges more slowly since the computational effort is increased. Moreover the values of the sigmas out of plasma usually remain close to the value 1 even when they are freed, as one should expect. It is even more significant that the values of the transfer rates do not change significantly in the two cases. Based on all these grounds, it seems reasonable to choose for our model the one represented by case A. The average values of each transfer rate together with their standard deviations are summarized for each model in the table 1. We have included the final estimates for the 6 normal cases. Table 2 represents a comparison of data with those obtained by others.

5. DISCUSSION AND CONCLUSIONS.

A few remarks on the values of the transfer rates and their statistics are in order. We note first of all that the parameters relative to plasma and extracellular fluid exhibit opposite behavior with respect to the one usually accepted, i.e. the rate out of plasma being higher than the rate into it. However the values we obtained have an estimated standard deviation larger than the value itself, which makes the estimate uncertain and leaves the situation fuzzy, except for the fact that as remarked earlier trying to reverse the proportions leads to poorer fits. Another problem we faced was

the impossibility of estimating the standard deviation for the parameter from the delay element into RBC's. By incorporating the delay element in the marrow itself, we observed the same problem for the rate out of marrow into RBC's. This suggests that it may be an intrinsic flaw of the code, which may not be able to give a proper statistics for a delay element. Another possible explanation could be given by the presence of the long delay line in the original model by Price et al. (1974). This acts like a negative feedback and therefore should have a stabilizing effect on the system, allowing more accurate statistics for the various parameters in that model, which is not the case in our situation.

We would like to point out a few limitations of our study compared with the previous work of Price et al. (1974). The essential difference between the two models is the time interval of the data acquisitions. While those data were taken over a span of a few months, here we had to deal with a span of 20 days at the very most. Since the number of data points in the two cases are of the same order of magnitude, it follows that the difference in time between two samples in the present model is smaller than the time lag between analogous samples for the longer term studies. This implies that the former "sees" more of the early details, while the latter is better in an overall sense. In other words, the present model is most useful to fit the data at early times after the dose injection. In the long term study for example, the plasma curve can be easily deleted, since probably not even the first measurement can take it into account. Indeed it was obtained at times when the plasma had already mostly cleared.

Finally one more point should be made, relative to the plasma curve. Statistical oscillations in the samples are observed, but it seems that around one day after administration of the dose, there appears a sort of upward kink and then afterwards the values drop again. Whether such a phenomenon represents a statistical fluctuation or instead has a biological interpretation is still an open question which needs further investigation. The model we studied here does not provide an answer and probably requires some further modifications, before we can address the question.

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Table 1.

Average values of kinetic parameters
for the different models tested: normal subjects.

Parameters	Model Type				
	A	B	C	D	E
L(5,1)	8.4+.33	8.15+.31	8.36+.29	8.24+.45	8.17+.42
L(13,5)	.21+.02	.20+.02	.2+.02	.21+.02	.20+.02
L(1,2)	2.7+28.8	2.5+17	4.3+24	2.48+3.48	2.2+8.27
L(2,1)	.25+.99	.22+.98	.16+.69	.01+.43	.01+.13
L(3,1)	2.31+.25	2.23+.22	2.27+.27	2.33+.26	2.29+.3
L(1,3)	.14+.02	.12+.02	.15+.02	.17+.01	.09+.02
L(4,3)	2E-5+.05	2E-5+.03	2E-5+.1	.001+.03	.002+.02
L(3,4)	.1+.53	.53+.46	.57+2.17	.07+.45	.04+.7
L(6,1)	.01+.005	.01+.01	-	.003+.006	-
L(6,13)	.03+8E4	.01+1E5	.001+1E5	.007+2E5	.036+3E4
DT(13)	.79+.06	.79+.09	.72+.1	.73+.2	.77+.14
K(1)	1.05+.09	1.03+.07	1.02+.07	1.04+.08	1.01+.09
Overall sum of squares	.355+2E-3	.356+2E-3	.531+3E-2	.371+2E-3	.65+2E-3

Table 2.

Values of Kinetic Parameters (transfer rates)
for normal subjects (with model A)

Parameters	Comparative Values		
	Model A	Price et al (1974)	Robertson (1983b)
L(5,1)	8.40+.33	8.00+.21	6.00
L(13,5)	.21+.02	-	-
L(1,2)	2.67+28.82	1.00 (fixed)	.91
L(2,1)	.25+.99	2.60 (fixed)	1.41
L(3,1)	2.31+.25	2.34+.15	2.00
L(1,3)	.14+.02	.20+.05	.33
L(4,3)	2.E-5+.05	.53+.11	.024
L(3,4)	.10+.53	.05+.01	.0018
L(6,1)	.01+.01	-	-
L(6,13)	.03	-	-
DT(13)	.79+.59	-	-

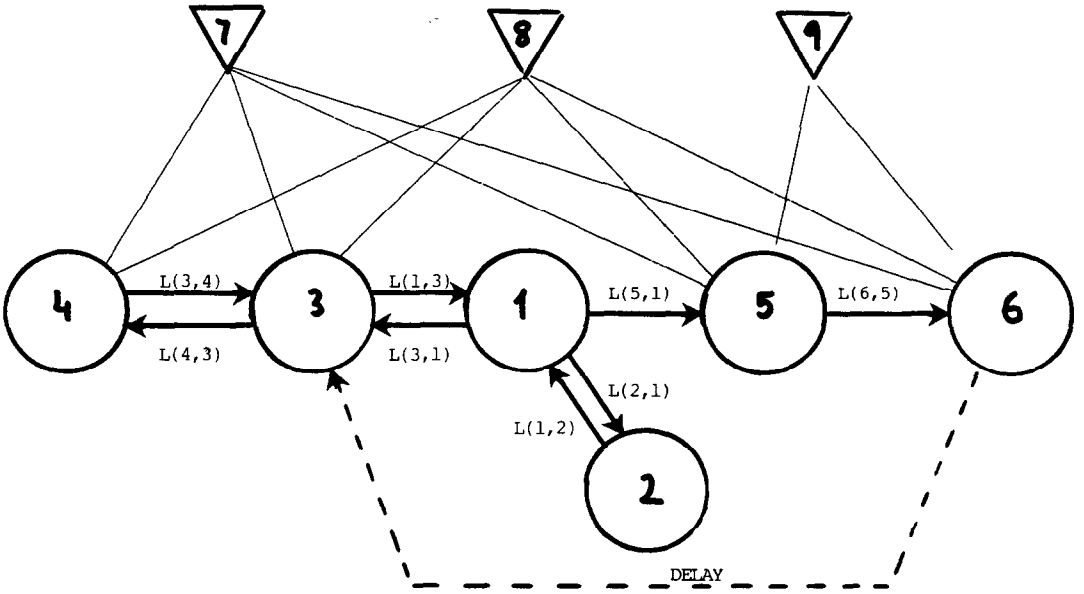

Fig. 1. Diagram of the initial model.

The compartments of the initial model are defined as follows: 1 = Plasma, 2 = Extracellular Fluid, 3 = Rapidly Equilibrating tissue, 4 = Slowly equilibrating tissue, 5 = Bone marrow, 6 = Red blood cells, 7 = Spleen, 8 = Liver, 9 = Sacrum. The heavy lines correspond to actual flow, the rate of which is given by the landas; the light lines represent the sigmas.

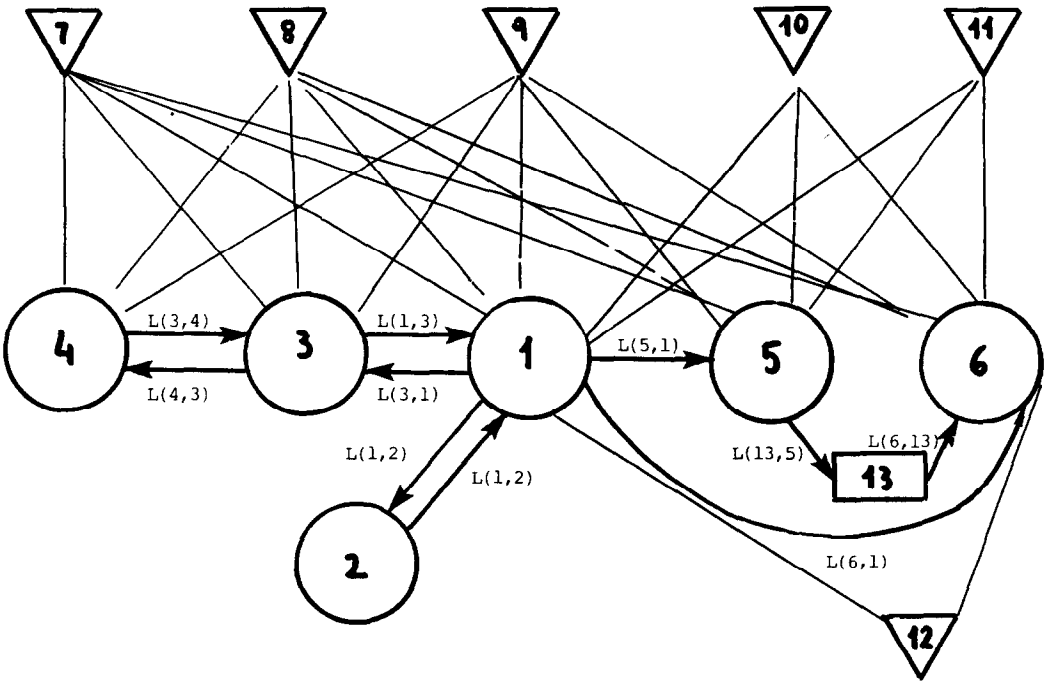

Fig. 2. Diagram of the final model.

The compartments of the final model are defined as follows: 1 = Plasma, 2 = Extracellular fluid, 3 = Rapidly equilibrating tissue, 4 = Slowly equilibrating tissue, 5 = Bone marrow, 6 = Red blood cells, 7 = Spleen, 8 = Liver, 9 = Sacrum, 10 = Thigh, 11 = Sternum, 12 = Blood. Light lines represent the sigmas.