II. BACKGROUND INFORMATION

Radioisotopes of iron have been used in studies of iron absorption and blood disorders for the last 35 years. The absorption of iron is known to depend on several factors notably the nature and the quantity of iron inherent in the food or the chemical form in which it is administered. Even when given in the same form, uptake may vary from day to day in the same subject. When investigating the absorption of iron in different forms of diets, it is advisable to use double tracer techniques, so that the uptake of iron in one specific kind of food to be investigated may be compared with that of a control or basal kind of food in the same individual. This is made possible by the pool concept (Fig.1) which is based on the assumption and later on supporting experiments that there is an isotopic exchange between food iron and inorganic salt added to food and that there is an isotopic exchange of iron between native food iron and extrinsic inorganic iron tracers in the nonheme iron pool. ^{55}Fe and ^{59}Fe are the two isotopes usually employed in such studies. ^{59}Fe with its 45 days half-life can readily be detected by means of its energetic gamma-rays (1.1 and 1.3 MeV) or by its beta-emissions (E_{max} 0.27 and 0.46 MeV). Samples can be measured in the conventional sodium iodide crystal scintillation counter directly without the need of special preparation. ^{55}Fe (half-life of 2.6 years), on the other hand, emits characteristic x-rays of only 25 per cent yield and of very low energy of 0.0059 MeV. The problem of self-absorp-

TABLE I. Physical Data on some radioisotopes used in Medical Diagnosis.

Radioisotope	Half-life	Decay process	Radiation Energy MeV	Radiation Intensity (%)	γ and x radiation Type and Energy MeV	γ and x radiation Intensity (%)
^{55}Fe	2.7 yr.	EC	-	-	Mn x rays (from EC)	-
^{59}Fe	45 days	β^-	0.46	54	1.29	44
			0.27	46	1.10	57
					0.19	3

tion of such weak radiation is formidable and requires elaborate processing of the sample. The problem of simultaneous measurement of ^{55}Fe and ^{59}Fe in the blood sample then is to overcome the difficulty in measuring ^{55}Fe.

Metabolized iron is found predominantly in the blood, as a constituent of haemoglobin. *PEACOCK and co-workers* (8) were the first to devise a method for differentially counting mixtures of the two isotopes. The estimation of ^{55}Fe in whole blood have, in the past, involved digestion and seperation of the iron by electroplating, and then the activity was determined by using the plated samples in conjunction with an X-ray Geiger-Müller counting technique. *DERN and HART* (9) used electrodeposition of the iron followed by dissolution and liquid scintillation counting. *PERRY and WARNER* (10) developed the method by making the iron to be a colourless complex in a mixture of ascorbic acid and hydrochloric acid, and then counting the activity of the mixture by a method.

A common factor in the methods was the low counting efficiency obtained for ^{55}Fe. Inorganic iron could form stable compounds with a variety of organic agents, but these complexes were generally strongly coloured. *LEFFINGWELL ET COLL.* (11) extracted up to 1 mg of iron from blood digests with orthophenanthrolene and have been able to count ^{59}Fe with fair efficiency in a liquid scintillation system. However, the colour-quenching produced precluded its application to the assay of ^{55}Fe.

DERN and HART [12] overcame this difficulty by using a method in which the iron was converted to its colourless perchlorate salt, which was then dissolved in ethanol. High counting efficiencies and good resolution of the two isotopes were obtained.

EAKINS and BROWN [13] introduced an improved method for obtaining a pure white compound of iron which enables good counting efficiencies to be obtained for ^{55}Fe and ^{59}Fe and permitted the two isotopes to be counted simultaneously using a Packard Tricarb 3214 series liquid scintillation spectrometer.

KATZ, et al [14] investigated the applicability of such an approach to the simultaneous assay of ^{55}Fe and ^{59}Fe and reported the method which avoided the need for electroplating.

THEORETICAL CONSIDERATIONS

For weak radiation emitters, liquid scintillation counter is the more convenient means of measurement; double isotope studies can be applied and such are the cases of tritium and carbon-14 or Fe-55 and Fe-59 in our case. The principles are outlined as follows:-

First the sample or the whole blood has to be processed in such a way that it becomes finally in the form of colourless solution which is chemically neutral in order to make sure that it has the least chemical as well as colour quenching effects interfering with the measurement. To make this type of measurement possible or to prevent self-absorption, next the sample such prepared needs to be not only to be closely contact with the scintillation fluors but also actually mixed with the scintillant itself. The mixture of the prepared sample and the scintillant can then be measured in a well regulated low temperature by co-incidence countings created in the opposing photomultipliers of the liquid scintillation system. The system of measurement has to be pre-calibrated for the particular measurement and the countings evaluated statistically before any interpretation. All these steps involve several factors which are briefly reviewed.

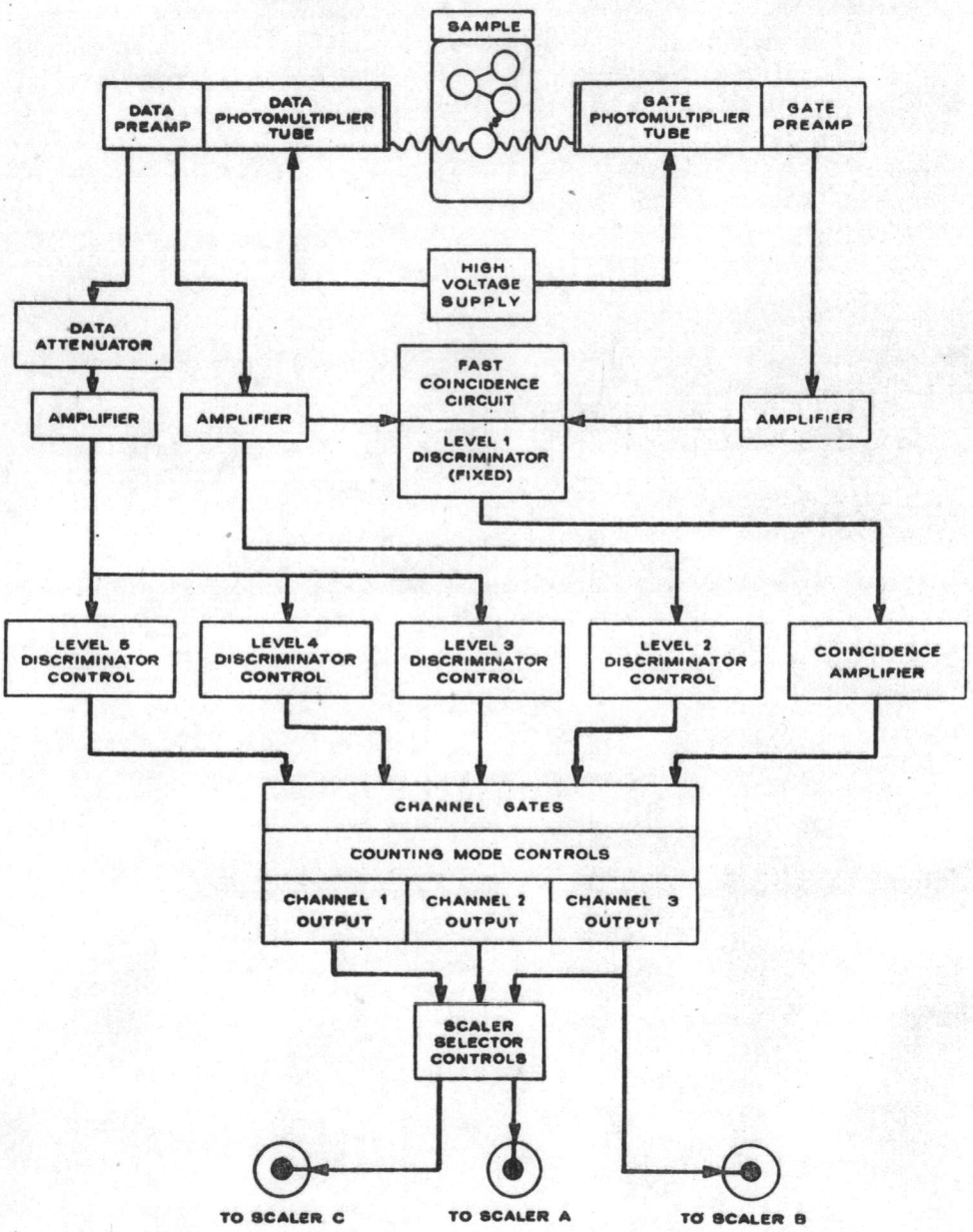

Fig. 2. Simplified block diagram of analyzer.

The blood sample is first digested or charred by caustic acids such as concentrated nitric and sulphuric acids and then decolourised with perchloric acid. After all the residual amount of acid has been removed, the quantity of inorganic iron is precipitated by ammonium hydroxide and ferric hydroxide powder is formed after drying in 95°C oven. The precipitate is then dissolved in ascorbic-hydrochloric acid and finally a clear and colourless solution of ferrous ascorbate is obtained.

Toluene is used as a solvent to dissolve the fluors or solute. The reagent should be of analytical grade which is free from quenching impurities as it comprises the bulk of the sample. Once the fluors have been added to the solvent, the solution should be protected from direct light to retard deterioration.

PPO and POPOP as primary and secondary solutes readily dissolved in toluene are added with triton X-100 to make a gel or emulsion system in counting vials made of glass of low K-40 content. The emulsion system is chosen because it can incorporate large quantities of inorganic salts in the form of mineral acids at high counting efficiencies. A further advantage is that aqueous mineral acids

can be counted at the same high efficiencies so that acidic solutions can be mixed with the scintillant[15,16].

COUNTING EFFICIENCY:

The efficiency with which a radioactive sample is counted in a liquid scintillation usually varies from sample to sample. The quenching, that is the decrease in counting efficiency which occurs, is produced by processes which interfere with the production of light in the liquid scintillant and its transmission to the photomultiplier tube of the liquid scintillation counter.

Quenching may take two forms, chemical quenching and colour quenching. In chemical quenching, compounds in the scintillant interfere with the transfer of energy from the emitted particle or radiation to the organic phosphor and the energy is degraded by processes which do not produce emission of light. In colour quenching, coloured materials in the liquid scintillant absorb light emitted by the organic phosphor and prevent it from being detected by the photomultiplier tube.

Three main methods of countings are used: internal standard, channel ratio and external standard. In the internal standard method a small volume of radioactive standard is added to the sample vial which is then recounted. It is assumed that the count of sample plus

standard is at the same efficiency as that of the sample alone and the latter can thus be determined, knowing the specific activity of the radioactive standard. In the channel ratio method the sample counts are monitored in two electronic channels. The total counts of each channel can then be expressed as a ratio of one another. The channels are selected so that a change in counting efficiency cause a shift of the electronic pulse-height " spectrum " of the sample and therefore a change in the ratio of the counts in the channels, or channels ratio.

Using a series of radioactive standards of different counting efficiencies a standard curve of counting versus channel ratio may be constructed. The counting efficiency of an unknown sample may then be determined from its channels ratio. The external standard method of determining counting efficiency depends on the use of a small gamma emitting source which is automatically positioned near the sample vial and whose gamma rays produce scintillations when absorbed in the liquid scintillant. The number of the liquid scintillant and, using radioactive standards as above, a calibration curve of external standard counts versus counting efficiency can be constructed. It is necessary for two counts to be taken, one for sample and one for the sample plus external standard. In addition, variations in the volume of the liquid scintillant, variations in the thickness of the glass vial and different liquid scintillants produce variations in the number of gamma-produced counts which lead to errors in the determination of counting efficiency.

Emulsion Counting

The emulsion counting technique was used by Patterson and Greene in 1965 for the counting of large quantities of aqueous samples of carbon-14 and tritium-labelled materials. Three liquid scintillant mixture were described containing different quantities of the surface-active agent " Triton X-100 " for various applications. Counting samples were prepared consisting of fluid emulsions or rigid gels. It gave good counting efficiencies for carbon-14 and fair counting efficiencies for tritium.

The emulsion were said to be unaffected by strong acid, alkali and concentrated phosphate buffer in the aqueous phase and were of the water-in-oil type. This type of system has advantages similar to the suspension counting system in that quenching agents in the sample are in a different phase from the liquid scintillant and exert less effect. Self-absorption would also be small if the droplet size of the emulsion was small in comparison with the range of the emitted-particles.

Internal-Sample Liquid Scintillation Counting

In internal-sample liquid scintillation counting techniques self absorption in the sample is avoided by mixing the latter with a liquid scintillating medium in a scintillation counter assembly.

During measurement the vial containing the sample rests directly in contact with the photocathode of a photomultiplier tube and is surrounded by an optical reflector which ensures good height collection. The whole assembly is enclosed in a lead shield to reduce background due to stray radiation. A light-tight shutter mechanism actuated by the lid of the shield enables the operator to change samples without exposing the photomultiplier to light, an essential provision if stable operating conditions are to be obtained. The associated electronic equipment is shown in Fig. 2 and comprises a high-voltage supply unit for the photomultiplier tube, a high-gain linear amplifier, single-channel pulse height analyser. The analyser and high-voltage supply are adjusted so that the greater part of the β-ray spectrum of the radioisotope of interest is recorded whilst unwanted background pulses of lesser or greater height are rejected. These " balance-point " operating conditions, which are best achieved by setting the analyser controls to appropriatevalues and then adjusting the photomultiplier voltage until the counting rate is at a maximum, give both high sensitivity and good stability.

The background may be both reduced and stabilized by cooling the counter assembly.

In instruments of this type, the sample vial is placed between two photomultiplier tubes in coincidence so that only pulses appearing simultaneously at both photomultiplier outputs are passed on to the recording equipment. A true scintillation consisting of several

photons is detected by both photomultipliers and fulfils this condition. A dark-current background pulse in either photomultiplier on the other hand will not be recorded unless it chances to be accompanied by a similar pulse in the other photomultiplier.