SHADOW - SHIELD WHOLE - BODY MONITORS AND THEIR APPLICATION IN NUCLEAR MEDICINE.

THESIS

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for the Degree of

MASTER OF SCIENCE

by

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PREFACE.

This thesis, composed by the author, contains an account of research conducted by her under the direct supervision of Dr. K. Boddy at the Scottish Research Reactor Centre, East Kilbride. The analysis of the results and their interpretation are those of the author. The thesis has not been accepted in fulfilment of the requirements of any other degree or professional qualification.

PUBLICATIONS.

The following papers have been published or submitted for publication with which the present author has been associated and in which her assistance has been acknowledged.

1. BODDY, K., KING, P.C., WILL, G. and PROVAN, G.: The measurement of phosphorous - 32 excretion using a whole-body monitor and the significance of excretion rates in therapy. Submitted for publication, 1967.

2. BODDY, K.: The development of a prototype shadow-shield whole-body monitor, Phys. Med. Biol., Vol. 12, No. 1, 43 - 50, 1967.

3. BODDY, K.: A high sensitivity shadow-shield whole-body monitor with scanning-bed and tilting-chair geometries, incorporated in a mobile laboratory. To be published in Brit. J. Radiol, 1967.

4. BODDY, K. and ADAMS, J.F.: The excretion rate and biological half-life of parenteral cobalamims and co-enzyme B_{12} in normal and B_{12} deficient subjects. Submitted for publication, 1967.

5. ADAMS, J.F. and BODDY, K.: Metabolic equilibrium of tracer and natural vitamin B_{12} - an experimental study. Submitted for publication, 1967.

6. WILL, G. and BODDY, K.: Iron turnover estimated by a whole-body monitor, Scot. Med. J., 12 157 - 162, 1967.

7. BODDY, K. and WILL, G.: Succinic acid and iron absorption, Scot. Med. J., 12, 183 - 185, 1967.

8. BODDY, K.: In vivo activation analysis of iodine in the thyroid gland - a preliminary study. Radioaktive isotope in Klinik and Forschung. Urban and Schwarzenberg, München - Berlin - Wein, 377 - 382, 1967.

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CONTENTS.

		Page
Chapter I.	INTRODUCTION.	1
Chapter II.	A DESCRIPTION OF THE MOBILE WHOLE-BODY MONITOR AND ITS PERFORMANCE.	10
	Description of MERLIN.	10
	Performance of MERLIN.	14
Chapter III.	A STUDY OF PHOSPHOROUS -32 THERAPY IN PATIENTS WITH POLYCYTHAEMIA RUBRA VERA.	23
Chapter IV.	THE MEASUREMENT OF TOTAL BODY POTASSIUM.	39
	1. Performance of MERLIN.	42
	2. The effect of the drug Biogastrone on serum and total body potassium levels in seven normal subjects.	49
Chapter V.	THE RETENTION AND EXCRETION RATES OF VITAMIN B_{12} IN PATIENTS WITH PERNICIOUS ANAEMIA AND HAEMATOLOGICALLY NORMAL SUBJECTS.	53

CHAPTER I.

INTRODUCTION.

There is no single branch of medicine or surgery today where isotopes have not found some application. They have been applied to the study of physiological processes in health and disease and have been used for assessing the results of various forms of treatment. Radioisotope techniques provide a valuable means of research for the medical scientist, they offer a constantly increasing range of valuable diagnostic procedures for clinicians, and they make available numerous technical improvements in therapeutic methods for radio-therapists.

Until 1946 radioactive isotopes were available to only a few workers, but in recent years these substances have become generally available as a result of a large-scale expansion of work on nuclear energy. The instigation of this is partly attributable to war-time advancement. Radioisotopes are finding increasing application in modern industrial practice, but since it was in the fields of biology and medicine that their value was first realised, it is in these fields that the greatest progress has been made to date.

Owing to this expansion of work on nuclear energy and to the increased availability of radioisotopes, it became essential to develop methods of measuring natural radioactivity in the body and of detecting the smallest possible increase that may occur from occupational or other causes. As the whole body counting technique developed, it became clear that a new tool for clinical research was emerging.

Table I. (SPIERS, 1962) illustrates the historical development of whole-body monitors and the improvement in sensitivity. The stimulus of the early work came from the use of radium salts medicinally and from the ingestion of radium by the early luminisers. The first measurements were made with ionization chambers, having volumes of only one or two litres, attached to a string of electrometers (SCHLUNDT et al., 1929). The modern development of accurate quantitative measurement was initiated by EVANS(1937), using a single Geiger-Müller counter at one metre from the subject, to minimise variations in response due to uneven distribution of the radioactivity in the skeleton. At this distance, however, there was poor geometrical efficiency and with the low intrinsic efficiency of the Geiger tube, this method was relatively insensitive.

The first apparatus with sensitivity approaching that

TABLE I

Early Development of Whole-Body Monitors.

Reference	TECHNIQUE	Subject Counting Time	Detection limit (or S.E.)	
			µg Ra equiv.	°/o total body K
Small Ionisation chambers:				
Schlundt et al (1929)	I.C. at 1 atm. + string electrometer	--	~5	
Schlundt et al (1933)	do.		~0.2	
Hess and McNiff (1947)	I.C. of 13 litres at 1 atm.		~0.03	
Geiger-Müller tube:				
Evans (1937)	1 metre arc.		0.1	
Large High Pressure Ionisation Chambers:				
Sievert (1951)	Circular array of 10 long chambers-411	2h	0.005(SE)	~50 (SE)
Burch and Spiers (1953)	do. + backing off chambers.	2h	0.003(SE)	~30 (SE)
Sievert (1956)	As before - underground	3-4h	0.001 (SE)	~10 (SE)
Scintillators:				
a) Organic				
Anderson (1956)	4π liquid scintillator	15 min	~0.0001(SE)	1 (SE)
Bird and Burch (1958)	Three unit plastic scintillator	15 min	~0.0001(SE)	1.5 (SE)
b) Inorganic				
Marinelli (1956)	4 in x 1.5 in NaI - chair geometry	15 min	~0.0003(SE)	3.5 (SE)

(SE) = standard error

µg Ra equiv. = µg Ra with all daughter products at equilibrium.

required for the measurement of natural gamma-ray activity of the body was developed by SIEVERT (1951). He attained geometrical efficiency by surrounding the subject, lying horizontally, with a circular array of ten long cylindrical high-pressure ionization chambers, which provide some compensation for uneven distribution of radioactivity in the body. Water-tank shielding to reduce the background was used. Later SIEVERT (1955,1956) installed similar equipment in an underground laboratory to reduce cosmic-ray background.

The break-through to the accurate measurements of natural gamma-ray activity of the body came with the development of large scale scintillation apparatus. Large liquid scintillators developed by ANDERSON et al. (1956), the sodium iodide apparatus pioneered by MILLER and MARINELLI (1956) and the three unit plastic scintillator apparatus evolved by BUNCH and BIRD (1958), are all capable of measuring natural body potassium with a statistical error of a few per cent or less in observation time of about 15 minutes. The advent of these methods has superseded both ion-chamber and Geiger-counter apparatus.

Many clinical investigations can be carried out using comparatively simple and inexpensive whole-body monitors costing £2,000 and less (BODDY, (1965))for example, for studing absorption of labelled iron or vitamin B_{12} in diagnosis and

treatment of iron deficiency anaemia or pernicious anaemia, while, less often, a high sensitivity monitor is required for long-term retention studies and the measurement of natural body radioactivity. The conventional type of high sensitivity monitor, requiring steel room housing, is of considerable cost, and so initial studies began at the Reactor Centre early in 1964 to develop a monitor of high sensitivity and low cost which could be incorporated in a mobile laboratory. Since no hospital in Scotland possessed or had easy access to a high sensitivity counter equal to that of a steel or lead room, the concept of a mobile unit which could serve numerous centres was particularly attractive.

To obtain high sensitivity, the background counting-rate which arises from cosmic radiation, local gamma-radiation and radioactive contamination in the detector itself must be minimal. Large pulses from cosmic-ray mesons entering a scintillator are excluded by a pulse-height analyser, but a considerable fraction of the residual count-rate is produced by lower-energy cosmic events. Local gamma-radiation can be considerably reduced by lead, steel, water or even chalk shielding. Owing to the monitor being made mobile, the weight of shielding which could be used was limited.

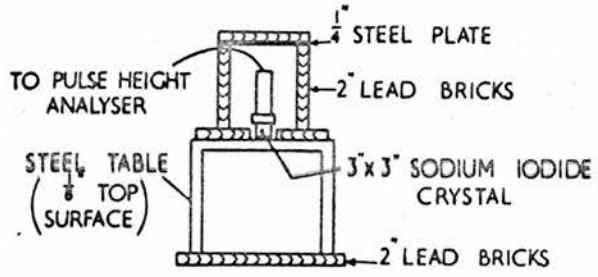
The stages in the development of the shadow shield are

shown diagramatically in Fig. I, and the present prototype which became operational in August, 1964, in Fig. 2. In Fig. 3. the unshielded and final shielded backgrounds are illustrated graphically. This initial programme was undertaken without direct financial aid and materials and equipment originally acquired for other purposes were used, consequently some improvision was necessary. The shadow shield comprises approximately $7\frac{1}{2}$ tons of commercial four inch lead bricks arranged as shown in Fig. 2. The shield is approximately 12 feet 8 inches in length, 3 feet 6 inches maximum width and 3 feet 2 inches maximum height.

The initial detector was a 3 inches diameter by 3 inches NaI (Tl) crystal located in the central turret and views the patient through a hole $5\frac{1}{2}$ inches diameter cut through the steel supporting plate. The distance from the crystal face to the patient mid-line is 9 inches. A single crystal was employed rather than a multiple array for ease of shielding. Another advantage of a single crystal is a counting rate equal to or higher in the photopeak and considerably lower at lower energies thanthose observed with multiple crystal systems of the same total crystal volume (MILLER, 1962). Relatively complex mixing and gating electronic circuits are needed when using two or more crystals. The main advantage of the multiple array

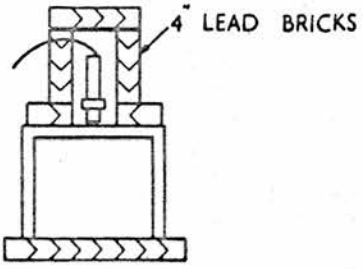
FIG 1.

STAGES IN THE DEVELOPMENT OF THE PROTOTYPE SHADOW SHIELD WHOLE BODY COUNTER



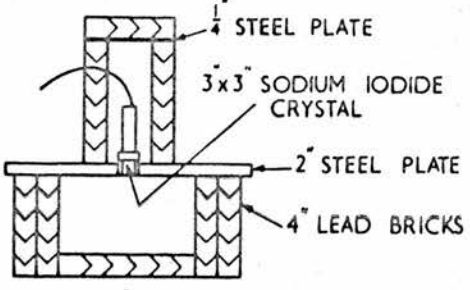
STAGE 1

% OF UNSHIELDED BACKGROUND : 13·7%

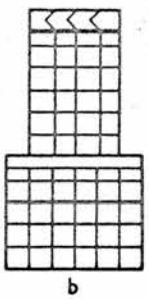


STAGE 2

% OF UNSHIELDED BACKGROUND : 7·2%



a



b

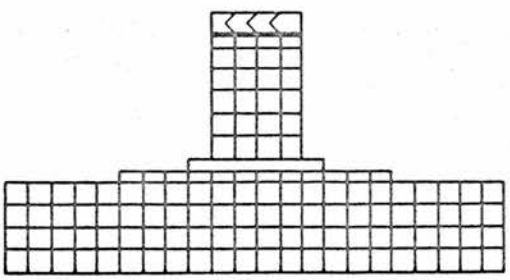
STAGE 3

% OF UNSHIELDED BACKGROUND : 4·8%

% OF PREVIOUS BACKGROUND : 67·4%

FULL BASIC % OF UNSHIELDED BACKGROUND : 2·5%

% OF PREVIOUS BACKGROUND : 51 4 %



STAGE 4

% OF UNSHIELDED BACKGROUND : 2·3 %

% OF PREVIOUS BACKGROUND : 92·9 %

FINAL

% OF UNSHIELDED BACKGROUND : 2·2 %

% OF PREVIOUS BACKGROUND : 96·1 %

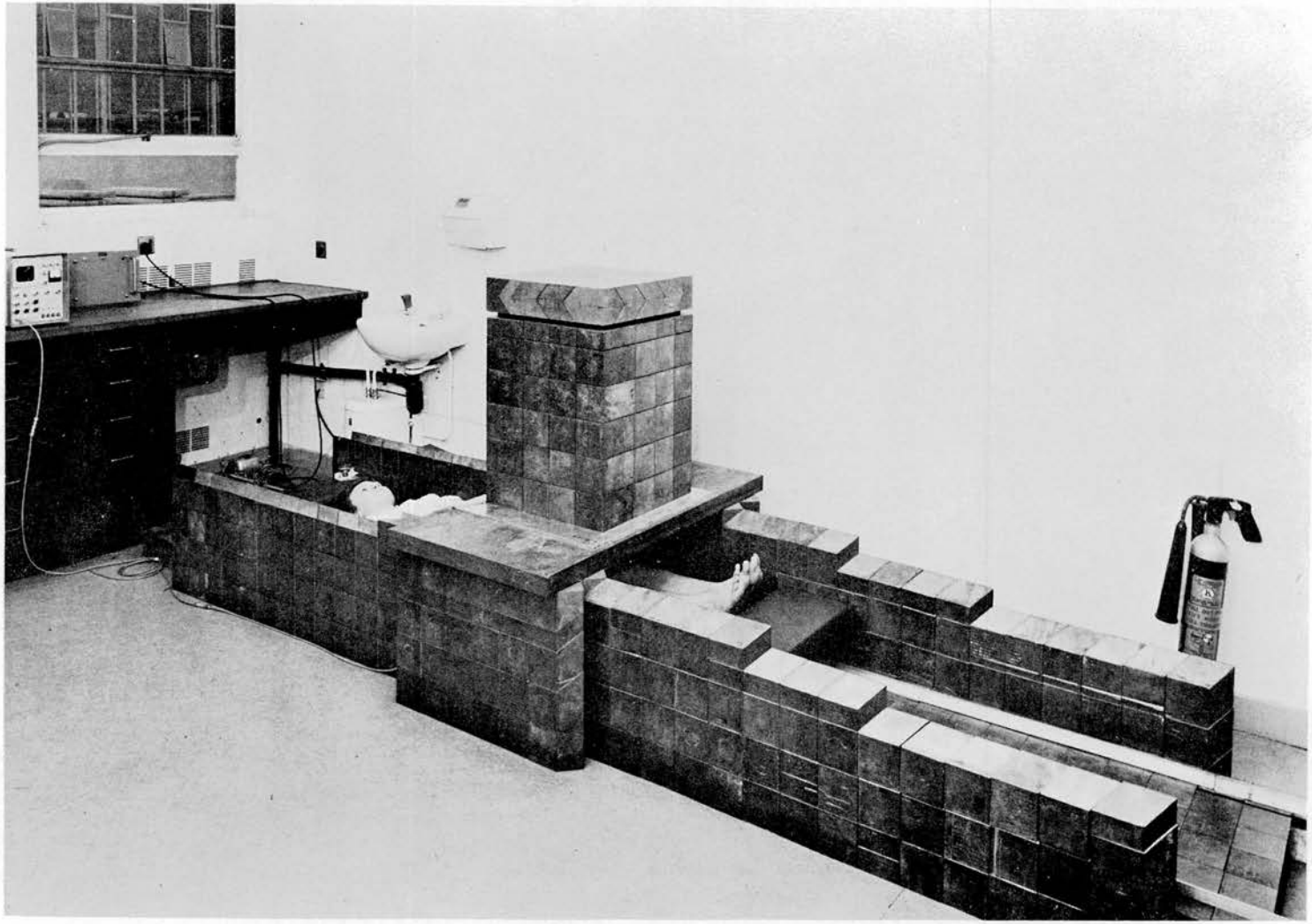
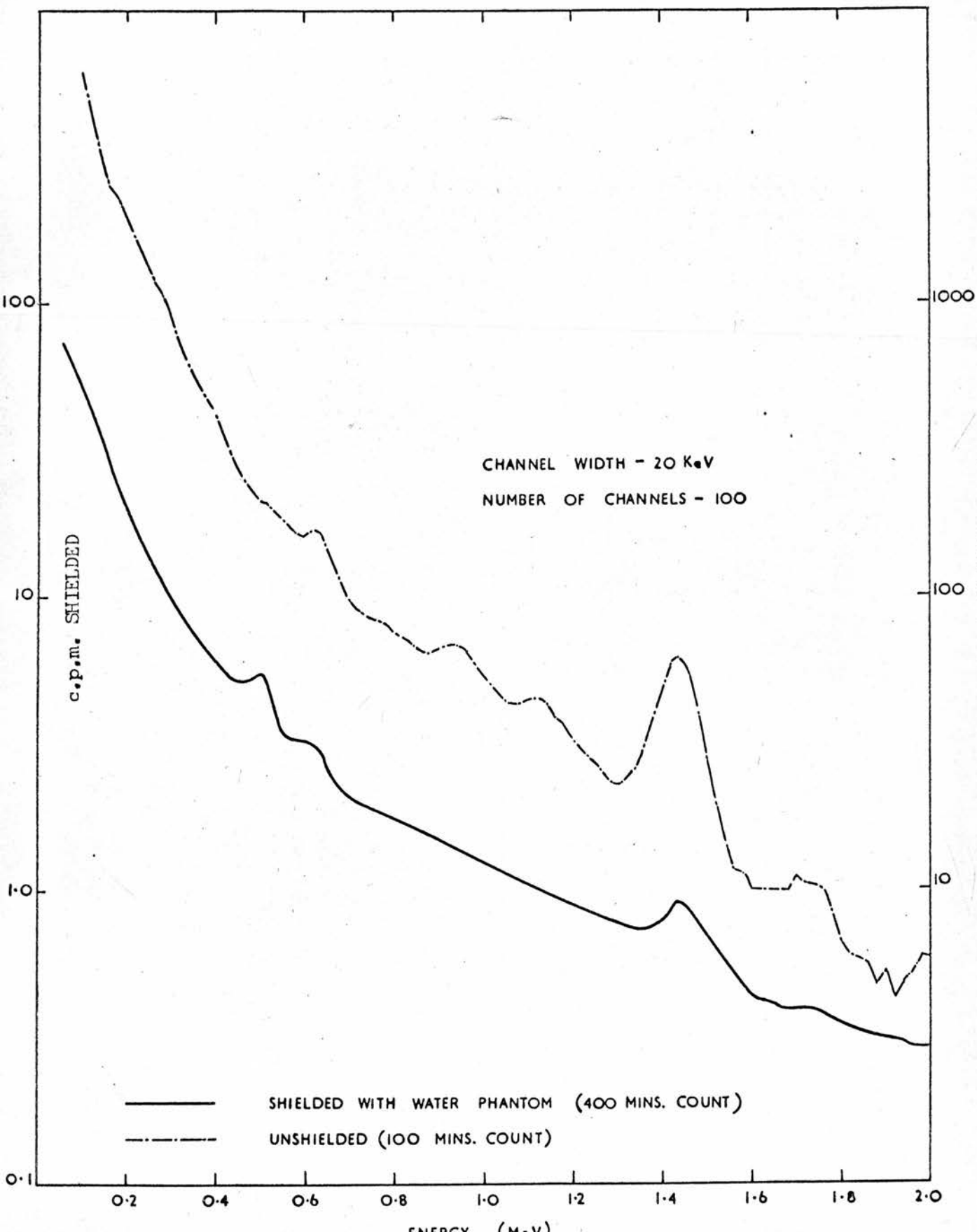


FIG. 2.

FIG 3.


CHANNEL WIDTH - 20 K.eV
NUMBER OF CHANNELS - 100
c.p.m. SHIELDED
c.p.m. UNSHIELDED
100
10
1·0
0·1
1000
100
10
0·2
0·4
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
ENERGY (M.eV)
SHIELDED WITH WATER PHANTOM (400 MINS. COUNT)
UNSHIELDED (100 MINS. COUNT)

system is the information about the distribution of the isotope in the body which can be obtained if the individual outputs of the crystals are sorted and recorded separately using either single channel analysis or separate sections of the analyser memory. In the prototype, however, indication of the distribution of the isotope in the patient can be obtained by plotting the variation in the dead-time meter reading of the analyser, which is proportional to the counting-rate. The output from the 3 inches X 3 inches crystal is analysed by a 100 channel T.M.C. Gammascope. Scanning-bed geometry is used in which the patient passes beneath the detector. The bed-drive mechanism operates "start-stop" micro-switches to control the analyser counting time. The performance of the prototype and its sensitivity, quantity of the isotope giving three times the standard deviation of the background in the same counting time (TROTT, 1965), are shown in Table II (BODDY, 1967, A).

The variation in the counting-rate due to redistribution of the isotope in the patient's body has been examined, (BODDY, 1967, A). The extent to which the counting-rate may be influenced was investigated using a sodium - 22 source placed consecutively at four inch intervals along the longitudinal axis and at three inch intervals along the lateral axis of the monitor at the patient mid-line height. The longitudinal variation was

TABLE II.

Sensitivity of shadow-shield prototype whole-body monitor from measurements on a polythene phantom.

Isotope	Energy range (Mev)	Counting-rate (cpm/μCi) with patient* or 'Active' phantom +	Background (cpm) with water phantom	Sensitivity (μCi) for 30 min scan.
Fe^{59}*	1.0 - 1.38	550	18.4	4.1×10^{-3}
	0.4 - 1.38	1670	103	3.4×10^{-3}
Co^{58}*	0.74 - 0.89	490	13.7	4.0×10^{-3}
	0.3 - 1.0	1800	112	3.2×10^{-3}
Co^{57}*	0.08 - 0.15	3430	330	2.9×10^{-3}
K^{40}*	1.36 - 1.56	0.03	8.0	
Na^{22}+	1.18 - 1.38	552	9.7	4.3×10^{-3}
I^{131}+	0.3 - 0.42	1515	64.4	2.9×10^{-3}

estimated as 3 per cent which could be reduced to approximately 1 per cent if the count was started with the head about 12 inches out from the crystal centre and stopped with the feet the same distance beyond the crystal centre. The lateral standard error was approximately 9.7 per cent which may be partly due to the small crystal diameter. The source was also placed at varying depths in a water phantom directly below the crystal and a depth standard error of approximately 16 per cent obtained by summing the counts in reciprocal positions, representing supine and prone scanning. Redistribution effects were also studied in five patients at times up to six hours after oral administration of iron - 59. The results are summarised in Table III, the maximum observed variation being $\pm$ 9.4 per cent (WILL and BODDY, 1967).

No sacrifice in sensitivity is involved in making the counter mobile or in using a shadow-shield. This is illustrated in Table IV where a comparison of sodium iodide crystal whole body monitors and shadow-shield monitors is made. The index of comparison is the product $CT^{\frac{1}{2}}$ which has the smallest value for the most sensitive counter.

$$CT^{\frac{1}{2}} = \frac{B^{\frac{1}{2}}}{D} + \left[\frac{B}{D^2} + \frac{1}{D}\right]^{\frac{1}{2}}$$

Table III. Variation in whole body counting rate in the post ingestion period and its effect on percentage iron absorption.

Case	Whole body counts				Percentage iron absorption				Mean Absorption	Variation ± o/o
	Immediate c.p.m.	2 hrs. c.p.m.	4 hrs. c.p.m.	6 hrs. c.p.m.	Immediate o/o	2 hrs. o/o	4 hrs. o/o	6 hrs. o/o		
1.	9721	8635	9699	9625	4.2	4.7	4.2	4.2	4.3 ± 0.4	9.3
2.	28831	-	33468	26810	15.1	-	13.0	16.3	14.8 ± 1.46	9.4
3.	34054	-	32700	-	15.1	-	15.7	-	15.4 ± 0.3	1.9
4.	11744	11696	10117	10010	11.9	11.9	13.8	13.9	12.9 ± 1.0	7.8
5.	6156	5327	5999	5825	18.1	20.9	19.1	19.2	19.3 ± 1.5	7.8
										Mean ± 7.2

TABLE IV. COMPARISON OF SODIUM IODIDE CRYSTAL WHOLE-BODY COUNTERS IN GREAT BRITAIN

AND

SHADOW SHIELD WHOLE BODY COUNTERS.

Expert in Charge.	Location	K^{40}					Cs^{137}				
		B Net background (cpm)	Net subject rate (cpm/gm)	D subject rate (cpm)	$CT^{\frac{1}{2}}$ Index of Comparison	C. Coeff. of variation per cent T=100 min	B Net background (cpm)	Net subject rate (cpm/μc)	D Subject rate (cpm)	$CT^{\frac{1}{2}}$ Index of Comparison	C. Coeff. of Variation per cent T = 100 min
Rundo. J.	Harwell	77	0.406	56.8	0.36	3.6	380	7.85×10^{3}	78.5	0.52	5.2
Vennart. J.	Sutton	90	0.200	28	0.73	7.3	325	4.25×10^{3}	42.5	0.88	8.8
Trott. N.G.	Sutton	83	0.11	15.4	1.24	12.4	340	2.1×10^{3}	21	1.78	17.8
-	Hammersmith	170	0.0218	3.05	8.6	86	342	381	3.81	9.74	97.4
Stott. G.	Dounreay	220	0.95	133	0.25	2.5	630	1.53×10^{4}	153	0.35	3.5
Peabody. C.O.	Winfrith	175	0.90	126	0.24	2.4	300	1.17×10^{4}	117	0.32	3.2
Hesp. R.	Windscale	141	0.872	122	0.23	2.3	275	1.32×10^{4}	132	0.28	2.8
Mobile[*]	Hanford	173	0.78	109	0.27	2.7	196	8.3×10^{3}	83	0.37	3.7
Prototype	E. Kilbride	8.1	0.05	7.0	0.95	9.5	25	1.0×10^{3}	10	1.1	11

[*] Data from Brady, D. N. 1964, (Private Communication)

Where B is the background counting-rate,

D is the net subject counting-rate

and T is the total counting-time available, (subject + background.

It can be seen that the Hanford Mobile Counter, which uses an 11½ inches diameter x 4 inches NaI (Tl) detector is of equal sensitivity to the Atomic Energy Association "steel room" Counters and more sensitive than the remainder.

It is possible to predict the approximate shielding weight and distribution and the crystal volume for a required sensitivity in a monitor similar to the prototype by using the data that has been presented, (BODDY, 1967 ,A). It can be assumed that the sensitivity and standard error are almost directly proportional to the square root of the background counting-rate for a given detector. In the Stage 1 shield, the background counting-rate is 13.7 per cent of the unshielded counting-rate, and in the final prototype is 2.2 per cent of the unshielded counting-rate. Hence, it can be calculated that the Stage 1 shield is about 13.7 / 2.2 = 2.5 times less sensitive than the final configuration. The sensitivity and standard error are roughly inversely proportional to the square root of the crystal volume. In the prototype, the standard error in measuring 140 gms of potassium is about

15 per cent, too large for clinical application. By using a detector of 11½ inches diameter x 4 inches instead of the 3 inches diameter x 3 inches detector, the standard error can be reduced to about 3 per cent. The standard error for a similar measurement in steel room monitors and in the Mobile Shadow-Shield Monitor at Hanford (PALMER and ROESCH, 1965) is of the same magnitude.

The prototype monitor was used when a study was made of phosphorous - 32 therapy on patients with polycythaemia rubra vera, which is discussed in Chapter III, and also for the long-term metabolic study of vitamin B_{12} turnover in patients with pernicious anaemia and in one normal subject in Chapter V. Part of the work also described in this chapter was performed in the Mobile Whole-Body Monitor (MERLIN - Monitoring Equipment for Radioactivity at Low-levels IN vivo). Chapters II and IV cover the description of MERLIN and its performance and total body potassium estimations measured by MERLIN respectively.

CHAPTER II.

A DESCRIPTION OF THE MOBILE WHOLE-BODY MONITOR AND ITS PERFORMANCE.

INTRODUCTION:

In May, 1965, the Scottish Hospital Endowments Research Trust awarded a capital grant of £19,745 for the construction of a high sensitivity shadow-shield monitor to be incorporated in a mobile laboratory. This monitor, MERLIN, completed in July, 1966, is intended for medical research in collaboration with Scottish hospitals.

DESCRIPTION OF MERLIN:

The overall length of the vehicle is 36 feet and the dimensions of the laboratory itself are approximately 24½ feet x 7½ feet x 8 feet high. The shield weight is supported by a 7½ ton reinforced Bedford chassis with a 54 inch extention. An instrument panel is recessed behind the cab and above this compartment the generator and thermostatically controlled air conditioner / heater unit are enclosed. The exterior of the vehicle is shown in Fig. I. and the interior in Fig. 2.

An increase in sensitivity of the monitor was obtained by increasing the detector volume to an 11½ inches diameter

FIG 1

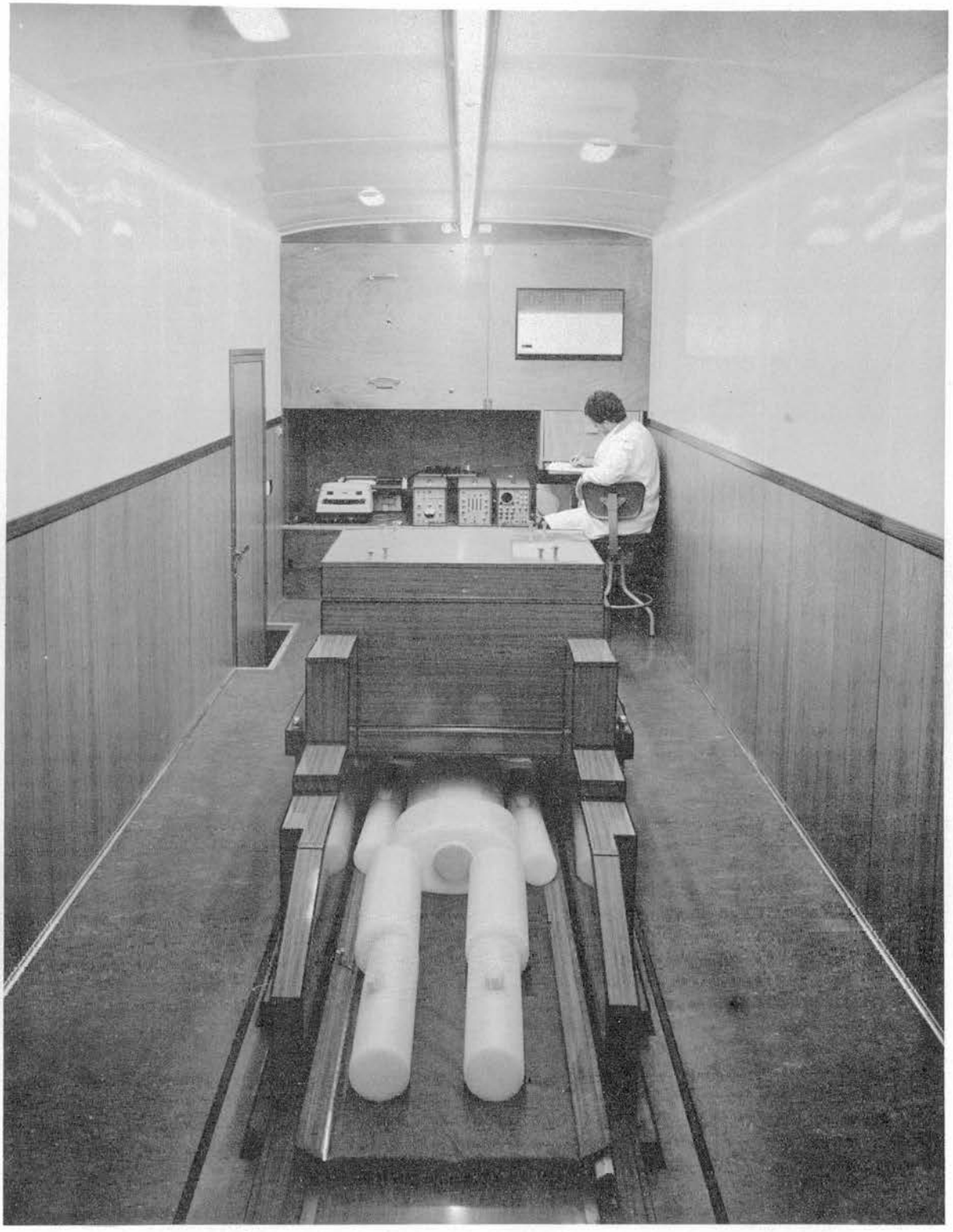


Fig. 2. MERLIN - Scanning-bed Geometry

x 4 inches sodium iodide crystal, see Fig, 3, above which are seven photomultiplier tubes, specially selected for low background and matched output. The improvement expected in the statistical accuracy of measurements with an increase in crystal size is mainly due to an increase in geometrical efficiency from the larger solid angle subtended, which is dependant on the diameter of the crystal, an increased probability of gamma-ray interaction with the greater crystal thickness and the enhancement of the total energy peak with a simultaneous reduction in the Compton - continuum region of the spectrum.

The results obtained in the construction of the prototype indicated that the extremities were "over - shielded", and since there had to be a reduction in the weight of lead to approximately 7 tons for MERLIN, the overall length of lead shielding was decreased to 8 feet 8 inches. Hardwood packing pieces extend the full length of the monitor to 13 feet, (Fig. 4.). Table I illustrates the average background reduction obtained at each stage of construction, the final addition of a 2 inch base and walls making no apparent difference to the counting-rate. Premonitored virgin lead bricks of 2 inch thickness were used.

The signals from the photomultiplier tubes are mixed and

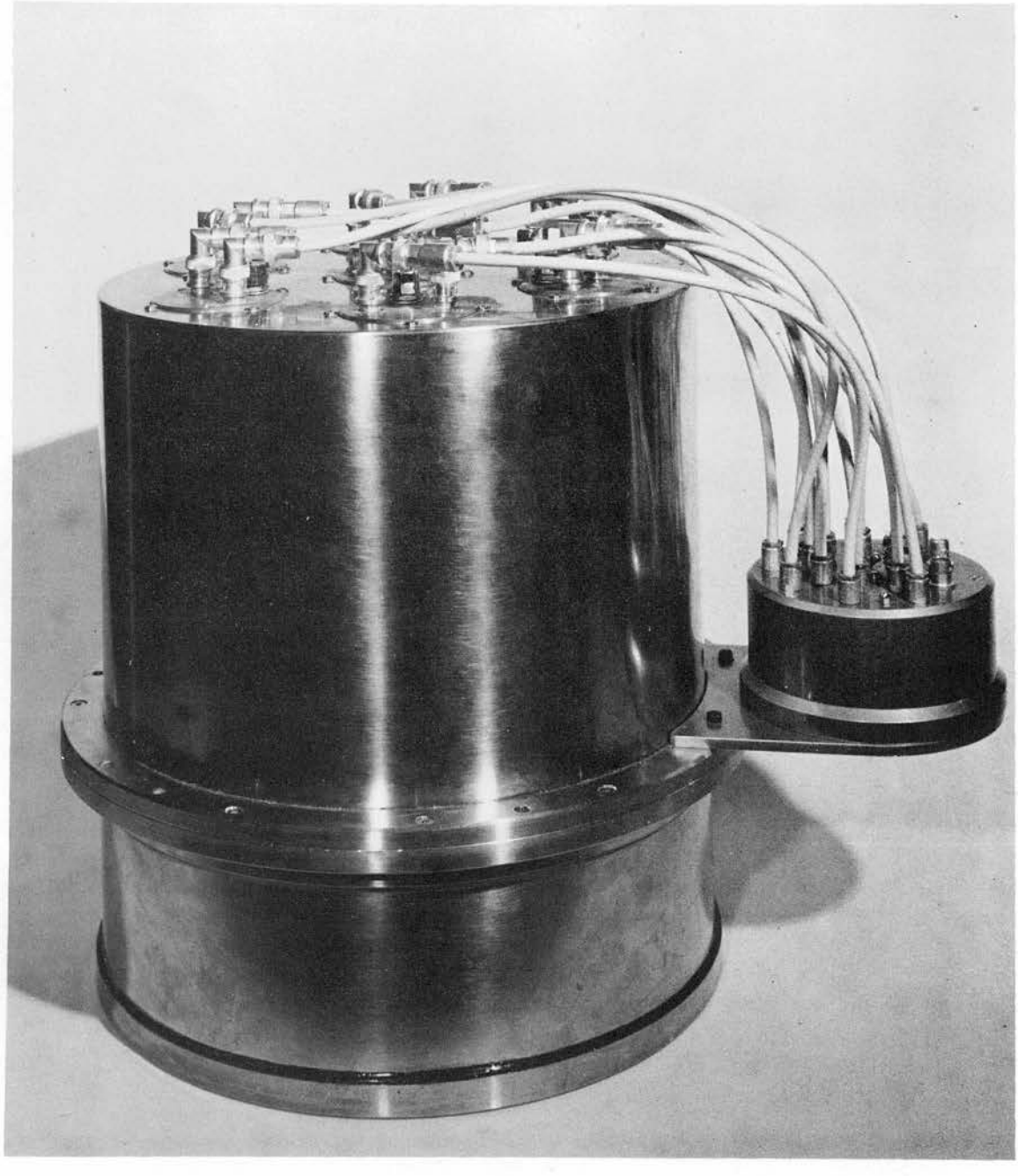
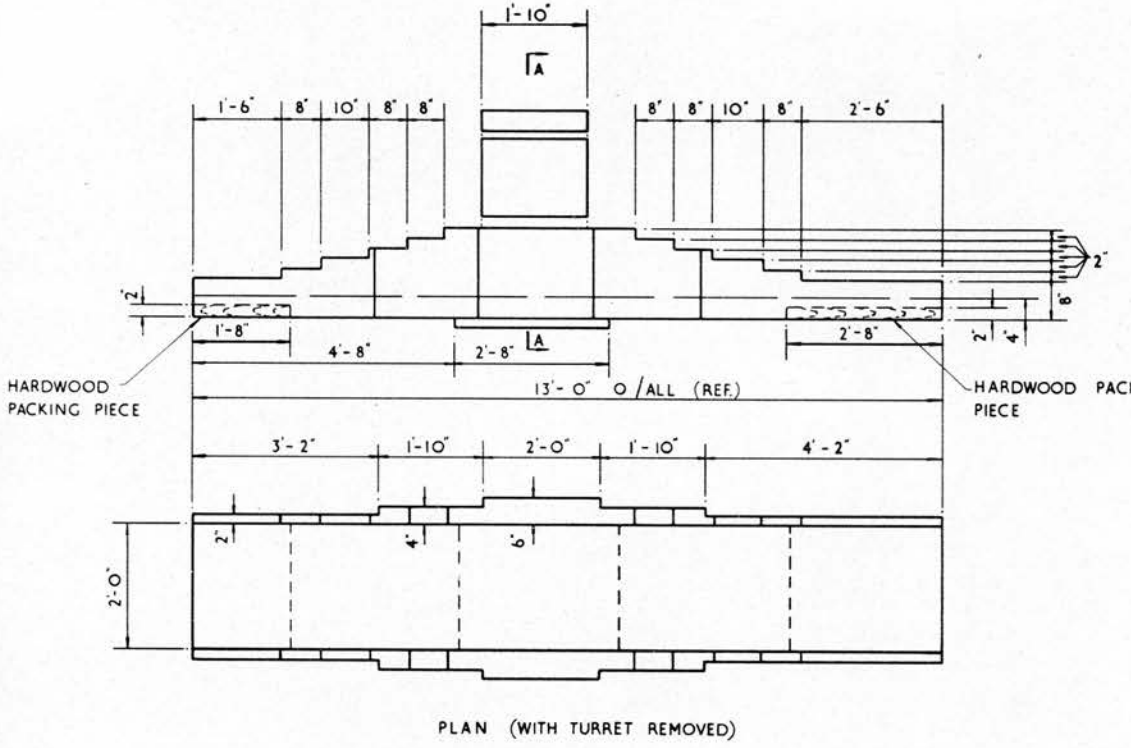


Fig. 3. Sodium Iodide Detector Assembly - $11\frac{1}{2}$ inches diameter x 4 inches

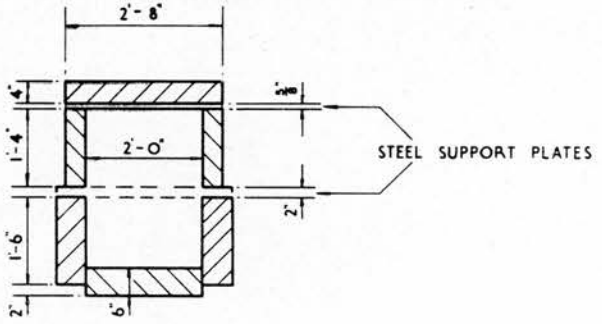
FIG 4a

SHADOW SHIELD

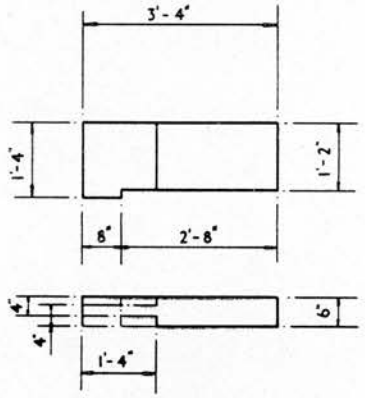
MATERIAL - PURE LEAD



PLAN (WITH TURRET REMOVED)



SECTION 'A-A'



TURRET EXTENSION

1 OFF AS DRAWN
1 OFF OPP. HAND (AS PER CHAIN DOT LINE)

FIG 4b

FINAL SHIELD FOR MERLIN

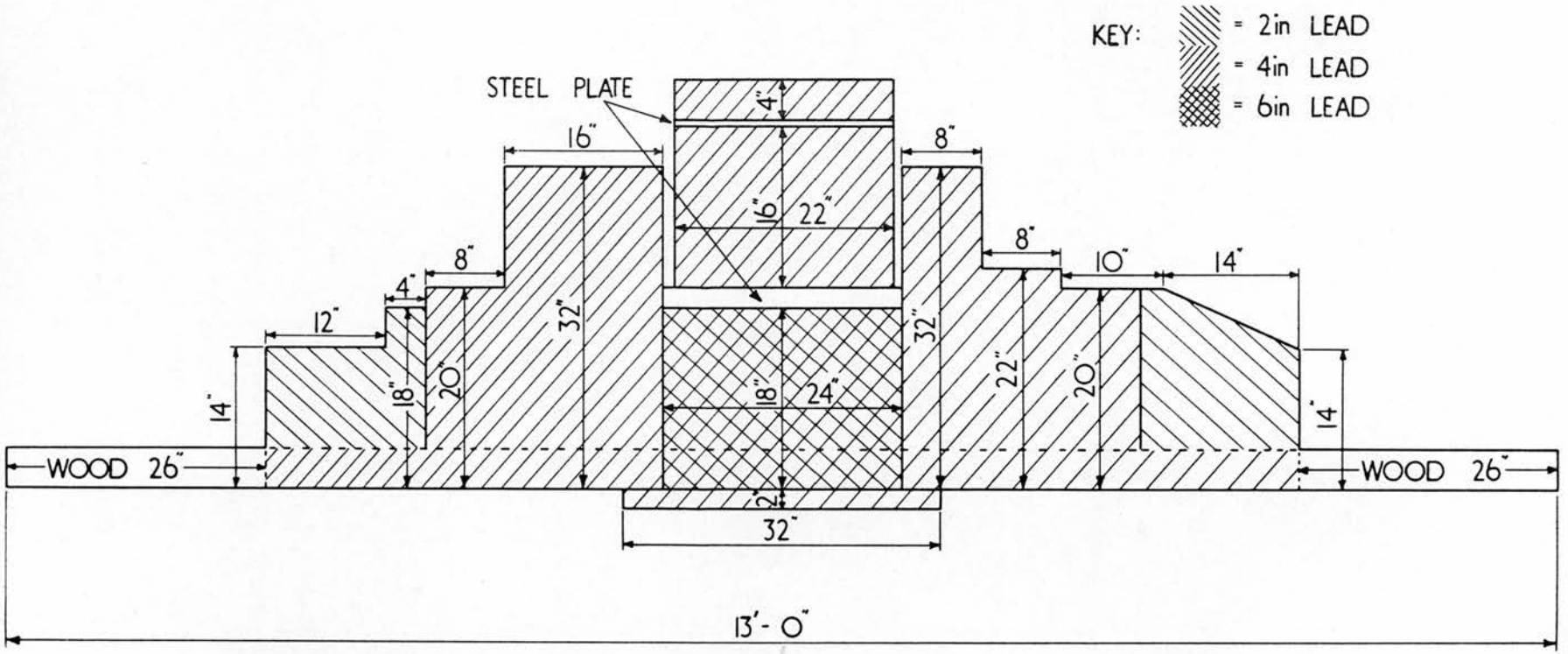


TABLE I.

Average reduction in background counting-rate at various stages of shield construction.

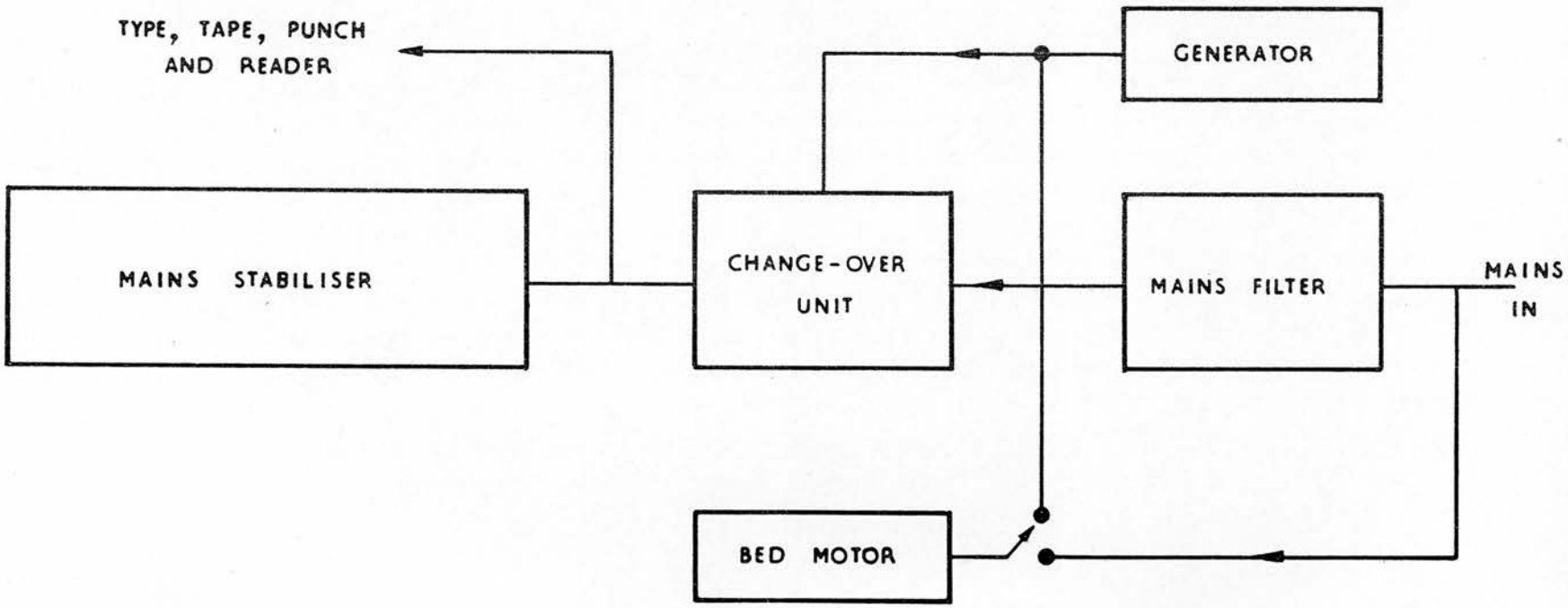
Stages in shield construction.	% of unshielded counting-rate.	% of counting-rate at previous stage.
1. Unshielded.	100	100
2. Turret and turret side walls.	13	13
3. Stage 2 + 6in, base.	5.3	38
4. Stage 3 + 4in. walls.	3.6	68
5. Stage 4 + 4in. base.	2.0	55
6. Stage 5 + 2in. base and walls.	2.0	100

fed into a T.M.C. 400 Channel 404 pulse height analyser. Other electronic equipment comprises a Resolver Integrator Unit, a Type - Punch - Read Control Unit including a high voltage power supply, an I.B.M. typewriter, and a tape punch readout and reader. Fluctuations and surges in the mains voltage are minimised by the use of filter and stabiliser units. To allow the change from the mains electrical supply to the generator, and vice versa, to be made without de-energising and therefore, de-stabilising the electronic equipment, a "change - over" unit has been installed. A diagrammatic presentation of the electronic equipment can be seen in Fig. 5.

The scanning - bed geometry is employed in which the patient, lying on a motorised couch, passes beneath the detector in the supine position and in the reverse direction in the prone position, with a bed to crystal face distance of 30 cms, when the detector is level with the steel supporting plate. With the detector in this "low" position a high sensitivity is obtained. Alternatively, the detector can be used in the "high" position, with a bed to crystal face distance of 41 cms, resulting in a reduction in redistribution effects due to the increased patient to detector distance. There is also, however, a decrease in

FIG 5

DIAGRAMMATIC PRESENTATION OF ELECTRONIC EQUIPMENT



sensitivity. The couch is attached to a screw 10 feet 3 inches in length which is driven by an $\frac{1}{8}$th H.p. A.C. motor, giving a scanning length of approximately 105 inches. The motor speed can be altered by changing pairs of gears, to give, for a patient of 5 feet, a minimum scanning time of 3.73 minutes and a maximum of 33.16 minutes. As in the prototype, the counting is automatically started and stopped by adjustable micro - switches which are set according to the patient's height.

The scanning bed geometry, which is normally used, can be changed, by modification of the shield, to a tilting-chair geometry, thus accomodating patients who cannot lie flat. This is achieved by raising the steel plate and turret 14 inches and, using additional lead bricks, by increasing the height of the supporting walls accordingly. A transportable, collapsible gantry with a lever action pull lift and metal yoke, which can be assembled and disassembled in the mobile laboratory, is used to lift the steel supporting plate and the turret. The bed is removed and a wall of 4 inch lead bricks is constructed across the monitor on one side of the turret, a similar wall on a moveable trolley being wheeled into position, behind the patient in the tilting - chair, on the opposite side of the

turret. The top of the patient's head and eyes are not enclosed, thus helping to avoid claustrophobic reactions. The geometry is shown diagrammatically in Fig. 6 and Fig. 7 illustrates the monitor with the gantry in position. The tilting - chair geometry was originally developed by MARINELLI (1957) and Miller (1956) in 1956.

By cutting a tapered slot across the steel supporting plate and by using a collimated detector of smaller volume than the 11½ inches diameter x 4 inches crystal, the facility of lateral scanning and individual organ counting is achieved.

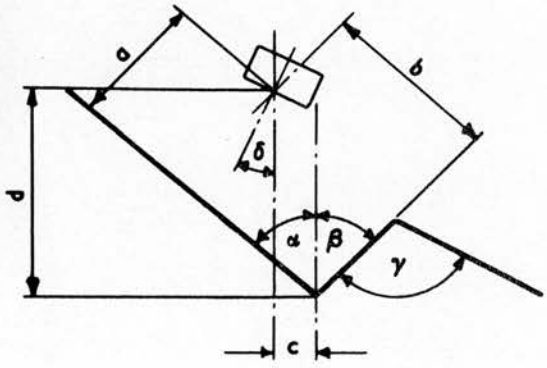
PERFORMANCE OF MERLIN.

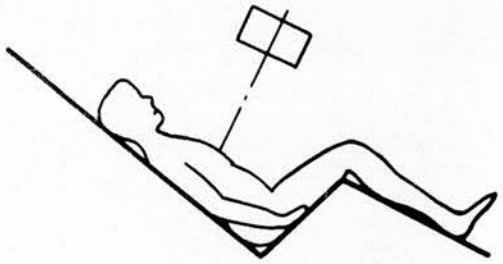
The Cs^{137} resolution and the Co^{60} peak: valley ratio were calculated for the 11½ inches diameter x 4 inches sodium iodide detector used in MERLIN and results of 9.27 per cent and 3.04 respectively were obtained.

By using the Background Indices, a comparison of the performance of MERLIN compared with that of other whole-body monitors in Britain has bee n made(BODDY, 1967 ,B). Background Indices, the counting rates over certain energy ranges per unit crystal volume, were calculated from the information given in the I.A.E.A. Directory of Whole-Body

Fig. 6.

MERLIN TILTING - CHAIR GEOMETRY





a = b = 40·4 cm
c = ZERO
d = 57·2 cm

α = β = 45°
γ = 45°
δ = ZERO

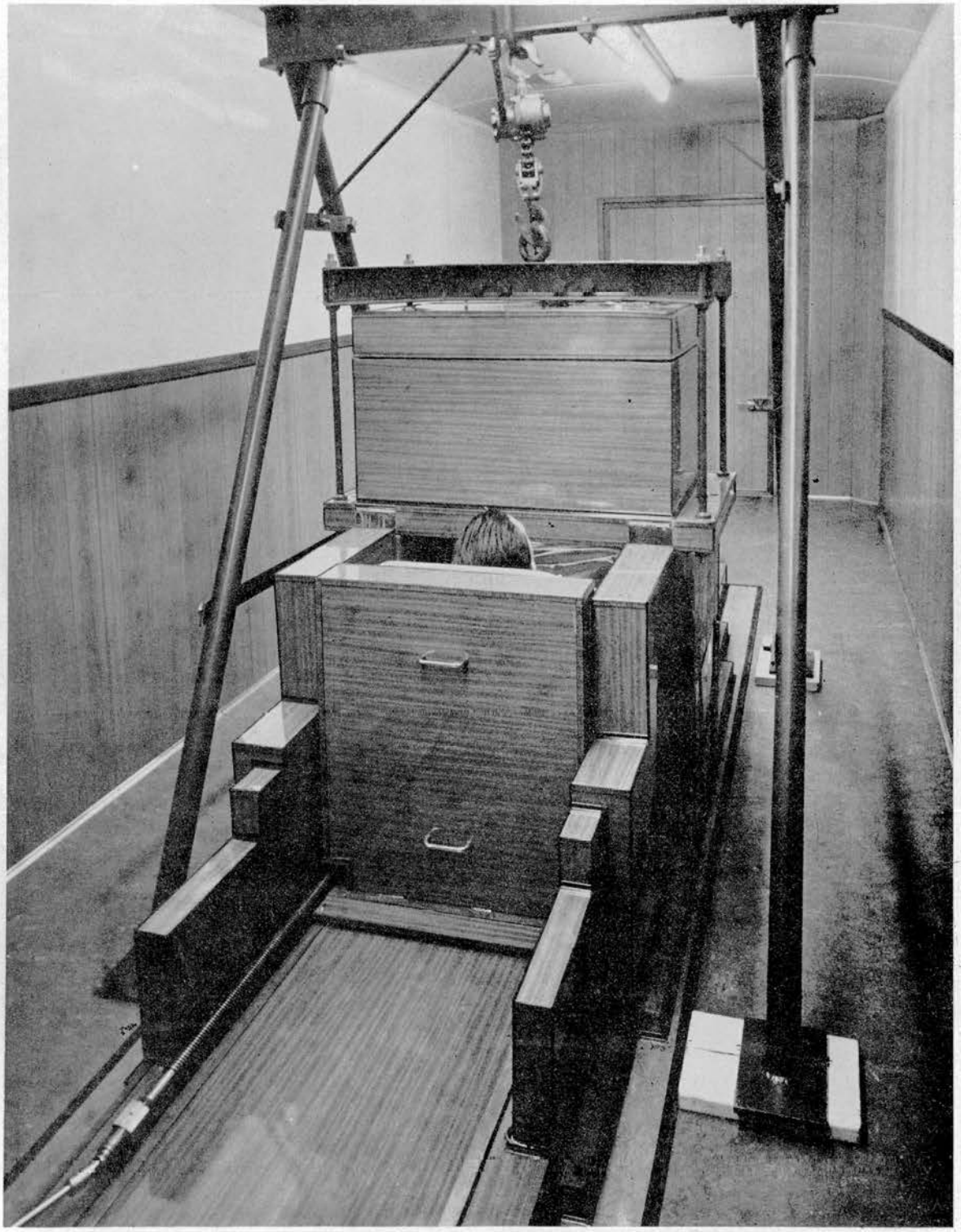


Fig. 7. MERLIN Chair Geometry
with Lifting Gantry

Radioactivity Monitors (1964) and from the data obtained from GLASS, POVER and BURKINSHAW (private communication). The results for both the higher and lower energy ranges are presented in Table II. It can be seen that the MERLIN shield is more effective than the shield of the other monitors including steel - rooms except for the Leeds and perhaps the Birmingham monitors and the lead room at Harwell. The location of the Leeds monitor contributes to its excellent performance. It is sited in a basement with a 30 inch block of concrete and a four - storey building above it, this being equilavent to at least 5 feet of concrete shielding. This comparison is not unequivocal as obviously the intrinsic background of the detector assembly will contribute to the total background counting-rate. Since, however, they are all high sensitivity monitors, with extensive steel or lead shielding, it would seem logical to assume that low background detectors would be used, and therefore that the intrinsic cpm per cc of all the detectors will be approximately constant. The results quoted for MERLIN were obtained with the monitor situated 50 yards from a 100 kW Reactor and 100 MeV Electron Linear Accelerator.

An indication of the monitor's sensitivity for iron - 59, cobalt - 58 and potassium - 40 is presented in Table III.

TABLE II

Comparison of Background Indices (cpm/cc) x 10^{-2}

MONITOR LOCATION	a) Higher Energy Range.			b) Lower Energy Range.		
	ENERGY range (MeV)	Monitor Background Index (cpm/cc)x 10^{-2}	MERLIN Background Index (cpm/cc)x10^{-2}	Energy range (Mev)	Monitor Background Index (cpm/cc)x10^{-2}	MERLIN Background Index (cpm/cc)x10^{-2}
R.P.S. Sutton C.L.	1.37-1.55	4.99	1.52	0.6-0.73	18.0	2.03
A.E.E. Windscale S.	1.295-1.625	2.99	2.48	0.52-0.80	7.88	5.88
A.E.E. Winfrith S.	1.28 -1.78	3.71	3.21	0.58-0.81	6.36	4.45
A.E.E. Dounreay S.	1.27 -1.65	4.66	2.75	0.51-0.86	13.3	7.12
Univ. of Birmingham S.	1.27 -1.61	2.60	2.58	0.44-0.58	5.28	4.51
A.E.R.E. Harwell L. -multiple	1.0 -1.4	3.35	3.74	0.32-0.4	3.48	3.03
-single	do.	3.79		do.	2.94	
Addenbrookes Hosp. Cambridge S.	0.95-1.4	5.03	3.87	0.27-0.45	12.9	6.88
Radcliffe Inf. Oxford L^x	0.4 -1.5	28.0	17.0	0.17-0.44	32.9	12.1
Royal Marsden Hosp. Sutton C.L.	0.4 -1.5	76.5	17.0	0.17-0.44	59.9	12.1
General Inf. Leeds S.L.	0.1 -2.0	13.9	38.0	0.27-0.45	2.00	6.88

Shield Key: C - Chalk Room
L - Lead Room
S - Steel Room
L^x - Lead Shadow-Shield

TABLE III.

Approximate performance data for MERLIN for scan on Patient[+] or phantom[*].

Isotope	Energy range MeV	cpm/μc	Background cpm
1. Detector at max. height:			
K-40[*]	1.36 - 1.56	0.66	126
Fe-59[+]	0.4 - 1.38	14,800	1055
	1.0 - 1.38	6,900	238
Co-58[+]	0.3 - 1.0	23,000	1104
	0.74 - 0.39	8,500	153
2. Detector in low position:			
K-40[*]	1.36 - 1.56	0.92	137
Fe-59[+]	0.4 - 1.38	27,900	1150
	1.0 - 1.38	13,300	260
3. Tilting-chair geometry:			
K-40[*]	1.36 - 1.56	1.17	133

[*] cpm/gm K. in phantom

The measurements for iron - 59 and cobalt - 58 were made on patients and for potassium - 40 on a phantom containing 140 gms of potassium. The phantom was scanned with the detector in both the "high" and "low" positions as well as being counted using the tilting - chair geometry. An approximate comparison of the performance of MERLIN with that of other whole-body monitors in the measurement of body potassium is made in Table IV. The total body content of potassium is assumed to be 140 gms and the total counting time to be 100 minutes, which is optimally divided between the subject and the background measurements. The monitoring data is taken from the I.A.E.A. Directory of Whole-Body Radioactivity Monitors (1964) and from the information obtained from NEWTON and BURKINSHAW (private communication).

The tilting - chair geometry was found to be useful for the measurement of approximately uniformly distributed radioisotopes, such as potassium and caesium - 137 (MILLER, 1959). However, this method has proved to be less useful for those non-uniformly distributed as reported by CEDERQUIST and LIDEN (1962) and PRICE et al. (1962) and other authors. In view of these findings, the chair geometry will generally be used only for monitoring body

TABLE IV.

Monitor Location	Background cpm	cpm per gmK	Subject cpm per 140 gm K	Standard error %
R.P.S., Sutton	90	0.20	28	7.3
Royal Marsden Hosp., Sutton	83	0.11	15.4	12.4
A.E.R.E., Dounreay	220	0.95	133	2.53
A.E.E., Winfrith	175	0.90	126	2.43
A.E.E., Windscale	141	0.872	122	2.30
General Infirmary, Leeds	80	1.33	186	1.36
A.E.R.E., Harwell	138	1.36	190	1.57
MERLIN - scanning	137	0.92	115	2.40
- chair	133	1.17	164	1.75

potassium.

The distribution of a tracer within a patient's body may vary considerably during the period of a study. Intravenously injected nuclides, for example, are often initially rather uniformly distributed in the circulatory system. They then may clear rapidly from circulation, accumulating in particular organs of the body. In order to determine the absolute body activity, regardless of its distribution in the body, it is desirable to have a detector arrangement with a counting efficiency that is independent of the site of the nuclide in the body. In MERLIN, when the redistribution of an administered isotope in the patient's body might significantly alter the counting - rate, the scan commences with the patient's head 18 inches out from the crystal centre and ends with the patient's feet the same distance beyond the crystal centre, the detector being used in the "high" position. Typical results obtained in patients following oral administration of iron - 59 in tablet form are given in Table V.

In this series, another patient was scanned immediately after administration of the radioiron and then at hourly intervals up to 6 hours post ingestion.

TABLE V.

Preliminary examination of redistribution effects in patients following oral administration of iron - 59 in tablet form.

Patient 1. Detector at maximum height.		Patient 2. Detector in low position	
Time after Administration	Retention	Time after Administration	Retention
0 hours	100%	0 hours	100%
1 hour	98.5%	1 hour	97.7%
2 hours	103.1%	2 hours	97.6%
3 hours	105.9%	3 hours	102.0%
4½ hours	104.6%	4 hours	98.2%
5½ hours	103.5%	5½hours	96.0%
6½ hours	103.5%	6½ hours	93.0%

The standard deviation in each of the above percentages based only on the counting statistics is about 0.3 per cent.

The total error on these results was 3.7 per cent. The detector was in the "high" position and the scan began with the patient's head 18 inches out from the centre of the crystal and overran the same distance. The patient was 5 feet 10 inches in height and 14 stones 10 pounds in weight, hence not a subject in which the redistribution effects would be expected to be minimal. By using the following formula an analysis of the total error was obtained:

$$\sigma_T^2 = \sigma_R^2 + \sigma_{LT+P}^2 + \sigma_S^2$$

where σ_T is the total error

σ_R is the redistributional error

σ_{LT} is the live - time error

σ_P is the positional error

and σ_S is the statistical error.

The positional error, which also includes a statistical and live - time error, was calculated on six repetative scans on the same patient. These measurements were made 70 days after the administration of the radioisotope.

$$\sigma_{LT+P+S} = 1.9\%$$

$$\sigma_{LT} = 0.52\%$$

and $$\sigma_{S} = 1.8\%$$

The statistical error, 1.8 per cent, was higher than that for the initial measurements, 0.45 per cent, owing to a decrease in the subject's counting rate. The positional error may be calculated using the formula

$$\sigma^2_{LT+P+S} = \sigma^2_{LT} + \sigma^2_{P} + \sigma^2_{S}$$

$$\therefore \quad \sigma^2_{P} = 0.1 \quad \text{and} \quad \sigma_{P} = 0.32\%$$

Where $$\sigma_{T} = 3.7\%$$

$$\sigma_{LT+P} = 0.61\%$$

and $$\sigma_{S} = 0.45\%$$

$$3.7^2 = \sigma^2_{R} + 0.61^2 + 0.45^2$$

$$\therefore \quad \sigma^2_{R} = 13.12 \quad \text{and} \quad \sigma_{R} = 3.62\%$$

Hence the total error of 3.7 per cent comprises

a live - time error of 0.52 per cent

a redistributional error of 3.62 per cent

a positional error of 0.32 per cent

and a statistical error of 0.45 per cent.

In order to obtain a more realistic idea of how much the counting efficiency varies with activity in different parts of the body, a direct approach to the problem was made by means of phantom studies. An Alderson phantom with a human skelton and compartments simulating various organs of the body were filled with dilute solutions of iron - 59. The crystal was used in the "high" position and both supine and prone measurements were made on the phantom, the scans starting with the head and ending with the feet directly beneath the detector. The results are presented in Table VI. In the whole-body measurement, the iron - 59 was uniformly distributed in the phantom.

The maximum variation in the counting rate observed with the radioiron in the seven compartments studied and in the whole body was about $\pm$ 4 per cent with a standard deviation of 3.0 per cent. It is unlikely, however, that redistribution of an isotope occurring in a patient would be as extreme as that studied.

A similar study was made by NAVERSTEN (1964) using the Alderson phantom to determine the differences in response for the scanning - bed and tilting - chair geometries in an iron room. In the latter geometry, two 5 inches diameter x 4 inches sodium iodide detectors are employed with a distance

TABLE VI.

ORGAN	NET COUNTING-RATE (cpm)
STOMACH and INTESTINE	14,450
LIVER	13,808
KIDNEY	13,530
SPLEEN	13,323
BLADDER	13,396
PANCREAS	14,026
THYROID	13,925
WHOLE BODY	14,470

of 45 cms between the bed and the face of the upper crystal and 70 cms between the faces of the crystals. With calcium - 47 in the bladder, the liver and then uniformly distributed in the phantom, a maximum variation of ± 6.1 per cent was observed and a standard deviation of 5.3 per cent. The results for MERLIN compare favourably with those obtained by NAVERSTEN, and it should be noted that no lateral organs, for example the spleen, and surface organs such as the kidneys were included in his measurements.

If to-wards the end of a study on a patient a higher counting sensitivity is required, the crystal may be changed from the "high" to the "low" position provided that inter-calibration measurements are obtained.

SUMMARY.

A high sensitivity shadow - shield monitor incorporated in a mobile laboratory was completed in July, 1966. It seems unlikely that every Scottish hospital which requires a high sensitivity monitor shall be able to obtain one in the foreseeable future. Hence, despite obvious shortcomings, a mobile unit can meet at least part of the requirements of several hospitals.

It was predicted from the performance of the prototype monitor that sensitivities at least comparable with those of steel - room monitors should be attainable with this design. This has been confirmed by the results for MERLIN which show that its sensitivity is better than that of some existing conventional monitors.

By adjusting the detector - bed distance, a very convenient facility for the reduction of redistribution effects is obtained without ultimate loss of sensitivity.

The introduction of a variable shield distribution to produce the tilting - chair geometry enables patients who cannot lie flat to be accommodated.

Redistribution effects described using the Alderson phantom and in patients following the oral administration of iron - 59 have not exceeded $\pm$ 7 per cent.

CHAPTER III.

A STUDY OF PHOSPHOROUS - 32 THERAPY IN PATIENTS WITH POLYCYTHAEMIA RUBRA VERA.

INTRODUCTION:

Polycythaemia rubra vera is characterised by hyperplasia of the erythroblastic, leucoblastic and megakaryocytic tissue of the bone marrow. The clinical picture is that of a greatly increased number of red blood cells in the peripheral circulation. Exhaustion of the marrow cells may occur, leading to aplastic anaemia, or the leucoblastic tissue may continue to proliferate as the erythroblasts die out, resulting in leukaemia.

A common treatment is that of an intravenous injection of an inorganic phosphate labelled with phosphorous - 32 in a dosage of 50 μCi to 100 μCi per kilogram of body weight, (VEAL and VETTER, 1958). Phosphorous - 32 is a beta emitting nuclide with a physical half-life of 14.3 days and a maximum beta energy of 1.71 MeV. The critical organ for the inorganic phosphate is bone, where it becomes incorporated in the rapidly dividing marrow cells. After a therapeutic dose of phosphorous - 32 there is usually a period of very satisfactory clinical remission of up to 2 years, as the production of red cells is depressed by the resulting dose

of radiation.

Treatment, however, is not effective in every case. One possible explanation of this could be a biological variation in the percentage retention of phosphorous - 32 and its rate of excretion. It is, therefore, of some importance to see how the excretion patterns vary. Another factor which could influence the effectiveness of the treatment is the distribution of the isotope in the skeleton. This may differ from one patient to another, but could not be investigated in the present study. VEAL and VETTER (1958) recommend that the same dose should be repeated in a patient who does not respond to treatment within 3 months. If it is suspected that the patient may haemorrhage or there is a possibility of a venous thrombosis, venesection of a few pints of blood should alleviate this danger.

The use of chemotherapeutic agents is an alternative method of treatment, resulting in drug - induced bone marrow depression.

LIDEN (1958) described experiments to optimise the external counting of phosphorous - 32 in vivo and showed that thin sodium iodide detectors were most effective. PALMER (1962 and 1966) reported measurements of the retention of phosphorous - 32 in parts of the body using thin crystals and proportional counters. However, using a whole-body

monitor with conventional detectors, as in routine use, causes least disturbance of other clinical investigations simultaneously requiring the monitor.

THEORETICAL CONSIDERATIONS:

For simplicity, and in view of the limited amount of published data, it may be permissible to describe the excretion of phosphorous - 32 by a single exponential term starting 24 hours post - administration.

The tissue dose - rate is then given simply by $D = D_o e^{-\lambda t}$ and the integrated dose by $D = D_o/\lambda$ where D_o, the initial dose - rate, is determined by the excretion during the first 24 hours and λ is the effective decay content.

Now $\lambda = \lambda$ physical + λ biological

For phosphorous - 32, λ physical = 0.05 days^{-1} approximately.

The effect on the integrated dose of the biological decay constant has been calculated and is given in Table I. If the range of the biological decay constant is from 0.01 to 0.05 days^{-1} the dose is reduced by a factor of up to 1.7 times. On the other hand, if the range is from 0.05 to 0.25 days^{-1} the dose is reduced by a factor of 3. The magnitude of the biological decay constant is, therefore, at least as important as the variation between individuals.

TABLE I.

Effect of biological decay constant on integrated dose.

Biological days $^{-1}$	Dose as per cent of dose due only to radioactive decay
0	100
0.01	83
0.02	71
0.03	63
0.05	50
0.10	33
0.25	17

MATERIALS AND METHOD.

Studies have been made on 3 patients; W.F. and J.M., both males, and E.M., female. Pre - administration measurements were made on each subject to determine the individual spectrum due to potassium - 40 and caesium - 137 already present in the body. Two energy ranges were chosen, somewhat arbitrarily, for the measurement of phosphorous - 32 in vivo, 0.16 - 0.27 MeV and 0.51 - 0.69 MeV, the lower range being close to the limit of non - linearity in the analyser and the higher range corresponding roughly to the energy of the maximum number of particles in the phosphorous - 32 beta spectrum.

In all cases, the phosphorous - 32 was administered intravenously. W.F. had a therapeutic dose of 3.25 mCi of phosphorous - 32 and a year later, a tracer dose of 50μCi was administered in the same way. This was to enable a detailed study of urinary and faecal losses, and loss rates to be made, as it was suspected at that time that the period of remission was shorter than anticipated after the therapeutic dose. Patient J.M. had 4 mCi and E.M. had 0.25 mCi followed in one year by 2.7 mCi of the isotope.

Phosphorous - 32 can be detected in vivo with a thallium - activated sodium iodide crystal by counting the Bremsstrahlung

resulting from the beta emission. These measurements were made using the prototype shadow-shield monitor described in Chapter I, and the total body content of the phosphorous - 32 estimated. The subjects were scanned in both the supine and prone positions. From the sum of the two counts, the whole-body retention figures were calculated for each energy range, and the mean obtained. Only three whole body measurements of patient J.M. could be made.

After W.F.'s second tracer dose of phosphorous - 32, 24 hour urine collections were made over a period of 5 days and over 3 days for faecal losses. On patient J.M. a 7 day urine collection was made. The total phosphorous content of the urine samples was obtained using liquid Geiger - Müller tube of 10 mls capacity, which was housed in a lead castle to reduce the background counting rate. The urine was untreated. The faecal samples were placed directly beneath the detector of the whole-body monitor and an estimation of their phosphorous - 32 content was obtained by counting a prepared standard source in the same geometry.

Patient W.F. had 540 mls of blood removed 4 days after the injection of the tracer dose. This was treated in the same way as the urine samples, and the amount of the isotope still circulating in the blood determined.

RESULTS.

The data obtained from the tracer doses of phosphorous - 32 on patients W.F. (50 μCi) and E.M. (250 μCi) are presented in Table II and Fig. I and from the therapeutic doses on W. F. (3.25 mCi), E.M. (2.7 mCi) and J.M. (4 mCi) in Table III. Fig. II illustrates graphically the whole-body retention after the therapeutic dose in W.F. and E.M.

From Table II and Fig I it can be seen that, in both cases, approximately 10 per cent of the phosphorous - 32 was lost in the first 24 hours after administration, whereas with E.M. (Table III and Fig.II), over the same period, there was a 19 per cent loss. In these two patients, by the 3rd and 4th day after injection of both the tracer and the therapeutic doses, there was about 80 per cent retention of the injected phosphorous and by the 14th or 15th day, this had fallen to about 65 per cent. In patient J.M. (Table III) the whole-body monitor results for day 3 also showed an 80 per cent retention. There appeared to be a continuing loss of phosphorous - 32 in both sets of results on W.F.

The best straight lines through the whole-body retention percentages for W.F. and E.M. from day 1 (Table II) were calculated using the least squares method, and the two lines obtained plotted in Fig. I. This was repeated with the results from Table III for W.F. from day 3 and for E.M. from

TABLE II

	E.M.		W.F.				
Day	W.B.M. Retention (%)	Cumulative Loss from W.B.M. (%)	W.B.M. Retention (%)	Cumulative Loss from W.B.M. (%)	Collection No.	Urinary Loss (%/24 Hrs.)	Faecal Loss (%/24 Hrs.)
0	100	-	100	-			
1	91	9	88	12	1	9.0	1.4
2	-	-	-	-	2	2.6	0.9
3	-	-	-	-	3	1.0	0.9
4	79	21	81	19	4	1.1	
5	-	-	-	-	5	0.7	
6	-	-	-	-			
7	74	26	-	-			
14	67	33	-	-			
15			64	36			
22			57	43			
29			53	47			

Collection no. 1 for patient W.F. refers to the first 24 hour urine and faecal collection, no. 2, the second, etc.

FIG I WHOLE - BODY RETENTION AFTER TRACER DOSE OF PHOSPHOROUS - 32

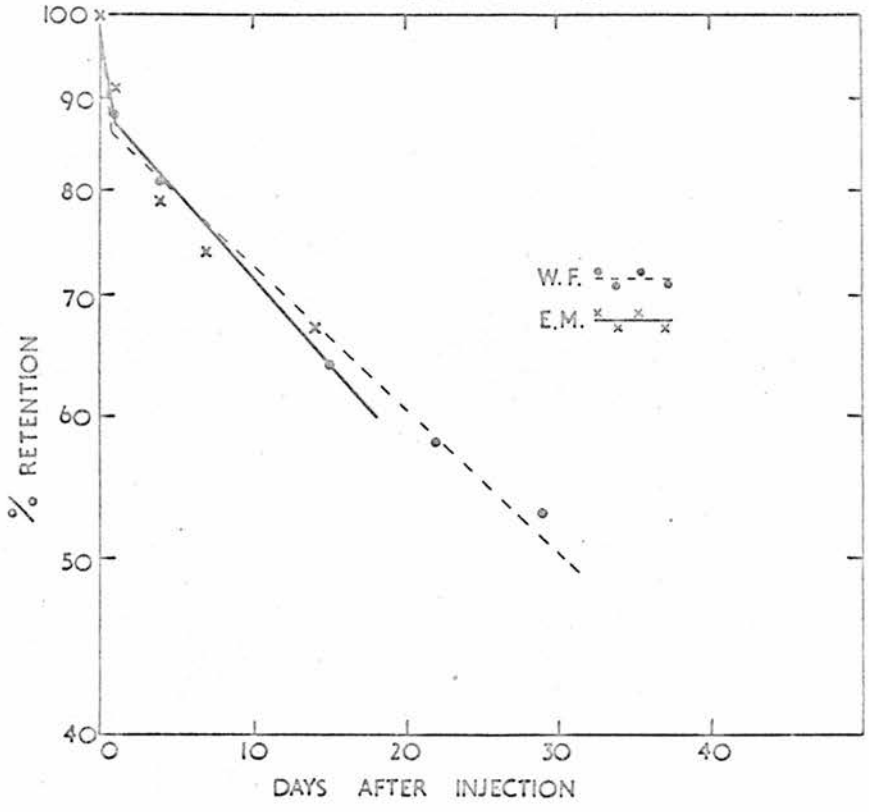


FIG II WHOLE - BODY RETENTION AFTER THERAPEUTIC DOSE OF PHOSPHOROUS - 32

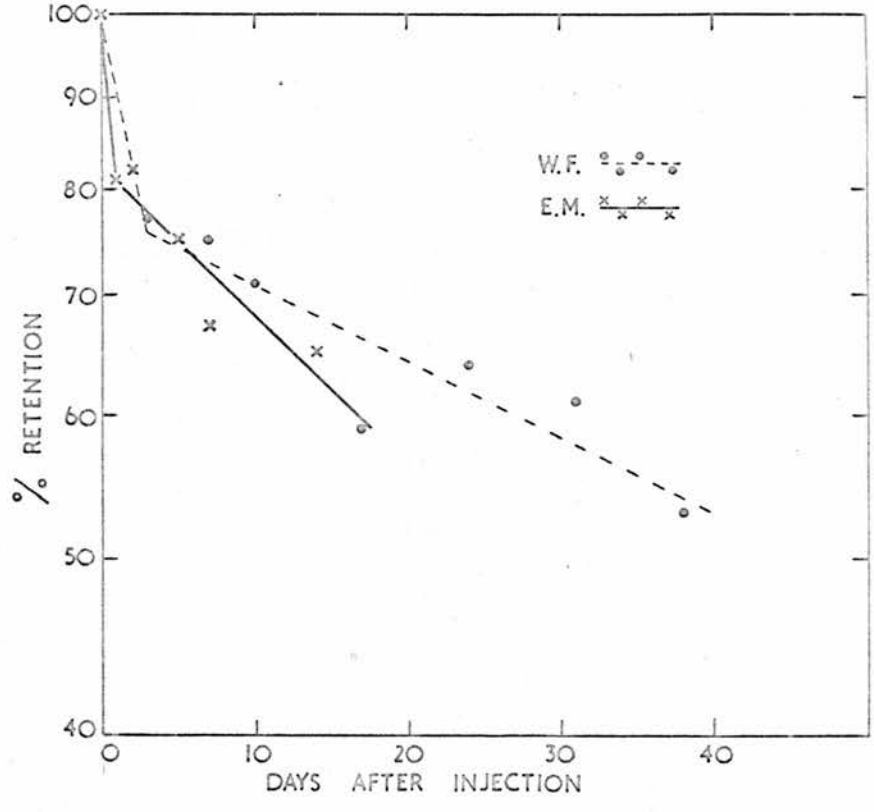


TABLE III

	W.F.		E.M.		J.M.			
Day	W.B.M. Retention (%)	Cumulative Loss from W.B.M. (%)	W.B.M. Retention (%)	Cumulative Loss from W.B.M. (%)	W.B.M. Retention (%)	Cumulative Loss from W.B.M. (%)	Collection No.	Urinary Loss (%/24 Hrs.)
0	100	-	100	-	100	-		-
1	-	-	81	19	95	5	1	19
2	-	-	82	18	-	-	2	27
3	77	23	-	-	80	20	3	36
4	-	-	-	-			4	42
5	-	-	75	25			5	48
6	-	-	-	-			6	51
7	75	25	67	33			7	57
10	71	29	-	-				
14	-	-	65	35				
17	59	41	-	-				
24	64	36	-	-				
31	61	39	-	-				
38	53	47	-	-				

day 1 and the results obtained presented in Fig. II. The variance on the slopes of the regression lines in Fig. II were calculated using the following formula:-

$$\sigma_v^2 = \frac{\Sigma(y^2) - \frac{\Sigma(y)^2}{n} - \beta^2 \Sigma(x-\bar{x})^2}{(n-2)\Sigma(x-\bar{x})^2}$$

where σ_v^2 is the variance,

y is the log per cent retention,

n is the no. of results,

β is the regression coefficient,

x is the no. of days,

$\bar{x}$ is the mean of the no. of days

and $(n-2)$ is the no. of degrees of freedom.

For E.M., $\sigma_v^2 = 2.96 \times 10^{-6}$

and $\sigma = 1.72 \times 10^{-3}$.

For W.F., $\sigma_v^2 = 4.53 \times 10^{-7}$

and $\sigma = 6.73 \times 10^{-4}$.

where σ is the standard deviation.

The error on the regression coefficient was obtained by expressing the standard deviation as a percentage of the coefficient.

Error on the regression coefficient for E.M. = 21.0%

and for W.F. = 16.2%.

A comparison of the regression coefficients was made and Student's t calculated to determine whether the difference in rates of loss of phosphorous - 32 was significant. The following formulae were used:-

$$r_1 = \frac{\Sigma (x-\bar{x})(y-\bar{y})}{\sqrt{(\Sigma (x-\bar{x})^2 \Sigma (y-\bar{y})^2)}}$$

where r_1 is the correlation coefficient for E.M.

Similarly for r_2, where r_2 is the correlation coefficient for W.F.

$$\sigma^2_{r_1} = (1-r_1^2)\left(\frac{\Sigma (y-\bar{y})^2}{n-2}\right)$$

where $\sigma^2_{r_1}$ is the residual variance about regression line for E.M.

Similarly for $\sigma^2_{r_2}$ where $\sigma^2_{r_2}$ is the residual variance about regression line for W.F.

$$\sigma^2_w = \frac{(n_1-2)\ \sigma^2_{r_1} + (n_2-2)\ \sigma^2_{r_2})}{(n_1 + n_2)}$$

where σ^2_w is the weighted average residual variance,

n_1 is the no. of results for E.M.

and n_2 is the no. of results for W.F.

$$\sigma_1^2 = \frac{\sigma_w^2}{\Sigma(x-\bar{x})^2}$$

where σ_1^2 is the weighted variance of regression coefficient for E.M.

Similarly for σ_2^2 where σ_2^2 is the weighted variance of regression coefficient for W.F.

$$\sigma_{12}^2 = \frac{\sigma_1^2}{\Sigma(x-\bar{x})^2} + \frac{\sigma_2^2}{\Sigma(x-\bar{x})^2}$$

where σ_{12}^2 is the variance of their difference.

$$t = \frac{m_1 - m_2}{\sigma_{12}}$$

where m_1 is the regression coefficient for E.M.

and m_2 is the regression coefficient for W.F.

$$t = 1.96$$

Degrees of freedon for this $t = ((n_1-2) + (n_2-2))$

$= 8.$

The urinary loss figures in patient J.M. were considerably higher than those detected by whole-body monitoring. This is the converse of what one would expect as incomplete collections often result in an underestimation of the amount of radioisotope excreted. Spectra from the whole-body measurements and urine samples and standard were examined for contaminent peaks such as iodine - 131 or chromium - 51, high background counting rates due to interference from the 100 kW reactor or the 100 MeV linear accelerator which are situated about 50 yards from the counting room, and any errors in dead-time corrections. Since no possible cause of the discrepancy in the results was found, they were included in Table III. However, the whole-body monitoring results are almost certainly more reliable, since there were difficulties about this time in obtaining accurately calibrated standard sources.

The blood content of injected phosphorous - 32 in patient W.F. was estimated as follows:-

Corrected cpm / 10 mls blood = 548

cpm / ml blood = 54.8

cpm / μCi standard = 22.6×10^4

$$\therefore \quad \mu Ci/ml = \frac{54.8}{22.6 \times 10^4}$$

$$= 24.2 \times 10^{-5}$$

Assuming 65 mls of blood / kilogram body weight, the total blood volume = 4550 mls.

Total blood content of phosphorous - 32 = $4550 \times 24.2 \times 10^{-5}$ μCi

= 1.1 μCi.

$$\% \text{ of test dose} = \frac{1.1 \times 100}{50}$$

$$= 2.2$$

% of total body content on day 4

$$= \frac{1.1 \times 100}{40.5}$$

$$= 2.7.$$

DISCUSSION.

There are certain difficulties in counting Bremsstrahlung by this method, resulting in a decreased counting efficiency. In patient W.F. however, the whole-body monitoring results and those from monitoring the urine and faeces (Table II) were in excellent agreement up to the end of the collection period; 19 per cent and 17 per cent respectively, which suggests that the technique is satisfactory.

The percentage losses after both tracer and therapeutic doses of phosphorous - 32 were similar except over the initial 24 hours. It is reported that there are 370 μgms of phosphorous per gm of whole blood, (BOWEN and CAWSE, 1963).

The injections that the patients received contained 1 mg of carrier per mCi of phosphorous - 32. It would therefore seem that this amount of carrier was insufficient to saturate the absorption mechanism. The initial losses, however, vary from 5 per cent to 20 per cent, which would appear to be due to the biological variation in absorption. In the calculation to determine whether the rates of loss of phosphorous - 32 in W.F. and E. M. after the injection of the isotope at the therapeutic dose level were significantly different, a value of 1.96 for Student's t was obtained. By examining the table of the significant values of t, it can be seen that for infinite degrees of freedom a value of t as large as 1.96 should only occur 1 in 20 times, ($P = 0.05$). Here, the degrees of freedom are 8, and for the 5 per cent level of significance a value of t of 2.31 would be needed. The present value of 1.96 is less than the value for the 5 per cent level but is greater than the value 1.86 for the 10 per cent level, ($0.1 > P > 0.05$). The significance of the present result is thus between 10 per cent and 5 percent and it can be concluded that there is no difference between excretion rates in these two patients. If the value of t had been a little larger, however, there would almost certainly have been a genuine difference in the loss

rates. EAKINS et al (1966) reported urinary loss figures up to day 20 (Table IV) on a patient with Gaucher's disease who had 3 mCi of phosphorous - 32 containing 3 mgs of carrier administered intravenously. By comparing the results obtained for the above patient with those for E.M. and W.F. (Table III), it can be seen that the two latter cases excreted the phosphorous - 32 at approximately twice the rate of the former. This would indicate that in the case of the patient with Gaucher's disease the treatment should be proportionally more effective. It has been shown that following parenteral injections of labelled Vitamin B_{12} the amount excreted in the urine varies widely, (Table V). The range of results may be different not only from patient to patient, but in the same patient at different times. If a similar biological variation applied to the percentage retention of the administered phosphorous - 32 then it could account for the fact that the period of remission varies from one case to another. In view of the limited data, further investigations are needed to determine the extent of the variation in retention which, if considerable, could be the reason for the treatment being ineffective in some patients.

ERF et al (1941) reported that normal individuals excrete radiophosphorous more rapidly than patients with

TABLE IV.

Percentage of intake of phosphorous - 32 excreted in urine.

Day	Cumulative urinary loss (%).
1	3.0
2.	4.4
3	5.5
4	6.8
5	7.9
6	8.7
7	9.7
8	10.6
9	11.8
10	13.1
11	14.4
12	15.6
13	16.9
14	17.8
15	18.3
16	18.9
17	19.4
18	20.0
19	20.8
20	21.3

TABLE V.

Estimates of the amount of vitamin B_{12} excreted in urine following parenteral injection of 50 µgms.

Parenteral dose µgms.	Amount excreted % dose.
50	6.4 - 8.2 (CHESTERMAN et al. 1951). 16.4 -22.0 (LANG et al. 1953). 8.8 - 29.4 (WATKIN et al. 1953). 7.6 - 28.0 (ESTRADA et al 1954). 14.5 - 31.5 (UNGLAUB et al 1954). 1.0 - 76.0 (BAKER et al. 1956). 9.8 - 19.5 (SHEELY et al. 1957).

leukaemia during the first 48 hours after administration due to the concentration of the isotope in the rapidly dividing leukaemic cells. This should also be the case in patients with polycythaemia where a higher percentage of the phosphorous - 32 will become incorporated in the bone marrow cells. In patients W.F., E.M. and J.M. (Table III) there would appear to be less than 20 per cent of the initial dose excreted in the first 48 hours, whereas the results of ERF et al.(1941) on two normals indicate a 32 per cent loss over the same period (Table VI).

From Table II, patient W.F., it can be seen that in the 24 hours after administration, 90 per cent of the excreted phosphorous - 32 was in the urine. From experiments with rats, ELLIS et al.(1951) found that after intra-cardiac injection of phosphorous - 32, a large percentage was excreted in the urine in the first 48 hours. After that time, however, they found that no phosphorous could be detected. The percentage urinary loss per 24 hours for patient W.F. over five days does not appear to be in agreement with this finding.

ERF et al.(1941) reported that after intravenous injection of labelled phosphate, faecal excretion is less than 2 per cent of the amount absorbed. This is in accordance with the results for patient W.F. who excreted about 1.6 per

TABLE VI.

Cumulative percentage of intravenously administered phosphorous - 32 excreted in urine.

No. of individuals.

Day	2 (normals) (ERF et al., 1941).
1	27.2 (27.3)*
2	31.5 (31.9)*
3	33.5 (34.0)*
4	35.1 (35.7)*
5	36.6 (37.2)*
6	37.7 (38.3)*

* Figures in brackets are for total excretion, including faeces.

cent in the first 24 hour faecal collection.

There is apparently little data on excretion beyond 6 days post - injection and yet there would appear to be a continuing loss shown by both sets of results for W.F. and by those for the patient reported by EAKINS et al. (1966),(Table IV). After this period of time, it is unlikely in the case of W.F. to be an artefact due to redistribution of the isotope, if, as is suggested, labelled phosphorous in blood is turned over with a half - life of about a day.

In patient W.F., 1.1 μCi of phosphorous - 32 was circulating in the blood on day 4, 2.7 per cent of the total body content on that day, or 2.2 per cent of the initial dose. This is in good agreement with the findings of EAKINS et al. (1966) who reported a rapid clearance of the isotope from the circulatory system, only 4 per cent remaining after first day.

SUMMARY.

The range of initial retention and the excretion rates obtained in the present study suggest that the physical decay constant and the initial retention of phosphorous - 32 are of principal importance in determining the radiation dose.

Biological variation in the absorption may account for

the differing periods of remission and possibly the ineffectiveness of treatment in some patients.

The majority of the administered dose is lost in the urine, facael excretion being less than 2 per cent of the amount absorbed, at least during the initial 24 hour period.

There is strong evidence, particularly for patient W.F., that significant losses continue even after 4 or 5 days.

Studies are continuing in further patients and in the patients so far investigated to determine whether the period of remission is different in these cases.

CHAPTER IV.

THE MEASUREMENT OF TOTAL BODY POTASSIUM.

INTRODUCTION.

Potassium is largely an intracellular ion and the extent to which it may be lacking is not easily measured. The plasma concentration of potassium is controlled by a number of factors in addition to the amount of potassium in the body, and although deficiency is often accompanied by a low level in the plasma, the relationship between the degree of deficiency and the plasma level is variable. The use of a whole-body monitor facilitates an accurate estimate of total body potassium to be made, upon which a precise diagnosis may depend.

The commonest cause of potassium deficiency in ambulant patients is the prolonged use of diuretics, which increase the amount of sodium reaching the distal tubules, where it is available for exchange with the potassium and hence increasing its excretion. If associated with this, there is a low intake of potassium, deficiency will develop. A practical problem is to decide which patients on these drugs require treatment with a potassium supplement. This would be indicated by a total body potassium measurement. Diuretics are often given for the treatment of patients with congestive cardiac failure.

These subjects would be unable to lie flat and would be monitored using the tilting - chair geometry.

A number of reports have appeared in recent years implying a constant relationship between body potassium content and a fundamental component of body composition described by BEHNKE (1941-42) as "lean body mass". Since this mass is largely potassium - rich muscle, its measurement is of considerable interest in muscle disease. It has been shown (BLAHD, 1965) that patients with muscular dystrophy have severe depression of body potassium concentration and, in general, that the level of body potassium was correlated with the severity of the muscular disease.

Since the colon can excrete potassium up to a concentration of 30 m Eq / litre (WYNN, 1965), chronic diarrhoea can lead to potassium depletion. Another cause may be that of repeated vomiting over a long period, due to potassium in the vomit and to resulting alkalosis necessitating loss of potassium in the urine. Excessive urinary losses may also be the result of pyelonephritis, a variety of congenital renal tubular defects and prolonged osmotic diuresis.

Hence it can be seen that there are numerous and varied causes of potassium deficiency and conditions where a total body potassium estimation would be of clinical value.

Any measurement of the radioactive content of a human, regardless of the type of whole-body counter, will contain an inherent uncertainty because internal absorption and scattering of gamma rays is inevitably greater in some subjects than in others. The magnitude of this inherent uncertainty will depend on the type of counting system, because body - build and the location of the isotope within the body will make more difference in some systems than in others.

Potassium - 40, with an abundance of 0.0119 per cent and a half-life of 1.25×10^9 years, is a naturally occurring radioisotope. The potassium - 40 activity in the human body amounts to about 10^{-8} Ci of gamma radiation of energy 1.46 MeV, but varies appreciably with the body - build because of its localised distribution in muscles. In the measurement of total body potassium, very precise calibration can be made with the artificial isotope potassium - 42, which emits a single gamma - ray of almost the same energy (1.51 MeV) as that of the potassium - 40 radiation and, very conveniently, has a short half-life of 12.45 hours. The amount of potassium - 42 administered orally - a few μCi resulting in a tissue dose of only a few mrad - attains a distribution very similar to that of the normal body potassium and produces an instrument response which can be used to allow for self- absorption of the

radiation and for body geometry. A comparison is made of the potassium - 42 response with that from a known amount of natural potassium using a phantom man.

While utilising MERLIN to measure potassium - 40 in a study of the effects of the drug Biogastrone on body potassium levels, an assessment was made of MERLIN'S performance and of the difficulties involved in the potassium - 42 technique.

1. PERFORMANCE OF MERLIN.

Total body potassium measurements have been made on 54 patients using the scanning bed geometry, with the detector in the "low" position. For a patient of 5 feet, the potassium - 40 scanning time was (2x18.65) minutes and the potassium - 42 (2x3.73) minutes.

After a background potassium - 40 measurement, the patients were given an oral dose of potassium - 42 of between 9 and 11 μCi. Approximately 24 hours after administration, the patients were scanned and an estimation of cpm / μCi of potassium - 42 obtained, appropriate corrections for radioactive decay and urinary loss being made. By 24 hours, the potassium - 42 has not necessarily equilibrated with the potassium - 40, but the spatial distribution is sufficiently similar to permit it to serve as a standard, (DELWAIDE et al., 1962). A polythene

phantom man was scanned containing 300 gms of potassium in the form of potassium chloride and then containing approximately 2.5 μCi of potassium - 42, this being the level of activity in the patient's body 24 hours after administration. The patient's total body potassium content is then calculated using the following equation.

$$K(\text{gms}) = A \times \frac{B}{C} \times \frac{D}{E}$$

where, for a given energy band,

A is cpm from K^{40} in the patient,

B is the mass (in gms) of potassium in the phantom,

C is cpm from K^{40} in the phantom,

E is cpm / μCi from K^{42} in the patient,

and D is cpm / μCi from K^{42} in the phantom.

Two energy bands are used, a Compton band from 0.77 to 1.27 MeV and a photopeak band from 1.36 to 1.56 MeV.

An indication of the way in which the counting - rate varies between individuals can be obtained by comparing the ratio of

$$\frac{\text{cpm / } \mu\text{Ci } K^{42} \text{ phantom}}{\text{cpm/ } \mu\text{Ci } K^{42} \text{ patient}} = \frac{D}{E} = g$$

in the photopeak and compton regions.

RESULTS.

Three visits have been made to the hospital at which this work was done, and total body potassium estimations made on 11 patients during the first visit, on 28 during the second and on 15 during the third. The results obtained are presented separately for groups I, II and III. A comparison has been made of the potassium content of the patients estimated using potassium - 42 and by using a constant calibration factor (0.9187 cpm / gm K (photopeak) and 0.6413 cpm / gm K (compton)) obtained by the measurement on the whole-body phantom containing natural potassium. The results for both the photopeak and compton energy ranges can be seen in Table I. Also included in Table I, for group I only, are the g - photopeak and g - compton values. An analysis of the deviations of the individual g values from the mean is given in Table II for groups II and III.

Table IV in Chapter II illustrates the potassium - 40 sensitivity of MERLIN. The statistical error on the potassium - 40 measurement is about 3.3 per cent for this measurement time.

DISCUSSION.

The potassium values for the first group of patients (Table I) are significantly lower using the constant factor than when using the potassium-42 calibration and the g factors are consistantly higher

TABLE I.

GROUP. I.

Subject	Gms K photo	Gms K comp.	Gms K photo	Gms K comp.	ε photo	ε comp.	Ht	Wt. kgms
	K^{42} calibration		Constant factor					
1	160	167	127	132	1.399	1.474	5'11½"	72.6
2	146	139	111	116	1.471	1.404	5'9¼"	78.9
3	130	124	109	96	1.321	1.394	5'6½"	64.3
4	99	93	84	86	1.325	1.255	5'1½"	70.3
5	191	197	138	132	1.543	1.503	6'3¼"	87.1
6	117	114	94	101	1.380	1.322	5'2½"	83.5
7	162	157	127	126	1.419	1.455	5'9¾"	71.2
8	169	160	131	131	1.446	1.436	5'11¼"	70.3
9	76	73	67	56	1.313	1.362	5'5¾"	61.7
10	187	199	140	113	1.486	1.495	6'0½"	79.4
11	117	118	96	100	1.352	1.373	5'5¾"	58.5

TABLE I. cont'd

GROUP II.

Subject	Gms K photo	Gms K comp	Mean	Gms K photo	Gms K comp	Mean
	K^{42} calibration.			Constant factor.		
1	87	84	85.5	92	97	94.5
2	99	91	95.0	92	104	98.0
3	129	114	121.5	134	123	128.5
4	115	116	115.5	112	120	116.0
5	128	123	125.5	128	127	127.5
6	133	128	130.5	123	127	125.0
7 *	159	151	155.0	137	143	140.0
8	135	144	139.5	136	147	141.5
9	160	157	158.5	153	155	154.0
10	111	95	103.0	107	97	102.0
11	135	127	131.0	131	131	131.0
12	138	134	136.0	136	142	139.0
13	130	134	132.0	119	128	123.5
14	81	75	78.0	79	81	80.0
15 *	63	59	61.0	77	71	74.0
16 *	156	145	150.5	134	134	134.0
17	101	80	90.5	99	82	90.5
18 *	87	85	86.0	95	95	95.0
19	130	112	121.0	129	111	120.0
20	102	85	93.5	103	91	97.0
21	139	125	132.0	127	122	124.5
22	89	77	83.0	83	86	84.5
23	103	91	97.0	102	110	106.0
24	65	66	65.5	75	67	71.0
25 *	76	72	74.0	83	82	82.5
26	119	122	120.5	116	131	123.5
27 *	95	77	86.0	89	62	75.5
28	100	93	96.5	94	96	95.0

TABLE I. cont'd

GROUP III.

Subject	Gms K photo	Gms K comp	Mean	Gms K photo	Gms K comp	Mean
	K^{42} Calibration.			Constant factor.		
1	140	138	139.0	136	129	132.5
2	73	74	73.5	71	71	71.0
3	93	88	90.5	88	88	88.0
4	99	104	101.5	95	102	98.5
5*	67	65	66.0	76	70	73.0
6	105	112	108.5	104	109	106.5
7	111	111	111.0	110	106	108.0
8	80	77	78.5	81	76	78.5
9	78	76	77.0	79	71	75.0
10*	164	164	164.0	131	126	128.5
11	79	71	75.0	76	70	73.0
12	96	93	94.5	92	90	91.0
13*	138	131	134.5	111	107	109.0
14	90	94	92.0	88	87	87.5
15*	145	145	145.0	126	120	123.0

TABLE II.

GROUP II.

Subject.	g photo	Δ g photo (%)	g comp	Δ g comp (%)	Avg. error (%)	Ht.	Wt. (kgms)
1	1.051	- 7.6	1.021	- 7.7	- 7.65	5'7"	49.9
2	1.198	+ 5.4	1.020	- 7.8	- 1.2	5'4"	99.8
3	1.068	- 6.1	1.081	- 2.3	- 4.2	5'6½"	61.2
4	1.148	+ 1.0	1.130	+ 2.2	+ 1.6	5'5½"	69.9
5	1.108	- 2.5	1.138	+ 2.9	+ 0.2	5'3½"	57.2
6	1.205	+ 6.0	1.180	+ 6.7	+ 6.35	5'7¼"	76.2
7	1.287	+ 13.2	1.231	+ 11.3	+ 12.25	5'11"	84.8
8	1.105	- 2.8	1.145	+ 3.5	+ 0.35	5'8"	70.4
9	1.169	+ 2.8	1.173	+ 6.1	+ 4.45	5'8"	72.1
10	1.147	+ 0.9	1.145	+ 3.5	+ 2.2	5'6"	70.8
11	1.149	+ 1.0	1.132	+ 2.3	+ 1.65	5'10"	78.0
12	1.128	- 0.8	1.105	- 0.1	- 0.45	5'6"	74.8
13	1.217	+ 7.0	1.213	+ 9.7	+ 8.35	5'10½"	73.0
14	1.148	+ 1.0	1.078	- 2.5	- 0.75	5'2½"	78.0
15	0.915	- 19.5	0.964	- 12.8	- 16.15	4'9"	39.9
16	1.299	+ 14.2	1.192	+ 7.8	+ 11.0	5'7½"	84.0
17	1.135	- 0.2	1.142	+ 3.2	+ 1.5	5'8½"	70.8
18	1.022	- 10.1	1.044	- 5.6	- 7.85	5'4"	51.5
19	1.124	- 1.1	1.187	+ 7.3	+ 3.1	5'8"	55.7
20	1.103	- 3.0	1.094	- 1.1	- 2.05	5'3"	62.1
21	1.229	+ 8.1	1.197	+ 8.2	+ 8.15	5'8"	78.5
22	1.208	+ 6.2	1.044	- 5.6	+ 0.3	5'3"	98.4
23	1.119	- 1.6	0.954	- 4.2	- 2.9	5'1"	93.4
24	0.999	- 12.1	0.999	- 9.7	- 10.9	5'2"	44.0
25	1.023	- 10.0	1.033	- 6.6	- 8.3	5'0"	39.5
26	1.142	+ 0.4	1.092	- 1.3	- 0.45	5'5½"	66.5
27	1.194	+ 5.0	1.109	+ 0.3	+ 2.65	5'7"	69.4
28	1.190	+ 4.7	1.133	+ 2.4	+ 3.55	5'10"	61.5
	Total 31.830 Mean 1.137		Total 30.981 Mean 1.106				

TABLE II. cont'd

GROUP III.

Subject	g photo	Δ g photo (%)	g comp	Δ g comp (%)	Avg. error (%)	Ht	Wt (kgms)
1	1.059	+ 0.4	1.081	+ 0.6	+ 0.5	5'8"	70.0
2	1.022	- 3.1	1.053	- 1.9	- 2.5	5'4½"	57.0
3	1.062	+ 0.7	1.002	- 6.7	- 3.0	5'2"	85.0
4	1.067	+ 1.1	1.024	- 4.6	- 1.75	5'2½"	83.5
5	0.886	- 16.0	0.925	- 13.9	- 14.95	4'10"	42.2
6	1.006	- 4.6	1.028	- 4.3	- 4.45	5'4¼"	50.8
7	1.010	- 4.3	1.045	- 2.7	- 3.5	5'5"	57.1
8	0.995	- 5.7	1.008	- 6.1	- 5.9	5'0"	50.2
9	0.986	- 6.5	1.061	- 1.2	- 3.85	5'2½"	51.0
10	1.257	+ 19.1	1.305	+ 21.5	+ 20.3	6'3¼"	87.1
11	1.029	- 2.5	1.012	- 5.8	- 4.15	4'10½"	63.5
12	1.036	- 1.8	1.032	- 3.9	- 2.85	5'3½"	61.2
13	1.244	+ 17.9	1.255	+ 16.8	+ 17.35	6'2¼"	74.1
14	1.022	- 3.1	1.082	+ 0.7	- 1.2	5'0½"	50.2
15	1.151	+ 9.1	1.200	+ 11.7	+ 10.4	5'10½"	73.0
	Total 15.832 Mean 1.055		Total 16.113 Mean 1.074				

than those in groups II and III (Table II). This is almost certainly due to errors in the dispensing of the potassium - 42 and the lower values are probably more accurate. The mean values of the remaining results, especially those of group III, are in remarkably good agreement, with the exception of the ones marked with an asterisk. The total body potassium estimations for 68 per cent of the group II subjects lie within ± 3 per cent, 90 per cent of these patients weighing from 50 to 80 kgms, and 78 per cent of the results lie within ± 4.5 per cent. In group III, 73 per cent of the results agree to ± 2.5 per cent, 82 per cent of these subjects weighing from 50 to 80 kgms. The discrepancy in some of the results appears to be associated with patients under 50 kgms and over 80 kgms in weight, and in others may possibly be connected with potassium - 42 dispensing errors. It can be seen that in patients under 50 kgms, subjects 1, 15, 24 and 25 in group II and subject 5 in group III, the use of a constant calibration factor overestimates the total body potassium. In three patients over 80 kgms, 7 and 16 in group II and 10 in group III, the total body potassium is underestimated when using the constant factor. However, there are three obese patients in group II, 2, 22 and 23, and two in group III, 3 and 4, all weighing over 80 kgms and giving results which agree to ± 4.4 per cent. Excluding subject 23, their results agree to

± 1.5 per cent. It would seem therefore that the cases in which the use of a constant factor underestimates the body potassium are the "heavy" normals rather than the obese subjects.

The variation in the g values obtained is due not only to variations in counting - rate from the body geometry but also to the variations in potassium - 42 dispensed to patients compared with that in the phantom, and these errors are almost inseparable. For the obese patients and for those between 50 and 80 kgms in weight in group II, the mean deviation was about ± 3.3 per cent and in group III about ± 4.5 per cent taking the average deviations for g photo and g compton. The lowest values of g ($g<1$ or close to 1.00) are associated with persons of low weight. This may be significant as a correlation of the g photo values against weight for group II yields a regression line of

$$g \text{ photo} = 0.850 + 4.114 \times 10^{-3} \text{ wt. (kilos)}$$

with a correlation coefficient of 0.779. The standard error of the regression coefficient (4.114×10^{-3}) is $\pm 6.77 \times 10^{-4}$ (or ± 16 per cent).

For the compton region however,

$$g \text{ compton} = 1.027 + 1.072 \times 10^{-3} \text{ wt. (kilos)}$$

with a correlation coefficient of only 0.241. The standard error of the regression coefficient (1.072×10^{-3}) is $\pm$ 8.9×10^{-4} (or $\pm$ 90 per cent).

The *g* value (cpm / μCi phantom / cpm / μCi subject) directly increases with the subject weight as the proportion of unscattered (photopeak) gamma rays emerging from the subject is proportionally decreased. On the other hand, in the compton region, absorption of gamma rays and scattering to energies outwith this energy band is presumably compensated by the primary photons which undergo compton collisions. Apparently two effects are almost balanced as there is significantly worse correlation of *g* compton and body weight than for *g* photopeak.

These conclusions would seem to confirm that where both *g* photo and *g* compton differ greatly from the mean *g* value, especially within the weight range stated, and if both are higher or both are lower, dispensing may be making an important contribution to the scatter of the results.

It should be noted that for three of the subjects studied, the total body potassium estimations were repeated. Patient number 6 in group I is also number 4 in group III, number 5 in group I is number 10 in group III and number 13 in group II is number 15 in group III. It can be seen that the total body potassium for the two measurements, estimated using a constant

factor, are in very good agreement, 97.5 and 98.5 kgms, 135.0 and 128.5 kgms and 123.5 and 123.0 kgms respectively. However, the potassium contents estimated using the potassium - 42 technique are 115.5 and 101.5 kgms, 194.0 and 164.0 kgms and 132.0 and 145.0 kgms respectively and would appear to support the above conclusions regarding dispensing errors.

On the basis of the patients studies, it would seem that for subjects weighing between 50 and 80 kgms and the obese subjects, the use of a constant calibration factor may be satisfactory. However, for those lying outwith the weight range stated, some correction factor is necessary. MENEELY et al. (1962) and LORIMER et al. (1965) use the following formula to calculate total body potassium which includes a correction factor for body build:

$$K \text{ gms} = F \times \text{subject's } K^{40} \text{ cpm},$$

where

$$F = \frac{A.e^{B\sqrt{W/H}}}{\sqrt{W.H}}$$

A and B are empirically derived constants for a particular counter,

W is the subject's weight,

H is the subject's height

and e is the base of natural logarithm.

A formula similar to this may be required if total body potassium estimations are to be made without using potassium - 42 for calibration purposes. However, a very large number of patient measurements would be required to provide a reliable sample size on which to derive such a formula.

SUMMARY.

On the basis of the patients studied, it would appear that for subjects of 50 to 80 kgms in weight and for obese subjects total body potassium estimations using a constant factor can be made to about ± 2 per cent of the value obtained using potassium - 42 for calibration purposes. For other subjects some correction factor is apparently necessary.

As expected g photopeak and body weight were correlated, whereas the correlation between g compton and weight was poor.

2. THE EFFECT OF THE DRUG BIOGASTRONE ON SERUM AND TOTAL BODY POTASSIUM LEVELS IN SEVEN NORMAL SUBJECTS.

Compounds of liquorice have been recognised for some time as having a beneficial effect in the healing of gastric ulcers. The active principle in liquorice has been identified as glycyrrhizic acid and from this was prepared the synthetic

substance glycyrrhetinic acid. The disodium salt of glycyrrhetinic acid hydrogen succinate is known as carbenoxolone sodium (Biogastrone) and has been shown to possess the ulcer healing properties of liquorice (DOLL et al. 1962, TURPIE and THOMSON 1965).

Carbenoxolone has a steroid-like structure and possesses certain recognised side effects, including fluid retention with weight gain, elevation of the blood pressure and lowering of the serum potassium, (DOLL et al. 1965 and TURPIE and THOMSON 1965). The hypokalaemia which is produced can be associated with levels as low as 2.5 m Eq/litre (HORWICH and GALLOWAY, 1965), weakness, hypotonia and electrocardiographic changes. In the study reported in this section, the effects of the drug Biogastrone on total body potassium and serum potassium levels for seven healthy normal male volunteers was examined.

METHOD.

For the seven subjects, ranging in age from 23 to 45 years, total body potassium estimations were obtained before and after therapy with Biogastrone, using the potassium-40 and -42 technique as previously described. A three week course of the drug was employed as it was thought that this period would suffice to demonstrate changes in the total body potassium levels. Subjects 3, 4 and 5 had a dosage of 100 mgs three

times daily and the remaining four 50 mgs three times daily. Throughout the period of treatment, the serum potassium, blood pressure and body weight were measured at three to four day intervals.

RESULTS.

The clinical data for the seven subjects are presented in Table III and the total body potassium estimations before and after treatment with Biogastrone are summarised in Table IV.

Student's t was calculated for the latter results and a value of 0.95 for t obtained. For each subject, t was also calculated for the serum potassium results, to determine whether there was any significant decrease in the serum levels. The regression coefficients (β) and the variance of the regression coefficients were calculated and a value for t obtained using the following formula:

$$t = \sqrt{\frac{\beta^2}{\mathrm{Var}\,\beta}} \qquad \text{with } (n-2) \text{ degrees of freedom.}$$

The value of t obtained for subject

1 = 0.22
2 = 1.83
3 = 2.43
4 = 2.00

TABLE III.

CLINICAL DATA

		1	5	8	11	12	13	15	16	17	19	20	21
Subj. 1	K^+	4.0	3.8	3.1		3.9		3.9			3.6		4.0
	BP	125/75	130/75	130/85		134/72		136/72			130/90		130/80
	WT	164.5	165.0	165.5		165.0		165.0			165.0		163.0
Subj. 2	K^+	4.6	4.0	3.6		3.9			4.0		3.5		
	BP	120/80	120/74	125/80		125/80		125/75		125/78	114/74		
	WT	161.0	160.0	159.0		158.0		158.0		158.0	158.0		
Subj. 3	K^+	4.0	3.8	3.5			3.6	3.5			3.4		3.7
	BP	116/80	135/75	130/90	135/75		128/90	145/90			125/85		150/115
	WT	160.5	163.0	164.0			163.0	163.0			162.0		163.0
Subj. 4	K^+	3.9	4.1			4.0		4.2			3.9		3.9
	BP	98/64	100/62	110/70		120/80		108/65			110/70		115/70
	WT	157.0	157.5	157.0		159.0		158.5			158.0		158.5
Subj. 5	K^+	3.7	3.7	3.3		3.7		3.6			3.4		3.4
	BP	105/75	116/70	105/70		120/70		120/78			120/75		130/85
	WT	172.0	174.0	173.0		174.0		172.5			175.0		176.0
Subj. 6	K^+	4.3	4.2	4.0		4.2		3.6				3.7	3.8
	BP	140/74	120/75	125/85		115/70		130/80				125/75	140/80
	WT	157.0	158.0	157.0		158.0		159.0				159.0	
Subj. 7	K^+	4.0	3.9	3.9			3.8	4.2				3.7	3.8
	BP	114/74	96/70	100/75	120/75		128/90	106/70					120/85
	WT	158	160.0	159.0	158.0		160.0	159.0					159.5

DAYS

K^+ is serum potassium (MEq/l)
BP is blood pressure (mm.Hg) and WT is weight (lbs).

TABLE IV.

Total body potassium, before and after treatment with Biogastrone.

Gms K (mean of photo and comp)	Subject						
	1	2	3	4	5	6	7
Before therapy	136	122	158	103	131	139	132
After therapy	136	116	150	105	138	135	128

5 = 0.91
6 = 3.92
and 7 = 0.65

DISCUSSION AND CONCLUSIONS.

There was no significant changes in the total body potassium ($t = 0.95$) in the seven normal subjects after a three week course of Biogastrone ($0.3 > P$). Only in subject number 6 was the decrease in serum potassium statistically significant, a value for t of 3.92 being obtained ($0.01 > P > 0.02$). No changes in blood pressure, body weight or electrocardiagrams were apparent.

Except for case number 4, who complained of mild heart-burn, there were no side-effects experienced by the subjects.

These results would appear to be in agreement with the findings of MONTGOMERY (1967) who reported clinical side-effects in only 4 per cent of the subjects studied, these all being initially hypertensive and all over 60 years old. Total body potassium estimations, however, were not measured by the above author.

CHAPTER V.

THE RETENTION AND EXCRETION RATES OF VITAMIN B_{12} IN PATIENTS WITH PERNICIOUS ANAEMIA AND HAEMATOLOGICALLY NORMAL SUBJECTS.

INTRODUCTION.

Pernicious anaemia is primarily a disease of the gastric mucosa, which in middle age or beyond fails to produce hydrochloric acid, pepsin and Castle's intrinsic factor. The cause of this failure is not known, but present evidence suggests that it is probably due to an inherited constitutional weakness, there being a significant familial incidence. In the absence of intrinsic factor from the gastric secretion the vitamin B_{12} in the diet is not absorbed. Treatment must continue for life since the gastric atrophy is permanent. Vitamin B_{12} is administered intramuscularly or intravenously, either in the pure form or in liver extract.

Administration of radio - vitamin B_{12}, labelled with one of the radioactive isotopes of cobalt, enables the retention and rate of loss of vitamin B_{12} to be measured by whole-body monitoring.

Whether administered radioactive cobalamin equilibrates matabolically with body stores of cobalamin is a matter of

practical as well as academic interest. The view that equilibration is established within a finite time has been supported by BOZIAN et al. (1963) and HEYSSEL et al (1966) while contrary opinions have been expressed by REIZENSTEIN et al (1962,1964), REIZENSTEIN (1966) and HEINRICH (1964). These latter authors suggest that the tracer and natural B_{12} excretion rates are not the same even after 3 years and that the tracer excretion rate changes and does not become constant until at least 250 days. The late excretion rates that they obtained are significantly different from those of other workers also using whole-body monitors.

A study of the literature on the subject was made (BODDY and ADAMS (1967a)) in view of the comparatively large amount of data supporting the assumption of effective metabolic equilibrium and the innumerable studies based on the validity of this assumption.

The development of the haematological and perhaps neurological symptons of B_{12} hypovitaminosis has been observed in patients from 1 to 14 years after total gastrectomy (MOLLIN and ROSS (1953), KELLY et al. (1954), PAULSON and HARVEY (1954), HARVEY (1956), WELBOURN et al (1956), M^{c}LEAN and SUNDBERG (1956), MARCHAL et al. (1961), COX et al. (1963) and GOLDBERG et al (1963)) with a mean of just over 4 years. A study of tissues

from untreated subjects in haematological relapse suggested that the whole-body content of vitamin B_{12} is reduced to less than 10 per cent and probably to less than 1 per cent of the original stores (SWENDSEID et al. (1957), ROSS and MOLLIN (1957), NELSON and DOKTOR (1958) and ADAMS (1962)). Hence, by assuming a simple exponential loss and using the equation $D = D_o e^{-\lambda t}$, the probable range of the excretion rates can be calculated. Taking the normal whole-body content of vitamin B_{12} to be between 2000 and 5000μgms (GRASBECK (1959) and ADAMS (1962)), the rate of loss for depletion to 10 per cent ranges from 0.045 - 0.63 per cent per day, and to 1 per cent from 0.09 - 1.26 per cent per day. The corresponding range for the mean depletion time of about 4.5 years is from 0.14 - 0.28 per cent per day. An excretion rate as low as 0.045 per cent per day would only be seen when hypovitaminosis occured at a depletion level of 10 per cent by 14 years, and a rate as high as 1.26 per cent per day when depletion to the 1 per cent level takes 1 year. Hence omitting these extreme cases, the most probable range may be from about 0.09 - 0.63 per cent per day.

In Table I, the final excretion rates observed by a number of workers are summarised. All of the data lies within the probable excretion range, except the final three results.

TABLE I.

Final rates of loss of vitamin B_{12}.

Authors.	Technique.	Final rates of loss % per day.
REIZENSTEIN et al. (1962)	W.B.M. - tracer	0.10
REIZENSTEIN et al. (1961)	W.B.M. - tracer	0.17
COHN et al. (1962)	W.B.M. - tracer	0.17
BOZIAN et al. (1963) (1964)	W.B.M. - tracer	0.09 - 0.23
HEYSSEL et al. (1966)	W.B.M. - tracer	0.09 - o.17
BODDY and ADAMS (1967 a)	W.B.M. - 5000µgms labelled.	0.09 - 0.19
BODDY and ADAMS (1967 b)	W.B.M. - tracer.	0.08 - 0.26
REIZENSTEIN (1959 c) and REIZENSTEIN et al. (1962)	non-labelled B_{12} - micro-biological, using tracer B_{12}.	0.03
REIZENSTEIN et al. (1964) (1966)	Kinetic analysis by computer	0.036
HEINRICH (1964)	W.B.M. - tracer	0.051

It is these values that are quoted as evidence disputing the assumption of tracer equilibrium with respect to excretion of vitamin B_{12}. HEINRICH (1964) incorrectly calculates a depletion time to the 10 percent level of 4.8 years using his estimate for the excretion rate of 0.051 per cent per day. He assumes a linear relationship between retention and time whereas, using the exponential relationship, the depletion time would be in excess of 12 years. The corresponding depletion time for an excretion rate of 0.036 per cent per day (REIZENSTEIN et al. (1966)) would be about 16 years.

It would therefore seem that the data assuming equilibrium is established shortly after tracer administration gives excretion rates which are in better agreement with the clinical observations of depletion times than the data which apparently refutes this assumption.

REIZENSTEIN et al. (1964), (1966) and HEINRICH (1964), on pooling data for several patients, found it necessary to use at least three exponential terms to describe the resultant excretion pattern, concluding that, since a steady loss rate is not attained until about 250 days after administration, equilibrium is not established until that time. It is inevitable that when summing exponentials a changing loss rate

would be obtained when using pooled data, unless the excretion rate for each patient is identical, whether equilibrium is established or not. Hence, these results cannot be interpreted, without enlargement, as an argument against supposition of tracer equilibrium.

REIZENSTEIN (1966) states that only for a single pool system is the assumption of isotopic equilibrium valid, this not being the case for vitamin B_{12}. However REIZENSTEIN et al. (1966) assume a three pool system to describe B_{12} metabolism, concluding that 99.3 per cent of the vitamin is in a single pool. This would seem to support the evidence that vitamin B_{12} is essentially a single pool system rather than being evidence to the contrary and therefore that the assumption of isotopic equilibrium is justifiable.

The study reported in section I was designed to elaborate this point in a practical fashion (BODDY and ADAMS (1967b)). Subjects with vitamin B_{12} deficiency to the point of megaloblastosis are known to have minute body stores of microbiologically active material. If such subjects, who are unable to absorb cobalamins from their diet, are given a large parenteral dose of labelled cobalamin then, for all practical purposes, the percentage of the dose retained after initial urinary losses represents their entire body stores.

If a steady state were established within a finite time and the subsequent daily loss could be adequately described by a single exponential term it would be reasonable to assume that the kinetics are those of a single pool system for which it is permissible to assume metabolic equilibration. Supporting evidence would be obtained if a normal subject, given the same intravenous dose, presented a late excretion rate, as per cent per day, of similar magnitude to the rate of loss of anaemic subjects.

Also reported in Section I are the results obtained on a small series of patients, comparing the percentage retentions after intramuscular injection of coenzyme B_{12} and cyanocobalamin.

Experimental data obtained from Section I did lend support to the view that a steady - state was attained with a finite time, and that the subsequent loss from the body could be described by a single exponential term. It seemed reasonable to expect that with tracer doses effective equilibrium might be established much earlier (BODDY and ADAMS (1967b)). If this were the case it would have practical application in the design of a method for measuring the influence of factors such as disease on cobalamin metabolism. A study using tracer doses has been made, and the results obtained

are presented in Section II.

SECTION I.

METHODS AND MATERIALS.

Pretreatment haematological data and treatment schedules can be seen in Table II for the fourteen subjects whose late excretion patterns were examined. One was a healthy male volunteer who was not anaemic, had a normal serum B_{12} level and ate a normal diet. Thirteen were initally B_{12} deficient all having subnormal serum B_{12} levels and megalobastic erythropoiesis on marrow biopsy smears. Some had presented with signs and symptoms of anaemia and others, who were not or only mildly anaemic, had presented with symptoms suggesting B_{12} deficiency such as glossitis, inflammation of the tongue. Additional investigations led to the diagnosis of Addison's pernicious anaemia in all but one subject (J.M.K.) who had post - gastrectomy megaloblastic anaemia. A parenteral dose of about 5000 μgms of vitamin B_{12}, labelled with either Cobalt - 57 or Cobalt - 58 was administered to each subject. In seven subjects the vitamin was in the hydroxocobalamin form, in two the cyanocobalamin form and in one co-ezyme B_{12} was administered. A double isotope study was made on the remaining four patients who received doses of cyancobalamin

TABLE II

Pretreatment Haematological Data & Treatment Schedules.

Subject	Sex	Age Years	Haemoglobin Gm. %	Serum B_{12} µµg/ml	Erythropoiesis	TREATMENT SCHEDULES
J.M.K.	M	71	10.1	58	M	5000 µg ^{57}Co OHB_{12} IV
M.B.	F	34	6.4	25	M	" "
A.B.	F	62	5.1	25	M	" "
M.O.	M	51	7.9	26	M	" "
J.P.	M	57	8.5	25	M	" "
H.M.	F	66	10.7	59	M	" "
T.M.	M	74	5.7	25	M	5000 µg ^{57}Co CNB_{12} IV
A.M.	F	41	11.1	25	M	5000 µg ^{57}Co CoE B_{12} IV (then 8/12 later 5000 µg ^{58}Co CN B_{12} IV)
J.H.	M	47	10.1	25	M	10 x 1000 µg ^{57}Co Oh B_{12} IM
J.K.	F	69	5.6	25	M	5000 µg ^{58}Co CN B_{12} IV then 1/52 later 5000 µg ^{57}Co Oh B_{12}IV
M.L.	F	51	12.0	25	M	" " " " "
R.A.	M	45	5.1	44	M	5000 µg ^{58}Co CN B_{12} IV then 2/52 later 5000 µg ^{57}Co Oh B_{12}IV
J.M.	F	70	6.9	25	M	10 x 1000 µg ^{58}Co CN B_{12} IM then 11/12 later 5000 µg ^{57}Co CN B_{12} IV
J.A.	M	42	14.8	35	-	5000 µg ^{57}Co Oh B_{12} IV

* M = Megaloblastic

labelled with cobalt - 58 and hydroxocobalamin labelled with cobalt - 57. In evaluating the results obtained in these latter patients, allowance was made for the contribution of the Compton region of the cobalt - 58 gamma ray spectrum to the photopeak of the cobalt - 57 spectrum. Although eight of the subjects had received the dose prior to the commencement of this study, long - term excretion rates were still obtainable. The retention of the administered B_{12} was measured in the prototype whole-body monitor for periods of up to 488 days after injection, the patients being scanned in the supine and prone positions where possible. For each subject the regression equation and coefficients were computed by the method of least squares assuming that the data after about 30 days post - injection were adequately described by a single term.

A double isotope study to determine whether there was preferential up - take between cyanocobalamin and co-enzyme B_{12} was made on five patients who had irrelevant diseases and were haematologically normal. The subjects were given 1000 μgms of cobalt - 58 labelled cyanocobalamin intramuscularly, then six days later, a similar dose of cobalt - 57 labelled co-enzyme B_{12}. The retention of the administered doses up to day 28 was measured in MERLIN, the patients being

scanned in both the supine and prone positions.

RESULTS.

The results are given in full in Tables III - VIII and in Fig. 1. Day 0 is the day of injection of the cobalamin and is the zero reference for the time scale.

DISCUSSION.

The largest variations in counting rate due to distribution and redistribution of the injected vitamin B_{12} may be expected within a few hours of administration. The maximum variation from 0 - $4\frac{1}{2}$ hours in the normal subject (Table III) was about 2 per cent and the standard error 1 per cent. Hence, the '100 per cent value' and measurements made shortly after intravenous administration can be accepted as being reasonably reliable.

It can be seen from the results presented in Tables IV to VII and Fig.1. that a single exponential term may be used to describe the late excretion pattern in the B_{12} deficient subjects and in the normal subject and therefore that the loss at this time can be only from a single compartment. The retained radioactive material in vitamin B_{12} deficient subjects will, for practical purposes, constitute the entire body stores.

TABLE III

Variation of whole-body counting shortly after intravenous administration of ^{57}Co vitamin B_{12} and comparison with urinary loss.

Time after initial whole-body count	Whole-body (supine and prone) cpm	Loss (% total injected)	Collection Period	Urine loss (% total injected)	Cumulative urine loss (% total injected)
0 hrs	20507	-	-	-	-
2½ hrs	20915	0 - 2.5%	-	-	-
4½ hrs	20902	-	-	-	-
6 hrs	9594	53.8	0 - 6 hrs	46.4	46.4
24 hrs	6719	67.7	6 -30 hrs	13.8	60.2
-	-	-	30 -48 hrs	3.3	63.5
3 days	4920	76.3	48.-72 hrs	2.6	66.1

TABLE IV

. Retention in anaemic subjects following a single intravenous dose of 5000 μg labelled vitamin B_{12}.

(H. -) Hydroxocobalamin (-. M) = Male
(C. -) Cyanocobalamin (-. F) = Female

Subject J.M.K. (HM)			Subject A.B. (HF)			Subject M.B. (HF)			Subject A.M. (EF)			Subject J.H. (HM)		
Days	cpm	% Retained	Days	cpm	% Retained	Days	cpm	% Retained	Days	cpm	% Retained	Days	cpm	% Retained
0	5774	100	14	2770	100	9	2595	100	8	1141	100	73	1405	100
2	1321	22.9	35	2400	88.8	27	2270	87.4	22	697	61.1	114	1394	99.2
7	915	15.8	88	2000	74.0	56	2109	81.2	50	516	45.2	135	1347	95.9
35	717	12.4	116	1958	72.5	105	1905	73.5	85	510	44.7	170	1204	85.7
69	612	10.6	137	1890	70.0	133	1845	71.1	113	390	34.2	206	1204	85.7
97	615	10.6	165	1780	65.9	168	1743	67.2	153	330	28.9	234	1197	85.2
132	577	10.0	193	1711	63.4	236	1648	63.5	176	338	29.6	268	1153	82.1
168	527	9.1	221	1730	64.1	253	1681	64.9	212	319	27.9	296	1115	79.4
196	495	8.6	263	1571	58.2	302	1524	58.7	325	306	26.8	334	1109	78.9
230	491	8.5	292	1523	56.4	330	1426	55.0	253	310	27.1	394	1123	72.8
258	484	8.4	320	1499	55.5	358	1445	55.7	281	307	26.9	432	954	67.9
296	435	7.9	354	1454	53.8	386	1392	53.7	309	301	26.4	488	1031	73.3
385	436	7.5	376	1472	54.5				337	302	26.4			
413	433	7.5												
441	445	7.7												
Subject H.M. (CF)			Subject M.O. (HF)			Subject J.P. (HM)			Subject T.M. (CM)					
7	467	100	7	2730	100	7	2820	100	61	784	100			
35	455	97.4	35	2281	83.6	35	2310	81.9	89	712	90.9			
66	426	91.2	63	2140	78.4	63	2070	73.4	117	765	97.5			
84	393	84.1	91	1938	71.0	91	2000	70.9	158	623	79.5			
112	401	85.9	112	1948	71.4	112	1872	66.4	179	634	80.9			
143	381	81.5	147	1878	69.1	147	1782	63.2	197	616	78.6			
156	365	78.1	176	1770	64.8	176	1758	62.4	225	623	79.5			
199	359	76.8	203	1773	65.0	203	1719	60.9	253	606	77.4			
227	378	81.0	218	1690	61.9	218	1662	58.9						
283	382	81.7	260	1675	61.4	260	1580	56.1						
304	334	71.5	281	1673	61.3	309	1498	53.1						
325	313	67.0	309	1582	58.0	337	1474	52.3						
			337	1573	57.6	365	1477	52.4						
			365	1535	56.2									

TABLE V.

Retention in a Normal Subject following a single intravenous dose of 5000 μg vitamin B_{12}.

SUBJECT J.A. (H.M).		
Days	cpm	% Retained
0	20774	100
1	6714	32.4
3	4920	23.7
14	3409	16.4
48	2342	11.3
70	2234	10.8
101	1982	9.6
129	1845	8.9
161	1769	8.5
192	1689	8.1
210	1609	7.7
238	1557	7.5
269	1528	7.3
282	1459	7.0
325	1457	7.0
381	1304	6.3
402	1363	6.6
430	1256	6.0

(H.M). = Hydroxocobalamin, male subject.

Retention in anaemic subjects following double isotope doses of 5000 μg labelled vitamin B_{12}.

	SUBJECT J.K. (F)				SUBJECT M.L. (F)			
Days	cpm C	% Retained	cpm H	% H Retained	cpm C	% C Retained	cpm H	% H Retained
0	991	100	1409	100	1172	100	1852	100
3	128	12.9	505	35.7	107	9.1	365	20.0
7	112	11.3	-	-	92.3	7.9	-	-
8	-	-	391	20.6	-	-	-	-
10	104	10.5	-	-	89.5	7.6	-	-
14	-	-	276	19.6	-	-	225	12.1
15	92.0	9.3	-	-	-	-	-	-
21	95.5	9.6	225	18.1	82.0	7.0	210	11.3
28	83.6	8.4	243	17.2	77.0	6.6	208	11.2
35	80.9	8.1	238	16.9	71.5	6.1	195	10.5
42	81.5	8.2	240	17.0	72.0	6.1	195	10.4
49	79.2	8.0	229	16.2	70.1	6.0	191	10.3
56	78.3	7.9	229	16.2	68.2	5.8	184	9.9
63	76.1	7.6	215	15.3	72.1	6.1	170	9.2
70	77.2	7.8	212	15.0	74.0	6.3	172	9.3
77	74.0	7.5	229	16.2	69.8	5.9	172	9.3
84	75.1	7.6	215	15.2	68.5	5.8	173	9.3
91	73.3	7.4	207	14.7	65.5	5.6	171	9.2
98	73.4	7.4	201	14.3	65.6	5.6	174	9.4
105	74.1	7.4	198	14.1	67.4	5.7	175	9.4
112	71.2	7.2	-	-	67.7	5.8	-	-
119	-	-	193	13.7	-	-	163	8.8
126	75.1	7.6	-	-	63.2	5.4	-	-
147	-	-	201	14.2	-	-	158	8.5
154	72.1	7.3	-	-	58.1	5.0	-	-
182	-	-	195	13.9	-	-	150	8.1
189	52.4	6.2	-	-	60.7	5.2	-	-

c = cyanocobalamin
h = hydroxocobalamin

TABLE VI cont'd

SUBJECT R.A. (M)						J.K. (F)					
Days	cpm C	% C Retained	Days	cpm H	% H Retained	Days	cpm C	% C Retained	Days	cpm H	% H Retained
0	1172	100	0	4896	100	25	271	100	0	11214	100
15	122	10.4	28	700	14.3	59	239	87.7	28	728	6.49
43	107	9.2	56	644	13.2	34	239	87.7	70	650	5.80
71	94.8	8.1	97	635	13.0	122	229	84.4	99	626	5.58
99	81.5	7.0	118	583	11.9	153	228	84.0	127	611	5.45
140	88.0	7.5	146	588	12.0	178	208	76.7	161	566	5.04
161	71.9	6.1	174	558	11.4	206	184	67.8	183	565	5.03
189	84.0	7.2	202	534	10.9	224	217	79.9			
217	73.3	6.2				255	182	67.1			
245	73.2	6.2				283	201	74.1			

TABLE VII.

Excretion rates and their standard errors.

Subject	Time period after administration	Biological $T_{\frac{1}{2}}$ days	Rate of loss % / day	Standard error (%) of rate of loss
Anaemic subjects, single isotope studies.				
J.M.K.	35 - 441	630	0.11	± 8.7
A.B.	35 - 376	495	0.14	± 7.4
M.B.	27 - 386	533	0.13	±11.2
H.M.	66 - 325	693	0.10	±13.8
A.M.	50 - 337	365	0.19	±18.3
M.O.	35 - 365	630	0.11	± 8.2
J.P.	35 - 365	533	0.13	± 6.8
T.M.	61 - 253	495	0.14	±20.8
J.H.	73 - 488	770	0.09	± 9.5
Anaemic subjects, double isotope studies.*				
J.K.(C.)	28 - 154	495	0.14	±12.1
J.K.(H.)	28 - 119	433	0.16	±15.6
M.L.(C.)	42 - 154	433	0.16	±14.8
M.L.(H.)	42 - 147	385	0.18	±22.9
R.A.(C.)	43 - 245	408	0.17	±26.1
R.A.(H.)	56 - 202	533	0.13	±14.4
J.M.(C.)	59 - 283	630	0.11	±27.0
J.M.(H.)	70 - 183	495	0.14	±10.5
Normal subject.				
J.A.	70 - 430	495	0.14	± 7.4

* (C.) Cyanocobalamin.

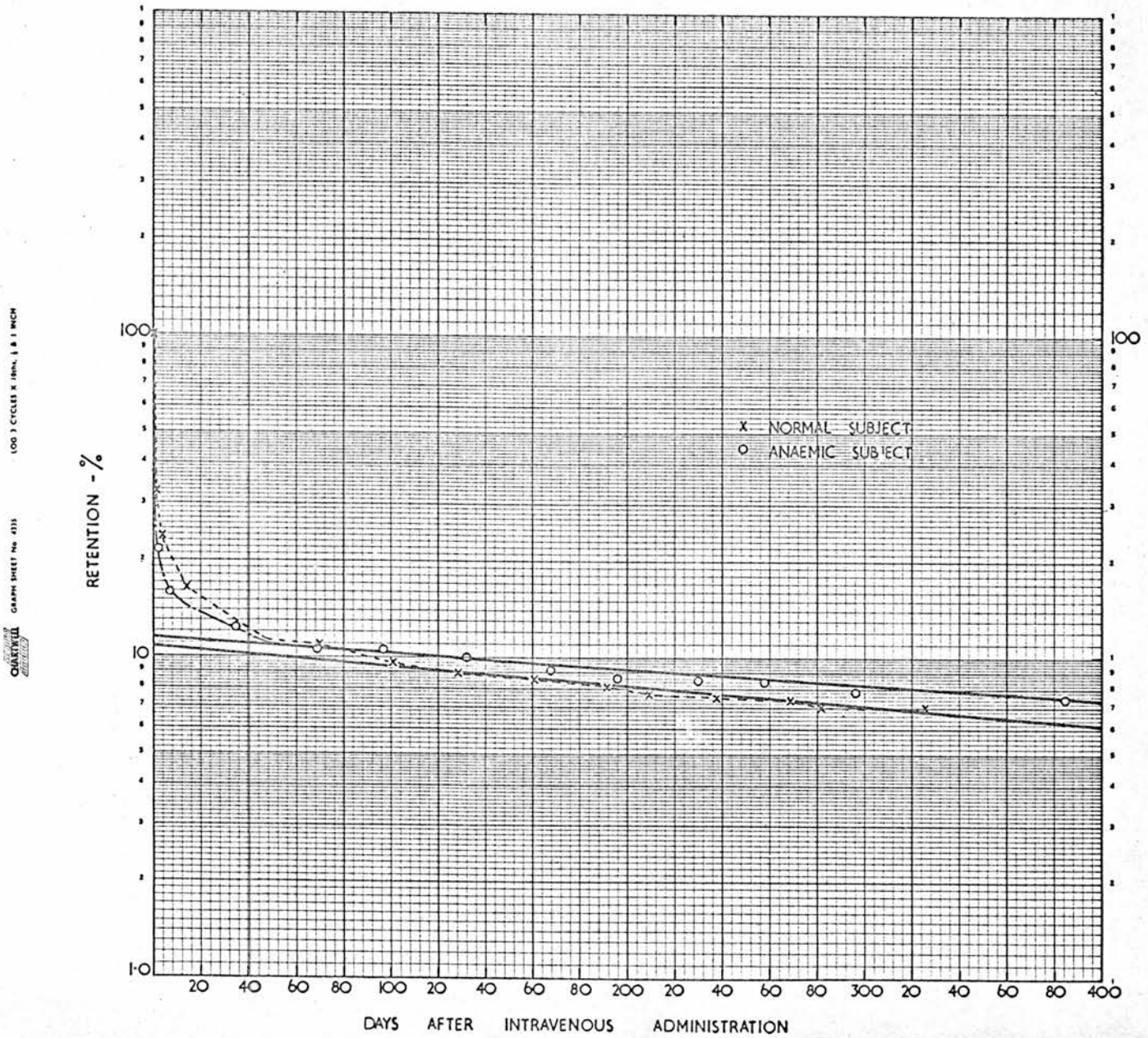
(H.) Hydroxocobalamin.

TABLE VIII.

Relative retention of cyanocobalamin and coenzyme B_{12}.

Subject.	% retention Co-enzyme B_{12}.		% retention Cyanocobalamin	
	Day 3	Day 28	Day 3	Day 28
1	40.2	17.1	12.3	11.9
2	55.6	16.8	13.8	11.6
3	41.9	19.3	19.4	16.7
4	36.6	17.2	15.6	14.0
5	37.0	22.0	16.0	15.0

FIG 1. PERCENTAGE RETENTION OF LABELLED VITAMIN B 12 IN A NORMAL SUBJECT AND AN ANAEMIC SUBJECT



In the normal subject, however, this will only be a fraction of the total body content and will be continually diluted by cobalamins absorbed from the diet. Since the results for these subjects are in good agreement and very similar to those reported by BOZIAN et al. (1963), (1964) it would seem reasonable to support the view that metabolic equilibration is attained within a finite time, a constant excretion rate being obtained by an interval of 4 - 8 weeks after dosing.

For the two patients on the double isotope study who were given the doses at short intervals, the total body stores, at the time of the second injection, were about 500 µgms (10 per cent of the previous dose). The late rate of loss from the material retained from the second injection did not differ significantly from that of the first, establishing additional evidence of the equilibration of the retained tracer and the natural B_{12}.

There are two theories regarding the form in which the injected cobalamins are utilised in vivo. Firstly that the cyanocobalamin and hydroxocobalamin are converted to the co-enzyme form and utilised as such, and secondly that they are made use of in their original form. The first possibility was supported by ROSENBLUM et al. (1960) and the studies reported by UCHINO et al. (1965) would seem to confirm this

theory. Hence, it would be expected that the turnover rate of retained cyanocobalamin, hydroxocobalamin and coenzyme would be the same. The late loss rate from the one patient given coenzyme B_{12} (0.19 per cent per day) did not differ significantly from that of patients given cyanocobalamin or hydroxocobalamin (0.09 - 0.18 per cent per day). The late loss components in the patients given cyanocobalamin and the patients given hydroxocobalamin and in the four subjects given both cobalamins were similar, indicating that the utilisation of the retained fraction is not different.

A frequent method of measuring post-injection loss is by the monitoring of urine within about 3 days of injection. With the dosage of 5000 µgms it can be seen that using this method the loss would be underestimated, since between day 3 and the point at which a steady - state is attained considerable B_{12} loss occurs in urine and faeces. It would probably not be detectable by the monitoring of daily urine collections.

A short term study of the relative retention of cyanocobalamin and coenzyme B_{12} was made and the results presented in Table VIII. It can be seen that the initial loss rate for coenzyme B_{12} is considerably slower than that for cyanocobalamin up to day 3, although the difference between the day 28 retention percentages is less significant. Site retention or

preferential uptake or the fact that in vivo utilisation of vitamin B_{12} is in the coenzyme form could account for the difference in the clearance time. If the coenzyme B_{12} is taken up by the stores, insecure binding would result in it being released and excreted at a later period. Further studies are planned to determine whether site retention is responsible for the initial higher retention of the coenzyme by means of surface counting and also to compare its initial loss rate with hydroxocobalamin.

SECTION II.

METHODS AND MATERIALS.

Six healthy male volunteers were studied, all having normal peripheral blood values, normal serum vitamin B_{12} levels and all on a normal diet. Each received a single intravenous dose of cobalt - 58 labelled cyanocobalamin 0.1 μgms 0.5 μCi in 3.0 mls of water shortly before the initial whole-body measurement. The retention of the administered B_{12} was measured in MERLIN, the subjects being scanned in both the supine and prone positions. For each subject the regression equation and coefficients were computed by the method of least squares. The calculations were repeated successively omitting earlier and later results to evaluate variations in

the excretion rate with time and to estimate when the early loss was essentially complete using a Univac 1107 computer.

RESULTS.

The data from the whole-body monitoring are presented in Table IX and the excretion rates and the standard errors at varying periods in Tables X and XI.

DISCUSSION.

It can be seen from the results in Table IX that the loss in the first few days after administration of the dose is greater than in subsequent periods. This is further shown in Table XI, the losses in the first week being considerably larger than the overall excretion rates. This initial loss is probably partly due to competition between binding sites and renal excretion. The loss rates observed after days 3 - 5 can be adequately described by a single exponential function, confirming the results obtained and the conclusions drawn in Section I.

It is of interest to note that subject 1 in this study had a rate of loss of 0.140 ± 0.011 per cent per day for days 70 - 430 after injection of 5000 μgms of hydroxocobalamin. This value is in excellent agreement with that of 0.158 ±

TABLE IX.

Retention of tracer B_{12} following intravenous administration.

Day	Subject 1. retention %	Subject 2. retention %	Subject 3. retention %	Subject 4. retention %	Subject 5. retention %	Subject 6. retention %
1	100	100	100	100	100	100
2	97.8	95.8	98.2	94.4	97.4	97.1
3	97.7	95.7	97.2	92.6	96.2	97.0
4	96.4	94.4	95.8	92.5	96.2	96.2
5	95.5	94.2	97.3	93.0	93.9	94.6
8	93.4	93.1	95.2	92.4	94.0	97.1
9	93.3	94.2	94.0	92.6	93.1	95.8
10	92.5	90.2	94.9	91.0	91.4	93.0
11	94.4	92.7	94.7	92.5	94.6	95.2
12	94.7	91.3	97.4	93.1	96.7	95.6
22	90.8	88.6	94.7	91.0	88.5	89.9
23	93.1	88.9	93.4	90.1	91.7	94.3
24	94.9	91.5	94.7	92.1	92.9	94.5
25	91.9	90.1	92.0	92.1	89.5	92.9
26	91.3	86.9	92.1	90.4	89.9	91.8
36	89.9	-	90.6	88.7	89.4	92.7
38	91.5	85.7	91.5	90.4	88.6	90.9
39	90.1	-	92.9	92.1	87.1	90.6
64	87.0	83.6	87.7	86.1	-	89.0
67	88.5	84.1	90.6	86.8	-	-
92	82.4	78.2	82.8	80.9	-	81.3
94	-	-	-	-	81.3	-
101	80.0	76.5	80.8	80.3	77.2	-

TABLE X.

Excretion rate and standard error from varying periods after injection until final day of study.

SUBJECT 1			SUBJECT 2			SUBJECT 3		
Days	Excretion-rate o/o per day.	Standard Error ± o/o per day.	Days	Excretion-rate o/o per day.	Standard Error ± o/o per day.	Days	Excretion-rate o/o per day.	Standard Error ± o/o per day.
1-101	0.174	0.013	1-101	0.216	0.016	1-101	0.171	0.012
2-101	0.168	0.012	2-101	0.207	0.013	2-101	0.166	0.012
3-101	0.164	0.012	3-101	0.204	0.013	3-101	0.164	0.012
4-101	0.160	0.012	4-101	0.200	0.013	4-101	0.163	0.013
5-101	0.158	0.012	5-101	0.197	0.014	5-101	0.165	0.014
8-101	0.157	0.013	8-101	0.195	0.014	8-101	0.163	0.014
9-101	0.159	0.014	9-101	0.193	0.015	9-101	0.164	0.015
10-101	0.163	0.015	10-101	0.187	0.015	10-101	0.169	0.016
11-101	0.169	0.014	11-101	0.191	0.015	11-101	0.173	0.017
12-101	0.170	0.016	12-101	0.187	0.016	12-101	0.177	0.018
22-101	0.169	0.017	22-101	0.187	0.019	22-101	0.171	0.019
23-101	0.177	0.018	23-101	0.191	0.020	23-101	0.169	0.021
24-101	0.177	0.018	24-101	0.195	0.023	24-101	0.170	0.023
25-101	0.168	0.018				25-101	0.165	0.025
26-101	0.172	0.021				26-101	0.174	0.028

TABLE X Cont'd

SUBJECT 4			SUBJECT 5			SUBJECT 6		
Days	Excretion-rate o/o per day.	Standard Error ± o/o per day.	Days	Excretion-rate o/o per day.	Standard Error ± o/o per day.	Days	Excretion-rate o/o per day.	Standard error ± o/o per day
1-101	0.154	0.014	1-101	0.209	0.017	1-92	0.179	0.017
2-101	0.144	0.011	2-101	0.202	0.016	2-92	0.172	0.016
3-101	0.143	0.011	3-101	0.198	0.016	3-92	0.171	0.017
4-101	0.145	0.011	4-101	0.195	0.016	4-92	0.169	0.018
5-101	0.148	0.012	5-101	0.192	0.017	5-92	0.169	0.019
8-101	0.149	0.013	8-101	0.193	0.018	8-92	0.174	0.020
9-101	0.151	0.013	9-101	0.192	0.019	9-92	0.169	0.021
10-101	0.153	0.014	10-101	0.194	0.021	10-92	0.167	0.023
11-101	0.160	0.014	11-101	0.200	0.021	11-92	0.176	0.023
12-101	0.163	0.015	12-101	0.197	0.023	12-92	0.177	0.026
22-101	0.165	0.016	22-101	0.183	0.020	22-92	0.174	0.029
23-101	0.168	0.017	23-101	0.191	0.018			
24-101	0.176	0.017	24-101	0.189	0.020			
25-101	0.176	0.022						

TABLE XI. Variation of excretion rate shortly after administration, for varying periods after injection.

Subject.	Days.	Excretion rate % per day.	Standard error ± %per day.	Days.	Excretion rate % per day.	Standard error ± % per day.	Days.	Excretion rate % per day.	Standard error ± % per day.	Days.	Excretion rate % per day.	Standard error ± % per day.
1.	1-101	0.174	0.013	5-101	0.158	0.012	8-101	0.157	0.013	9-101	0.159	0.014
	1-92	0.165	0.015	5-92	0.142	0.014	8-92	0.140	0.015	9-92	0.143	0.016
	1-67	0.159	0.021	5-67	0.122	0.018	8-67	0.117	0.019	9-67	0.120	0.020
	1-64	0.179	0.024	5-64	0.136	0.022	8-54	0.130	0.023	9-54	0.135	0.025
	1-39	0.196	0.033	5-39	0.128	0.032	8-39	0.116	0.035	9-39	0.123	0.039
	1-38	0.200	0.037	5-38	0.122	0.037	8-38	0.108	0.041	9-38	0.115	0.046
2.	1-101	0.216	0.016	5-101	0.197	0.013	8-101	0.195	0.014	9-101	0.193	0.015
	1-92	0.217	0.020	5-92	0.193	0.017	8-92	0.189	0.018	9-92	0.186	0.019
	1-67	0.221	0.027	5-67	0.185	0.024	8-67	0.178	0.025	9-67	0.173	0.027
	1-64	0.250	0.033	5-64	0.207	0.030	8-64	0.199	0.032	9-64	0.194	0.035
	1-38	0.328	0.043	5-38	0.268	0.047	8-38	0.260	0.054	9-38	0.257	0.062
3.	1-101	0.171	0.012	5-101	0.165	0.014	8-101	0.163	0.014	9-101	0.164	0.015
	1-92	0.160	0.015	5-92	0.150	0.016	8-92	0.147	0.017	9-92	0.148	0.018
	1-67	0.145	0.019	5-67	0.126	0.020	8-67	0.119	0.021	9-67	0.120	0.022
	1-64	0.172	0.020	5-64	0.154	0.022	8-64	0.147	0.023	9-64	0.149	0.025
	1-39	0.177	0.027	5-39	0.149	0.032	8-39	0.135	0.034	9-39	0.138	0.039
	1-38	0.198	0.029	5-38	0.171	0.034	8-38	0.158	0.037	9-38	0.164	0.042
4.	1-101	0.154	0.014	5-101	0.148	0.012	8-101	0.149	0.013	9-101	0.151	0.013
	1-92	0.148	0.018	5-92	0.139	0.015	8-92	0.141	0.016	9-92	0.143	0.017
	1-67	0.129	0.022	5-67	0.110	0.016	8-67	0.110	0.018	9-67	0.111	0.019
	1-64	0.133	0.027	5-64	0.108	0.021	8-64	0.108	0.108	9-64	0.110	0.025
	1-39	0.125	0.037	5-39	0.076	0.027	8-39	0.072	0.031	9-39	0.071	0.035
	1-38	0.152	0.040	5-38	0.105	0.026	8-38	0.103	0.029	9-38	0.105	0.033

TABLE XI Cont'd

Subject	Days	Excretion rate o/o per day.	Standard Error ± o/o per day.	Days	Excretion rate o/o per day.	Standard error. ± o/o per day.	Days.	Excretion rate o/o per day.	Standard Error ± o/o per day.	Days.	Excretion rate o/o per day.	Standard Error ± o/o per day.
	1-101	0.209	0.017	5-101	0.192	0.017	8-101	0.193	0.018	9-101	0.192	0.019
	1-94	0.202	0.023	5-94	0.174	0.022	8-94	0.175	0.024	9-94	0.173	0.026
	1-39	0.264	0.037	5-39	0.212	0.044	8-39	0.220	0.050	9-39	0.221	0.056
	1-38	0.260	0.042	5-38	0.198	0.051	8-38	0.205	0.058	9-38	0.205	0.066
	1-92	0.179	0.017	5-92	0.169	0.019	8-92	0.174	0.020	9-92	0.169	0.021
	1-63	0.158	0.024	5-63	0.132	0.027	8-63	0.137	0.029	9-63	0.125	0.030
	1-39	0.181	0.031	5-39	0.147	0.039	8-39	0.159	0.043	9-39	0.139	0.045
	1-38	0.181	0.036	5-38	0.140	0.046	8-38	0.154	0.051	9-38	0.130	0.054

0.012 per cent per day for days 5 - 101 in this present study.

The calculated excretion rates have a standard error of ± 10 per cent which is considered acceptable. BOZIAN et al. (1963) concluded that although large variations in their initial counting rate occurred, cobalamin excretion could be described by a single exponential term. In the study reported in this section, initial losses of up to 5 per cent were observed, these being statistically insignificant (Table X) and hence explaining and confirming the conclusions drawn by these authors.

Short term measurements of cobalamin turnover using a whole-body monitor have not previously been considered practical due to the initial large redistribution effects. As a result of this, very little is known of the effects of disease or of drugs and dietary constituents on cobalamin metabolism. It would seem that a study similar to this could be used to investigate these effects. If an error of about 20 - 30 per cent is acceptable measurements of whole-body retentions from 7 to 30 days would suffice, and if an error of 10 per cent is necessary, measurements of up to 60 days would be required.

The human requirement for cobalamins is the amount which must be absorbed to maintain body stores. With a loss of

0.1 - 0.2 per cent per day, the daily requirement will range from 1 - 10 μgms, 0.1 - 0.2 per cent of the body stores, assuming a total body content of between 1000 and 5000 μgms. These values are in agreement with those suggested by HEYSSEL et al. (1966) of 3 - 5 μgms daily. This gives rise to questions about the availability of food bound cobalamins and the quantity which must be ingested to ensure absorption of the required amount. Further work on these problems is necessary.

SUMMARY AND CONCLUSIONS.

The excretion rates following intravenous injection of 5000 μgms of vitamin B_{12} were constant after an interval of 4 - 8 weeks and the late excretion pattern adequately described by a single exponential term. This implies that metabolic and isotopic equilibrium has been established.

Similar excretion rates were seen in the normal and anaemic subjects suggesting that vitamin B_{12} deficiency in these patients is not attributable to a metabolic defect.

The late excretion rates after dosing with hydroxocobalamin, cyanocobalamin and coenzyme B_{12} did not differ significantly. These results would appear to be in agreement with the theory that the two cobalamins are converted "in vivo" to the coenzyme

form for utilisation.

A considerable difference in clearance time of coenzyme B_{12} compared with cyanocobalamin was noted and studies are continuing, endeavouring to explain the initial higher retention of the coenzyme.

With tracer doses of 0.1 µgms of vitamin B_{12} effective equilibrium is established between 3 and 5 days after injection and the subsequent excretion rates observed were in good agreement with those after a dosage of 5000 µgms.

Short term measurements to investigate the effects of disease, drugs and diet on cobalamin metabolism would appear practical.

The human requirement for cobalamins is from 1 to 10 µgms daily. Further investigation is required to determine the availability of food bound cobalamins.

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