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The following documents were submitted by Clement Finch, M.D., principal investigator for the contract. These documents have been patent-cleared by the RL Representative of the Chicago Patent Group:

RLO-2048-2 "Effect of Altitude on Erythropoiesis" by Jose Faura, Jose Ramos, Cesar Reynafarje, Eugenia English, Per Finne and Clement A. Finch

RLO-2048-3 "Effect of Altitude on Oxygen Binding by Hemoglobin and on Organic Phosphate Levels" by Claude Lenfant, John Torrance, Eugenia English, C.A. Finch, C. Reynafarje, Jose Ramos and Jose Faura

RLO-2048-5 "The Precision in Vivo Measurement of Radioiron by Whole-Body Counting" by H.E. Palmer, J.D. Cook, K.G. Pailthorpe and C.A. Finch

RLO-2048-6 "The Measurement of Iron Absorption by Whole-Body Counting" by J.D. Cook, H.E. Palmer, G.K. Pailthorpe, and C.A. Finch

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RLO-2048-2

Effect of Altitude on Erythropoiesis

By JOSE FAURA, JOSE RAMOS, CESAR REYNAFARJE, EUGENIA ENGLISH, PER FINNE AND CLEMENT A. FINCH

ERYTHROPOIESIS is stimulated by ascent to high altitude and suppressed after return to sea level. The resulting changes in the erythron have been previously described.[1,2] The present study was undertaken to characterize the role of erythropoietin in mediating this marrow response.

MATERIALS AND METHODS

Volunteer subjects participating in this study were inhabitants of Lima and Morococha. *Group I* was composed of 7 healthy males between the ages of 18 and 30 living in the sea-level city of Lima, where baseline studies were performed. The group was then transported by car over a 4 hour period to Morococha (4530 meters above sea level). They remained there for 7 days, confined to living quarters in the building where further measurements were made. All these subjects experienced some disability for the first 48 hours at high altitude and subjects B, C, and E were sufficiently symptomatic to be confined to bed for the first day.

Group II was composed of male inhabitants of Morococha between the ages of 20 and 39. They were first studied at high altitude and then transported to Lima where the observations were continued over the following week. One individual (L) suffered contusions from a car accident on the second day in Lima.

The procedure for collecting, processing and assaying urinary erythropoietin has been described elsewhere.[3] All urine specimens were refrigerated at once after voiding at 4 C. A 25 per cent aliquot of the preceding day's collection was dialyzed within 24 hours, concentrated to less than 100 ml., and frozen. At the time of altitude change 8 hour collections were processed. All aliquots were further concentrated in Seattle to 25 ml. and assayed in polycythemic protein-starved female, Swiss-Webster mice, 11 to 12 weeks old. The assay procedure was identical to that previously reported except that urinary concentrate was injected twice daily for only 2 days. Results were calibrated against a known erythropoietin standard* and the activity was expressed as units/24 hours.

Plasma iron turnover (PIT) measurements were made between 9 and 11 a.m. in each subject. ^{59}Fe citrate (2 to 5 μc. and less than 1 μg. of iron) was injected intravenously and 4 samples were taken beginning at 10 minutes after injection and at intervals of 15 to 30 minutes depending on the anticipated rate of disappearance of radioiron. Activity was measured in a liquid scintillation vial counter to an accuracy of less than $\pm$ 2 per cent.

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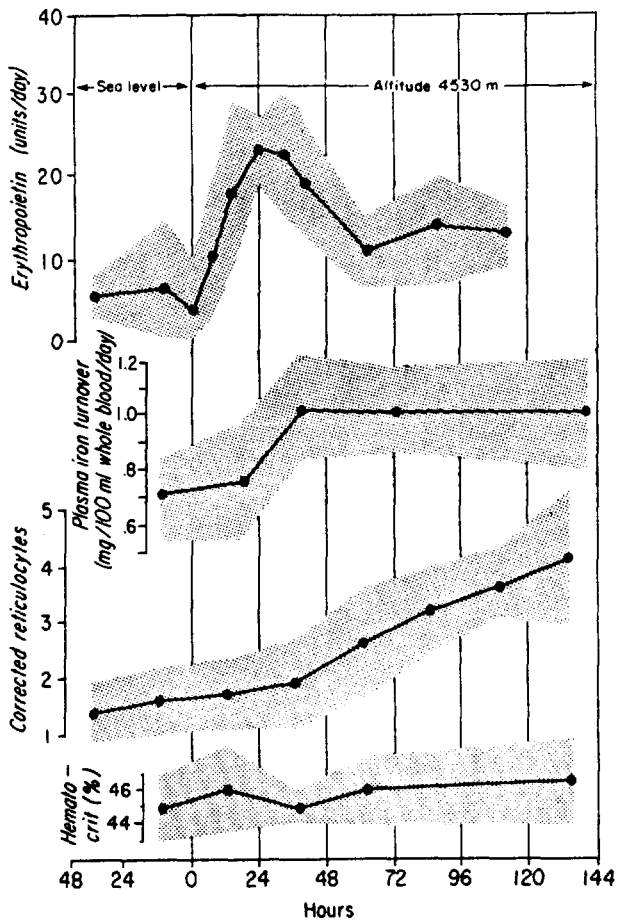

Fig. 1.—Erythroid response to high altitude anoxia. Mean value of the seven subjects is shown by the solid line; shaded area indicates 1 standard deviation. Urinary erythropoietin levels increased within 12 hours of high altitude exposure and became maximal at 24 hours. Plasma iron turnover increased maximally within 24 hours of the increase in erythropoietin and showed no further increase over the following 4 days. Reticulocytes increased threefold, but half of this increase would appear to be related to the prolongation in maturation time. Hematocrit values remained constant over the 7 day period.

PIT was calculated by the following formula:

$$\frac{\text{Plasma iron } (\mu\text{g. } \%) \times 100 - \text{Hct} \times 0.9}{\text{T } 1/2 \text{ (min.)} \times 100}$$

On a different date for each subject, ^{55}Fe citrate (15 μc.) was injected intravenously so that the marrow transit time as well as plasma iron turnover could be determined. Samples were then drawn every 12 hours for 4 days and at the end of 2 weeks for digestion, electroplating and differential counting.[4] The marrow transit time was calculated as the interval after injection required for 50 per cent of the 14 day red cell radioactivity to appear in circulation.

All samples for erythropoietin, isotope and iron determinations were shipped in the frozen state from Lima to Seattle where the analyses were carried out. Plasma iron was measured by the method of Bothwell and Mallet,[5] iron binding capacity by a modification of the method of Morgan[6] and by the immunodiffusion technic.[7] Reticulocyte smears

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Table 1.—Ferrokinetic Measurements of Group I

Subject	Time (hrs.)	Plasma iron (μg.%)	Iron disappearance (T 1/2-min.)	Plasma iron turnover*
A.	Basal	113	65	1.02
	14	91	69	0.72
	36	64	39	1.00
	60	49	33	0.88
	132	44	30	0.85
B.	Basal	80	79	0.60
	14	42	45	0.55
	36	76	48	0.95
	60	58	40	0.87
	132	42	40	0.64
C.	Basal	64	53	0.76
	14	43	31	0.86
	36	79	34	1.37
	60	54	26	1.24
	132	42	25	1.01
D.	Basal	79	58	0.80
	14	103	60	1.05
	36	87	51	1.00
	60	63	37	1.01
	132	75	33	1.31
E.	Basal	83	74	0.66
	14	95	78	0.70
	36	161	78	1.22
	60	59	38	0.87
	132	55	33	0.93
F.	Basal	53	55	0.54
	14	141	85	0.96
	36	139	77	1.07
	60	174	92	1.14
	132	79	40	1.09
G.	Basal	114	110	0.63
	14	72	90	0.47
	36	107	80	0.81
	60	131	67	1.07
	132	98	52	1.14
Average	Basal	84	71	0.715
	14	84	65	0.917
	36	102	58	1.06
	60	84	48	1.01
	132	62	36	0.995

* mg./100 ml. whole blood/day.

were coded and 4000 cells counted blind. Hemoglobin was determined by the cyanmethemoglobin method and the packed cell volume by the Wintrobe technic.

Results

I. *Ascent to High Altitude*

The effect of altitude on erythropoiesis in Group I is summarized in Figure 1 and Table 1. Over a 7 day period at high altitude, the mean hematocrit changed little despite the removal of approximately 400 ml. of blood. Reticulocytes showed an increase from a mean value of 1.5 to 4.2 per cent with

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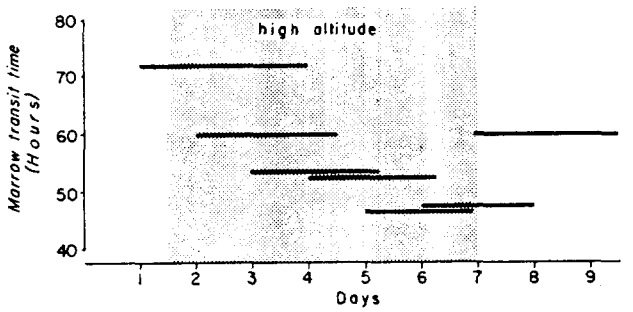

Fig. 2.—Marrow transit time of subjects exposed to high altitude. The ordinate indicates the duration of marrow transit of radioiron (time between injection and appearance of 50 per cent of activity in the red cell mass). The horizontal line also indicates by its beginning the time when radioiron was injected and by its end the time when 50 per cent of the red cell activity had appeared in circulation. Each measurement is in a different subject. The mean value for normal subjects is 90 hours.

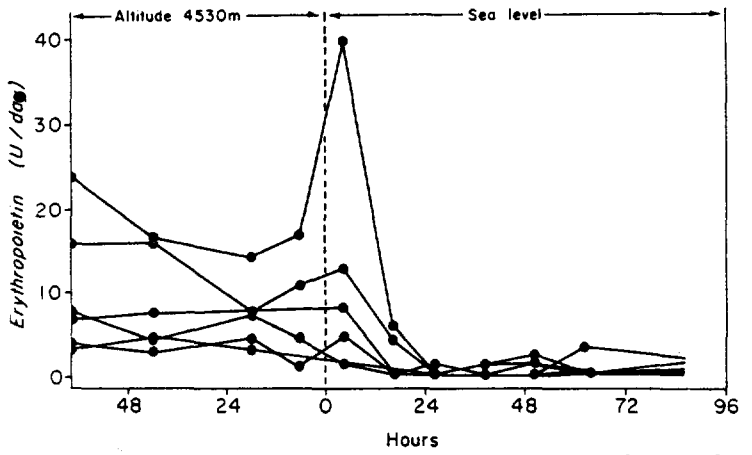

Fig. 3.—Urinary erythropoietin response to low altitude. High altitude dwellers were moved to sea level. Original erythropoietin levels were not significantly elevated in 4 of the 6 subjects. In the two individuals with elevated levels, there was an unexplained increase at or just before the descent. In all subjects erythropoietin decreased rapidly after descent.

the greatest increase occurring during the second and third day after exposure to anoxia. The PIT increased abruptly from 0.76 at 12 hours of high altitude exposure to 1.06 at 36 hours; thereafter it fell slightly. Mean erythropoietin output did not change during the first 6 hours at high altitude from the baseline of about 7 units per day, but by the second day, it had risen to 20 units per day. Over the subsequent 3 days, it fell to a level of between 10 and 15 units per day. Marrow transit time (Fig. 2) shortened progressively when subjects were at high altitude. The shortening occurred whether the iron was injected before ascent (subject A) or immediately before descent (subject C).

II. *Descent to Low Altitude*

Hematologic values of the second group were more heterogeneous than the

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Table 2.—Hematologic Measurements of Group II

Subject	Time (hrs.)	Hematocrit (%)	Corrected reticulocyte (%)	Plasma iron (μg.%)	Iron disappearance (T 1/2-min.)	Plasma iron turnover*	Erythropoietin (units in preceding 24 hr.)
H.	Basal	54	1.9	68	48	0.72	7
	16	53	1.5	55	38	0.75	2
	40	53	1.4	58	45	0.67	< 1
	64	51	2.0	83	56	0.79	< 1
	88	48	2.3	94	78	0.68	< 1
I.	Basal	72	2.2	40	14	0.94	18
	16	72	1.9	88	18	1.70	27
	40	70	2.2	56	24	0.86	3
	64	70	1.5	138	50	1.05	2
	88	67	1.6	212	101	0.82	< 1
J.	Basal	54	3.6	89	41	1.03	7
	16	57	3.0	92	38	1.18	9
	40	55	3.2	160	38	2.13	< 1
	64	55	2.3	73	41	0.93	< 1
	88	48	1.7	128	77	0.94	< 1
K.†	Basal	64	2.8	128	50	0.99	4
	16	64	2.8	101	48	0.89	< 1
	40	61	1.8	144	48	1.35	1
	64	61	1.7	127			< 1
	88	56	2.2	128	62	1.02	< 1
L.	Basal	73	3.0	64	16	1.30	13
	16	71	3.2	118	15	2.84	21
	40	74	2.6	197	37	1.78	2
	64	74	3.0	157	41	1.38	< 1
	88	70	2.3	349	99	1.30	< 1
M.	Basal	60	2.8	216	78	1.14	4
	16	60	2.8	97	59	0.76	3
	40	59	2.8	113	83	0.64	< 1
	64	59	2.3	130	88	0.70	1
	88	55	2.4	234	145	0.81	1
Average	Basal	63	2.7	101	41	1.05	9
	16	63	2.5	92	36	1.11	11
	40	62	2.3	121	46	1.17	1
	64	62	2.1	118	55	0.98	< 1
	88	58	2.1	191	94	0.98	< 1

* mg./100 ml. whole blood/day.

† This subject traumatized on the first day at sea level.

first. Two subjects had basal hematocrits of 72 and 73 per cent and elevated erythropoietin levels. The other four subjects had initial hematocrits between 54 and 64 and erythropoietin levels similar to those of Group I. The effect of moving the inhabitants of Morococha to sea level is shown in Figure 3 and Table 2. During the two days at high altitude and four days at sea level, approximately 450 ml. of blood were removed and their mean hematocrit fell from 63 to 58 per cent. Reticulocytes decreased from 2.6 to 2.1 per cent. The mean PIT of 1 mg./100 ml. of whole blood per day at high altitude actually rose during the first 42 hours at low altitude and thereafter decreased. The rise was largely attributable to subjects I, J, and L who showed increases in erythropoietin on the preceding day. During the following three days PIT

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showed a minimal fall. Erythropoietin levels fell very rapidly after descent to sea level and were undetectable within 12 hours.

There was an increase in iron binding capacity as measured by both charcoal and immunologic methods from a mean value of 361 to 392 in Group I and a decrease in Group II from 376 to 359 μg. per cent.

Discussion

Previous studies by Huff et al.[1] and Reynafarje et al.[2] have defined the general response of the erythroid marrow to changes in altitude. The measurements of myeloid:erythroid ratios, reticulocytes, and erythroid iron turnover indicate an augmentation of blood production to approximately twice normal when sea level dwellers moved to high altitudes, whereas erythropoiesis decreased to about one-third normal when high altitude dwellers move to sea level.

This study was focused on the erythropoietin output and its immediate effect on erythropoiesis with such altitude changes. Upon exposure to altitude anoxia, there was a lag period of about 6 hours, followed by a marked increase in erythropoietin output over the next 24 hours and then a decline to twice basal levels. These results are consistent with previous data on erythropoietin response in animals, which have been shown to have a peak response at 8 to 18 hours, and a fall to normal after 48 to 72 hours.[8-14] The more limited studies in man also indicate demonstrable erythropoietin activity in plasma only during the first 3 days at increased altitude.[15-17] The reason for the initial exaggerated response and secondary fall is not clear. The slight increment in marrow activity makes the speculation of marrow utilization less likely. A more likely possibility is the reduction in anoxia by another mechanism such as the shift in the oxygen dissociation curve which occurred during the first 2 days.[18]

In subjects in Group II, who were transported from high to low altitude, response occurred immediately, so that erythropoietin was not measurable within 12 hours. This rate of disappearance is more rapid than would be expected from some clearance studies in man.[19] The conspicuous rise of erythropoietin observed in two subjects at the time of descent and the increase in plasma iron turnover in all subjects during the first 2 days at low altitude is unexplained; it is possible that the increased oxygen supply actually reduced blood flow and led to tissue anoxia.[20]

Information concerning the mode of action of erythropoietin on the erythroid marrow is provided by the ferrokinetic measurements. Radioiron taken up by the erythroid marrow is known to be incorporated within minutes into hemoglobin.[21,22] Thus, radioiron uptake by the marrow is assumed to represent the hemoglobin synthesizing capacity of that organ. In the stimulated erythropoiesis of high altitude, the amount of iron going to the erythroid marrow and appearing in circulating red cells increases from about 80 per cent at sea level to 90 per cent or more.[2] There is then an increase in both the proportion of iron turnover concerned with erythropoiesis and the absolute amount. This increase in PIT occurred in the Group I subjects between 12 and 36 hours after altitude exposure or within 24 hours of the erythropoietin

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response. The same time relationship was observed in the two subjects of Group II who were most polycythemic: a temporary increase in erythropoietin at the time of descent was followed by an increase in PIT. Such an effect on marrow iron uptake is too rapid to be related to stem cell proliferation; the maximum effect of stem cell differentiation and proliferation of normoblast progeny requires several days.[23] The rapid increase in PIT must then be taken as evidence of a direct action of erythropoietin on hemoglobin synthesis by the existing erythroid cells. This is in agreement with in vitro studies which have indicated an enhancement of hemoglobin synthesis within 24 hours through the action of erythropoietin on DNA-dependent RNA synthesis.[24-28]

Another effect of erythropoietin is to shorten the marrow transit time of radioiron.[29] The time relationships of this effect are illustrated in Figure 2. When radioiron was administered at sea level and the subject later moved to a high altitude, transit time was shortened from the normal of 90 hours to 84 hours, suggesting a slight effect on marrow cells labeled with iron before anoxic stimulation. The shortening of marrow transit time also persisted in the subject injected at high altitude but moved 8 hours later to sea level, suggesting that the action of erythropoietin on marrow transit time did not require continued erythropoietin stimulation.

The removal of the erythropoietin stimulus in Group II did not interfere with completion of maturation of those cells already synthesizing hemoglobin. PIT and reticulocytes remained near normal levels over the following 3 days. This response resembles the wave of erythropoiesis in the polycythemic animal given a single injection of erythropoietin, where cells converted to the stage of hemoglobin synthesis by the injection continue to mature despite the absence of further erythropoietin.[30]

This short study permits only an approximation of the rate of erythropoiesis which followed erythropoietin stimulation. However, it appears from the increase of reticulocytes to 1.6 times normal* and the plasma iron turnover to 1.4 times normal that production was only moderately increased and that the iron going to the marrow may actually have decreased after the second day at high altitude. The red cell balance cannot be calculated in the absence of blood volume measurements; however, considering the decrease in plasma volume which usually occurs,[31] the stable hematocrit with removal of 400 ml. of blood over a week also suggests a production rate of less than 2 times normal. Any of these estimates falls far short of the production rate expected in hemolytic anemia. It has been suggested that the reason for this difference may be the limitation of iron supply to the bone marrow in nonhemolytic states.[32] In the present study (Group I) the decrease in mean plasma iron to 62 μg. per cent and the fall in mean clearance time of radioiron from 71 to 31 minutes are consistent with a restricted iron supply.

Summary

Measurements were made to characterize the relationship between erythropoietin output and erythropoiesis in two groups of subjects, one moved from

*The absolute increase of three times normal in reticulocytes should be reduced to 1.6 by a factor of 47/90 to correct for the longer maturation time of "shift" reticulocytes.[32]

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a sea level habitat to high altitude, and the second moved from a high altitude habitat to sea level. In the first group, there was a latent period of 6 hours followed by a rapid increase in erythropoietin, and a secondary fall to a level of approximately twice normal. The increased erythropoietin stimulus was also reflected in a shortened marrow radioiron transit time. In the second group, there was an initial unexplained rise, after which erythropoietin fell within 8 hours to undetectable amounts.

Elevated erythropoietin was associated in Group I with an increased iron uptake within 24 hours of the stimulus, suggesting a direct action of erythropoietin on hemoglobin synthesis by the existing marrow population. Limitation in erythropoiesis to a rate of less than twice normal was tentatively explained by a restricted iron supply. In the second group, marrow activity continued for 3 days despite a marked fall in erythropoietin, indicating that cells in the maturation phase completed their normal development.

SUMMARIO IN INTERLINGUA

Esseva effectuate mesurationes pro characterisar le relation inter le rendimento de erythropoietina e le erythropoiese in duo gruppos de subjectos, i.e., un gruppo que habeva essite transferite ab domicilios al nivello del mar ad un ambiente de grande altitude e un secunde gruppo que habeva essite transferite ab domicilios in grande altitudes ad un ambiente al nivello del mar. In le prime gruppo, un periodo latente de 6 horas esseva notate, sequite de un rapide augmento in le erythropoietina e un declino secundari a un nivello de approximativemente duo vices le norma. Le augentate stimulo erythropoietinic esseva etiam reflectite in un reducite tempore de transito medullari de sero radioactive. In le secunde gruppo, un inexplicate augmento initial esseva notate, sequite de un declino de erythropoietina intra 8 horas ad nivellos non plus detegibile.

Elevate nivellos de erythropoietina esseva associate in Gruppo I con us augmentate acceptation de sero intra 24 horas post le stimulo, suggestionante un effecto directe de erythropoietina super le synthese de hemoglobina per le existente population medullari. Le reduction del erythropoiese a un intensitate de minus que duo vices le norma esseva tentativemente explicate como effecto de un restringite provision de ferro. In le secunde gruppo le activitate medullari continuava durante 3 dies in despecto de un marcate declino in erythropoietina, indicante que cellulas in le phase de maturation completava lor disveloppamento normal.

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Effect of Altitude on Oxygen Binding by Hemoglobin and on Organic Phosphate Levels

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ABSTRACT The relationship between oxygen dissociation and 2,3-diphosphoglycerate (2,3-DPG) in the red cell has been studied in subjects moving from low to high altitude and vice versa. Within 24 hr following the change in altitude there was a change in hemoglobin affinity for oxygen; this modification therefore represents an important rapid adaptive mechanism to anoxia. A parallel change occurred in the organic phosphate content of the red cell. While this study does not provide direct evidence of a cause-effect relationship, the data strongly suggest that with anoxia, the observed rise in organic phosphate content of the red cell is responsible for increased availability of oxygen to tissues.

INTRODUCTION

At high altitude the affinity of hemoglobin for oxygen is decreased to make hemoglobin-bound oxygen more available to body tissues (1). There is little information on the manner in which this effect is accomplished. Recently Benesch and Benesch (2), Benesch, Benesch, and Yu (3), and Chanutin and Curnish (4) demonstrated that the affinity of a hemoglobin solution for oxygen may be decreased by its interaction with organic phosphates, particularly 2,3-diphosphoglycerate (2,3-DPG) (5). Since this compound is a major component of red cell organic phasphate, it may play an important role in controlling the equilibrium of oxygen with hemoglobin in vivo. The present study was undertaken to examine precise changes of the oxygen dissociation curve as an early adaptive mechanism to changes in environmental oxygen partial pressure, and to determine the relationship between oxygen dissociation and red cell organic phosphate concentration in subjects moved from one altitude to another.

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METHODS

Seven subjects who resided at sea level in Lima, Peru, were taken to Morococha (elevation 4530 m, $P_{O_2} = 84$ mm Hg) where they stayed for 144 hr before returning to sea level. Before ascent, baseline values for O_2Hb dissociation of intact red cells and hemolysates, blood organic phosphates, and standard hematologic measurements were obtained. Hematologic values are reported elsewhere.[1] Further values were obtained during the 6 day period at high altitude and again at sea level. A second group of six subjects who resided in Morococha were studied there and after their descent to sea level. Venous blood collected in heparin was used in all measurements.

Oxygen dissociation curve. The O_2Hb dissociation curve was measured by the mixing technique (6) whereby saturation is predetermined and P_{O_2} is the unknown parameter. Each sample was divided into two equal portions, of which one was totally reduced and the other fully oxygenated by equilibration at 37°C. The samples were then mixed to achieve approximately 50% saturation, and the partial pressure of oxygen and the pH were measured in this mixture. To standardize the results, two corrections were made. One consisted of converting the measured P_{O_2} to that of a saturation of 50% using Hill's expression ($\log S/100 - S = n \log P_{O_2}$, in which $n = 2.65$) and the other of correcting P_{O_2} for a pH of 7.4 us-

[1] Faura, J., J. Ramos, C. Reynafarje, E. English, P. Finne, and C. A. Finch. Effects of altitude on erythropoiesis. In preparation.

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ing the factor $\Delta \log Po_2/\Delta pH = -0.48$ (7). All the results given in this paper are expressed as the oxygen tension in millimeters of mercury required to obtain an oxygen saturation of 50% at 37°C and pH 7.4 [P_{50} (7.4)]. This P_{50} was also determined in hemolysates prepared by the addition of saponin prior to equilibration.

The Po_2 and pH were measured using a Beckman 160 gas analyzer equipped with a Beckman macro Po_2 electrode (Beckman Instruments, Inc., Fullerton, Calif.) mounted in a microcuvette (0.03 ml) and a micro pH assembly.

Organic phosphates. 4 ml of blood were pipetted into centrifuge tubes, and the cells were washed three times with cold saline. The white cells were not removed with the supernatant since it was established that white cells contain only about 3% of the 2,3-DPG in normal blood. The acid-soluble phosphates in packed cells were extracted and separated according to the method of Robinson, Loder, and deGruchy (8). The method was modified so that four fractions were collected. These fractions contained the following compounds:

(*a*) 50 ml water + 50 ml 0.02 N HCl	-inorganic phosphate, AMP, ADP hexose monophosphates, TPN, MPG,
(*b*) 100 ml 0.1 N NH_4Cl	-hexose diphosphates,
(*c*) 100 ml 0.2 N NH_4Cl	-2,3-DPG,
(*d*) 100 ml 0.5 N NH_4Cl	-ATP

The fractions were analyzed for total phosphate content using the method of Bartlett (9). Using this technique we were able to recover 85–90% of 2,3-DPG or ATP added to a hemolysate before extraction. The range of values for 2,3-DPG and ATP in 12 normal subjects residing in Seattle was for 2,3-DPG, 186–309 μgP/ml packed cells (mean 229, SD 34); and for ATP, 71–100 μgP/ml packed cells (mean 80, SD 7.6). These values are similar to those reported by Robinson et al. (8).

RESULTS

Group I. The P_{50} sea level value of 26.6 mm Hg (SD ±0.3) of the first group increased to 28.6 ±0.6 within 12 hr after ascent to Morococha and to 31.0 ±0.4 at 36 hr. This increase was sustained during the 5 days at high altitude but fell after descent and was found to be 27.3 ±0.7 by 60 hr (Fig. 1).

The mean concentration of 2,3-DPG at sea level was 90 ±11 μg phosphorus/ml of blood. This increased to 142 ±8 at 36 hr after moving to high altitudes; at 132 hr it was 140 ±10. After return to sea level this value fell to 87 ±13.

Group II. Natives of Morococha had a P_{50} of 30.7 ±0.6 mm Hg and a 2,3-DPG concentration of 155 ±14 μg phosphorus/ml of blood. On descent to sea level the Po_2 decreased to 28.6 ±0.5 mm Hg of 12 hr and to 27.3 ±0.8 at 36 hr. The corresponding level of 2,3-DPG at 36 hr was 109 ±13. The final measurements made 84 hr after descent showed a Po_2 of 28.3 ±0.6 and a 2,3-DPG level of 87 ±14. These changes are shown in Fig. 2.

Changes in the same direction occurred in the ATP content of the red cell, but proportionately to 2,3-DPG, these amounted to only 1/10 in group I and 1/3 in group II. Changes in the phosphate content of fractions 1 and 2 were negligible. In both groups the P_{50} of hemolyzed blood was parallel to, but much lower than changes in whole blood (Tables I and II).

The mean reticulocyte count of subjects in group I was less than 2% during the first 2 days at high altitude and less than 5% during the 1st week.[1] Thus the changes observed could not be ascribed to any significant ingress of young erythrocytes.

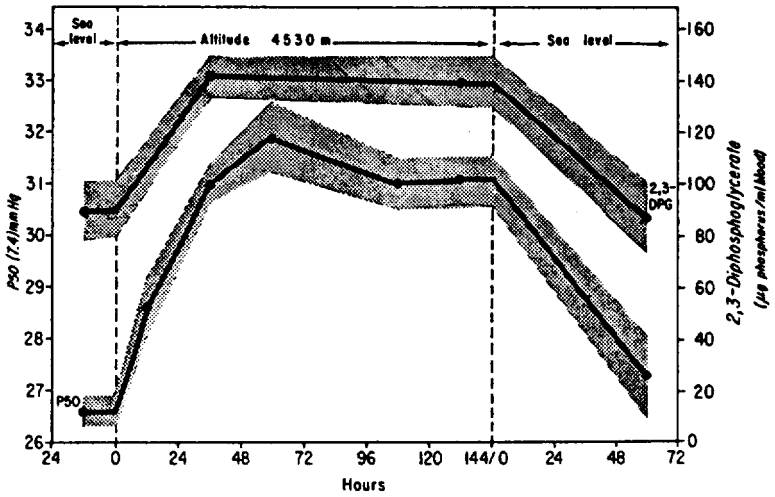

FIGURE 1 Changes in P_{50} (7.4) and 2,3-DPG of whole blood induced by high altitude. Mean values are shown by the lines, one standard deviation is indicated by the shaded area. Parallel increases with exposure to high altitude and decreases on return to sea level were observed.

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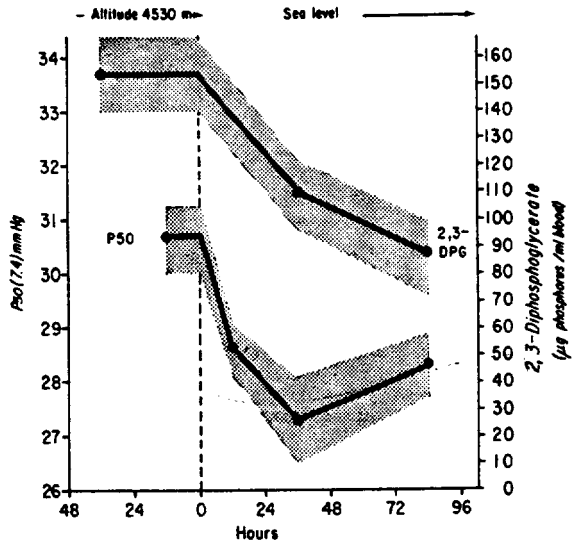

FIGURE 2 Changes in P_{50} (7.4) and 2,3-DPG of whole blood in natives of high altitude on descent to sea level. The shaded area represents one standard deviation.

DISCUSSION

The present study shows that in response to high altitude hypoxia, the shift of the O_2Hb dissociation curve occurs rapidly. Within 2 days after ascent to 4530 m, the P_{50} of lowlanders reached the value prevailing in residents of the highlands. This change provides an important physiological advantage by making more oxygen available to the tissues at any given oxygen tension (1). There is consequently less need for increasing the cardiac output, a mechanism which would serve the same purpose but at a greater cost to body economy. Conversely the suppression of the hypoxic stress in highlanders moved to sea level causes a rapid decrease in the P_{50}. The response therefore appears to relate to oxygen supply and tissue oxygen tension and does not correlate with the level of hemoglobin.

The similar changes in oxygen dissociation of intact red cells and hemolysates suggests that the effect is not a function of the red cell membrane. It may, however, be related to the concomitant changes in red cell organic phosphate. Benesch et al. (3) and Garby and deVerdier (10) have shown that 2,3-DPG forms a complex with purified deoxyhemoglobin and in doing so, reduces the affinity of hemoglobin for oxygen. The parallel changes in 2,3-DPG and oxygen dissociation found in subjects moved from one altitude to another might well represent the same phenomenon. Other examples of this relationship exists. Akerblom, deVerdier, Garby, and Hogman (5) found a linear relationship between P_{50} and the sum of the molar concentrations of 2,3-DPG and ATP in stored blood; others have observed progressive decreases in organic phosphate and increases in hemoglobin affinity for oxygen during storage (11–14). Likewise, blood transfused after storage shows a return to normal of both oxygen dissociation (2) and organic phosphate (4) in vivo. In anemic states oxygen dissociation curves shift to the right (5–7) and in at least one type of anemia the organic phosphate has been reported as increased (8).

Benesch and Benesch (9) have suggested a

TABLE I
Group I

Time at high altitude, *hr*		−12	+36	+132	+204
P_{50} (7.4), *mm Hg*	whole blood	26.6 ±0.3	31.0 ±0.4	31.1 ±0.5	27.3 ±0.8
	hemolysate	18.5 ±1.2	20.7 ±1.2	22.2 ±1.2	20.3 ±0.9
Organic phosphate					
2,3-DPG	µgP/ml blood	90 ±11	142 ±8	140 ±10	87 ±14
	µgP/g Hb	588 ±74	912 ±80	870 ±98	554 ±107
	Molar ratio	0.61 ±0.07	0.94 ±0.08	0.90 ±0.10	0.57 ±0.11
ATP	µgP/ml blood	36 ±6.7	45 ±3.6	47 ±4.6	37 ±3.3
	µgP/g Hb	238 ±41	285 ±20	302 ±42	234 ±23
	Molar ratio	0.16 ±0.03	0.20 ±0.01	0.21 ±0.03	0.16 ±0.01
Fraction 1	µgP/ml blood	14 ±2.2	17 ±0.9	15.7 ±1.0	14.0 ±2.4
Fraction 2	µgP/ml blood	12 ±2.6	13 ±2.0	12.8 ±1.7	12.0 ±4.0

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TABLE II
Group II

Time at low altitude, *hr*		−36	+12	+36	+84
P_{50} (7.4) mm Hg	Whole blood	30.7 ±0.6	28.6 ±0.5	27.3 ±0.8	28.3 ±0.6
	Hemolysate	24.1 ±0.8	22.0 ±1.1	21.1 ±1.5	22.5 ±1.0
Organic phosphate					
2,3 DPG	μgP/ml blood	155 ±14		109 ±13	87 ±14
	Molar ratio	0.80 ±0.08		0.58 ±0.03	0.49 ±0.08
ATP	μgP/ml blood	70 ±17		48 ±13	38 ±12
	Molar ratio	0.23 ±0.05		0.16 ±0.03	0.13 ±0.04
Fraction 1	μgP/ml blood	20 ±1.6		18 ±0.2	12 ±1.9
Fraction 2	μgP/ml blood	16 ±2.2		16 ±1.3	10 ±2.6

sequence of events to account for the increase in 2,3-DPG in the anoxic state. Since organic phosphate reacts only with reduced hemoglobin, the greater the anoxia the greater the amount of reduced hemoglobin present and the larger the amount of 2,3-DPG bound. This binding depletes the soluble organic phosphate in the cell, and glycolysis is stimulated to produce more 2,3-DPG. Increased glycolysis and organic phosphate production has been shown to occur in vitro in deoxygenated blood (*20*). Such a mechanism would automatically control the balance between oxyhemoglobin and deoxyhemoglobin and thus would counteract the effects of an increase in reduced hemoglobin.

Although an increase in pH has been found to cause an increase in 2,3-DPG (21), the changes in 2,3-DPG observed in our two groups of subjects cannot be accounted for by the acid-base status usually observed in subjects going to altitude or in natives before and after descent to sea level. While it is a common finding that alkalosis develops rapidly and persists in lowlanders subjected to altitude hypoxia, it is also recognized that natives of the highland differ by maintaining a normal pH (22, 23). Therefore, the increase in 2,3-DPG in lowlanders and the prevailing high level of organic phosphate in natives cannot be ascribed to the pH. Furthermore, as natives do not become acidotic upon descending to sea level, their decrease in 2,3-DPG cannot be explained by such a mechanism.

ACKNOWLEDGMENTS

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September 11, 1969

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RLO-1375-15	"Low Temperature Resistometric Properties of Titanium Alloys" by T.S. Luhman, R. Taggart and D.H. Polonis
RLO-1925-28	"Construction of Commuting Quasiparticle and Phonon Operators" by A. Goswami, O. Nalcioglu and A.I. Sherwood
RLO-1925-29	"Pushing-Up Effect in Particle (Hole) Spectra Near Closed Shells" by A. Goswami
RLO-2048-4	"An Investigation into the Factors Regulating Erythropoiesis in Subjects Residing at Various Altitudes" by C. A. Finch, M.D., (Progress Report)

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Appendix 1

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Effect of Altitude on Oxygen Binding by Hemoglobin and on Organic Phosphate Levels

CLAUDE LENFANT, JOHN TORRANCE, EUGENIA ENGLISH, CLEMENT A. FINCH, CESAR REYNAFARJE, JOSE RAMOS, and JOSE FAURA

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At high altitude the affinity of hemoglobin for oxygen is decreased to make hemoglobin-bound oxygen more available to body tissues (1). There is little information on the manner in which this effect is accomplished. Recently Benesch and Benesch (2), Benesch, Benesch, and Yu (3), and Chanutin and Curnish (4) demonstrated that the affinity of a hemoglobin solution for oxygen may be decreased by its interaction with organic phosphates, particularly 2,3-diphosphoglycerate (2,3-DPG) (5). Since this compound is a major component of red cell organic phasphate, it may play an important role in controlling the equilibrium of oxygen with hemoglobin in vivo. The present study was undertaken to examine precise changes of the oxygen dissociation curve as an early adaptive mechanism to changes in environmental oxygen partial pressure, and to determine the relationship between oxygen dissociation and red cell organic phosphate concentration in subjects moved from one altitude to another.

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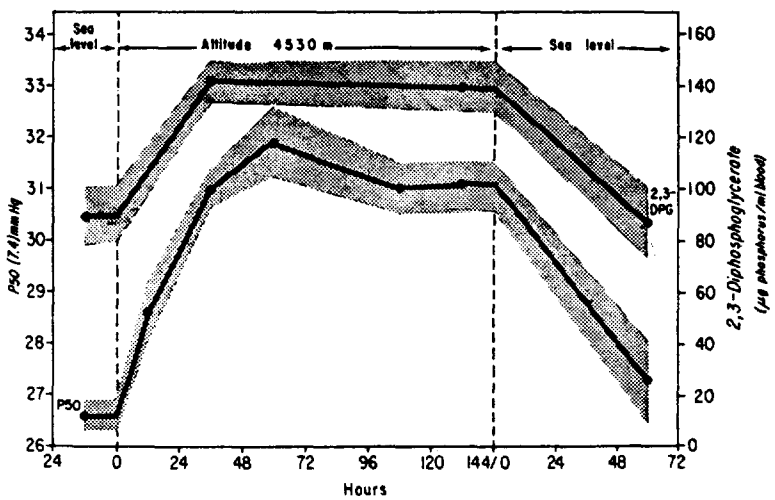

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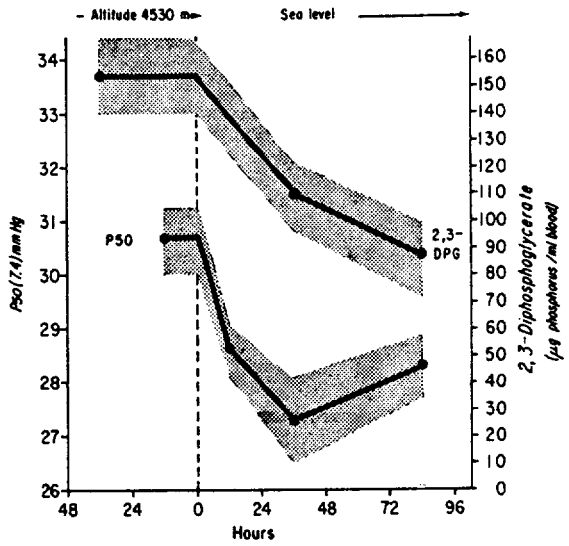

FIGURE 2 Changes in P_{50} (7.4) and 2,3-DPG of whole blood in natives of high altitude on descent to sea level. The shaded area represents one standard deviation.

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	µgP/g Hb	238 ±41	285 ±20	302 ±42	234 ±23
	Molar ratio	0.16 ±0.03	0.20 ±0.01	0.21 ±0.03	0.16 ±0.01
Fraction 1	µgP/ml blood	14 ±2.2	17 ±0.9	15.7 ±1.0	14.0 ±2.4
Fraction 2	µgP/ml blood	12 ±2.6	13 ±2.0	12.8 ±1.7	12.0 ±4.0

1190371

TABLE II
Group II

Time at low altitude, *hr*		−36	+12	+36	+84
P_{50} (7.4) mm Hg	Whole blood	30.7 ±0.6	28.6 ±0.5	27.3 ±0.8	28.3 ±0.6
	Hemolysate	24.1 ±0.8	22.0 ±1.1	21.1 ±1.5	22.5 ±1.0
Organic phosphate					
2,3 DPG	μgP/ml blood	155 ±14		109 ±13	87 ±14
	Molar ratio	0.80 ±0.08		0.58 ±0.03	0.49 ±0.08
ATP	μgP/ml blood	70 ±17		48 ±13	38 ±12
	Molar ratio	0.23 ±0.05		0.16 ±0.03	0.13 ±0.04
Fraction 1	μgP/ml blood	20 ±1.6		18 ±0.2	12 ±1.9
Fraction 2	μgP/ml blood	16 ±2.2		16 ±1.3	10 ±2.6

sequence of events to account for the increase in 2,3-DPG in the anoxic state. Since organic phosphate reacts only with reduced hemoglobin, the greater the anoxia the greater the amount of reduced hemoglobin present and the larger the amount of 2,3-DPG bound. This binding depletes the soluble organic phosphate in the cell, and glycolysis is stimulated to produce more 2,3-DPG. Increased glycolysis and organic phosphate production has been shown to occur in vitro in deoxygenated blood (20). Such a mechanism would automatically control the balance between oxyhemoglobin and deoxyhemoglobin and thus would counteract the effects of an increase in reduced hemoglobin.

Although an increase in pH has been found to cause an increase in 2,3-DPG (21), the changes in 2,3-DPG observed in our two groups of subjects cannot be accounted for by the acid-base status usually observed in subjects going to altitude or in natives before and after descent to sea level. While it is a common finding that alkalosis develops rapidly and persists in lowlanders subjected to altitude hypoxia, it is also recognized that natives of the highland differ by maintaining a normal pH (22, 23). Therefore, the increase in 2,3-DPG in lowlanders and the prevailing high level of organic phosphate in natives cannot be ascribed to the pH. Furthermore, as natives do not become acidotic upon descending to sea level, their decrease in 2,3-DPG cannot be explained by such a mechanism.

ACKNOWLEDGMENTS

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1190373

R-L 2048-5

THE PRECISION IN VIVO MEASUREMENT OF RADIOIRON BY WHOLE-BODY COUNTING

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RUNNING TITLE: Precision Whole-Body Counting

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1190374

Summary

The in vivo counting system described will accurately measure the absolute level of total body ^{59}Fe regardless of body size or isotope distribution. These results are accomplished by (1) counting only the Compton scatter events emitted from the body rather than the full energy events from unscattered photons, (2) employing an optimum arrangement of multiple NaI crystal detectors, and (3) surrounding the subject with tissue equivalent plastic pellots. This counting system provides an accurate and efficient means for studying iron absorption or loss in man.

1190375

1. Introduction

During the past two decades, ^{59}Fe has been used extensively to measure body iron exchange in man. The use of ^{59}Fe requires a method that will accurately reflect the total body isotope content regardless of its distribution. Whole-body counters can provide an accurate measure of the body content of an isotope provided the isotope reaches a constant distribution within the body and provided body size can be standardized or allowance made for differences. ^{40}K, ^{137}Cs and ^{24}Na are good examples of isotopes that can be measured reliably by most total body counters regardless of the type of geometry or radiation detector used because of their uniform body distribution. Other isotopes such as ^{59}Fe and ^{65}Zn do not reach a fixed distribution until one to several weeks after their administration. Thus changes in counting efficiency have occurred due to changes in self-absorption of gamma rays within the body. Warner and Oliver (1966) have described a whole-body counting method for ^{59}Fe in which errors due to absorption of gamma rays within the body were minimized by using two opposing collimated detectors and employing the combined count of the Compton scatter and photopeak region of the energy spectrum. Appreciable changes in detector response still occurred, however, between a localized and distributed source of activity. Our objective has been to develop a whole-body counter designed specifically for iron absorption measurements using the principles of the Warner-Oliver counter but attempting to overcome the residual geometrical and absorption errors in measuring subjects of varying body size.

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2. Preliminary studies

Several counter arrangements have been developed which give a constant count rate from a point source in air when placed in any position normally occupied by the body, but when the source is placed in various positions within a water-filled phantom, count rates may vary by as much as a factor of 2. However, studies with such a counter (Palmer and Langford 1967) show that if the Compton scatter region of the gamma ray spectrum is used rather than the photopeak region which is normally used, the count rate becomes independent of the position of the source. Table 1 shows a comparison of counts in the Compton scatter region versus the photopeak region for a ^{137}Cs source (gamma ray energy = 0.663 MeV) placed in various positions from the center to the outside of a 10" diameter phantom. All the counts in the Compton scatter region were within ± 1.6 per cent, whereas with photopeak energies, the outside position counted 83 per cent higher than the center position.

Since the body is not circular in shape but more nearly elliptical, an ellipsoidal water phantom was constructed from styrofoam plastic to provide phantom walls of low mass. The water-filled phantom had a minor axis of 9", a major axis of 15-1/2" and was 12" high. Four 6" diameter by 4" thick NaI(Tl) detectors were placed around the phantom as shown in figure 1, and their optimum position was empirically determined such that a point source of ^{59}Fe placed anywhere in the phantom on a plane parallel to the center of all four detectors gave the same count rate in the Compton scatter

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region. The optimum arrangement of the detectors for ^{59}Fe gamma rays is 17" between the faces of the two vertical sets of opposing detectors and 15" between the sides of the detectors in the two horizontal sets.

3. Whole body counter description

The detectors in the arrangement described above were placed in a shadow shield type (Palmer and Roesch 1965) whole-body counter shown in figure 2. This counter was designed to minimize the lead brick shielding required. The subject is transported in a mobile carrier through and completely beyond the major viewing area of the detectors.

The shielding weighs about 5 tons. The center section of 4" thick lead shielding is 39" high, 16" wide and 36" long. The 2" thick shadow shielding extends out 24" from each side of the center section and is constructed so that background gamma rays from the surrounding room cannot directly enter any of the detectors without going through at least 3" of lead. The opening through the lead bricks for the patient and carrier to pass through the counter is centered between the four detectors and is 20" wide and 12" high. The total length of the counter is 15-1/2'. The carrier is driven by a stepping motor and chain drive that is controlled by a constant speed pulser, or when a multi-channel analyzer is used, the speed can be controlled by pulses from the live time scaler of the analyzer so that errors caused by dead time of the analyzer are eliminated (Sheen, Palmer and Stringer 1967). The carrier is made of 1/8" thick aluminum and the inside dimensions are 8" deep, 18-1/4" wide and 7' long. The radius

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of curvature of bottom corners of the carrier is 3". The cost of the counter as shown in figure 2 without the four detectors and counting instruments was about $3,000 and the cost of the detectors was approximately $10,000.

The rather small tunnel through which the patient travels during a traverse count has not presented any problems. However, the patients would feel less confined during the counting procedure if the tunnel dimensions were enlarged with the addition of more lead bricks to provide a proportional increase in shadow shielding.

4. Characteristics of the counter

The best results with patients would be expected if they were of the same size and had the same general body contour. This same effect can be accomplished, however, by layering small pellets of a heavy plastic called Delrin (manufactured by dePont Chemical Company) over the person until level with the top of the aluminum carrier. The Delrin plastic has a density of 1.4 but the bulk density of the small 1/8" pellets is about 1.0 or about the same as the human body. A point source of ^{59}Fe placed within a Delrin phantom provides a useful means of assessing the distributional effects of ^{59}Fe activity as they might occur in vivo.

When a source of ^{59}Fe was placed anywhere in the Delrin-filled carrier, the accumulated count rate during a 10 minute traverse of the carrier was within ± 4 per cent for all positions in a Delrin thickness of 8-1/2". This was similar to the results obtained

earlier in the water phantom even though the shape and width of the carrier was slightly different. Figure 3 shows the relative count rate of the Compton scatter events as a point source is placed in various positions in a vertical cross section of Delrin pellets. As the thickness of Delrin is increased up to as much as 11-1/2", the average count rate of a point source positioned within the Delrin decreases slightly while the variation in count rates at various positions increases slightly.

Figure 4 compares the energy spectra of two measurements of a point source of ^{59}Fe placed at two extreme positions in Delrin. When the source was placed on top and near the side of the Delrin phantom curve (a) was obtained, and when the source was in the center, curve (b) was obtained. A large reduction in photopeak counts was obtained as the source was moved into the center because of increased absorption of the gamma rays whereas the area under the Compton scatter region remained nearly the same. For the latter energy, a slight reduction in the region of 800 keV was offset by a comparable increase in counts below 500 keV with the result that the integral count rate between 90 and 960 keV remained constant. This explains why the use of the Compton scatter photons provides a count that is independent of the location or distribution of the radioactivity.

Figure 5 shows a spectrum from a 10 minute count of 1 μCi of ^{59}Fe in a human subject packed in Delrin after the background of the counter and the subject have been subtracted. The Compton scatter region of energy used in all studies includes all events between 90

and 960 keV and is shown by the shaded region of the spectrum. The 10 minute net count rate in the Compton scatter region was 144,000 with a background of 30,500 whereas photopeak measurements gave values of 43,000 and 4,200 respectively. The sensitivity with photopeak counting is about 1.6 times higher than that using the Compton scatter events, but the greater absolute accuracy obtained with the Compton scatter counts makes their use more attractive. The background count of a subject added from 1,500 to 2,000 counts to the counter background depending on the body size. The counter background remained quite constant from day to day within a three-week period. Background counts measured during a 10-minute traverse on 40 occasions over a one-month interval gave a standard deviation which was 2.2 times the theoretical standard deviation for a single background count as determined by the Poisson distribution for radioactive decay, indicating excellent day-to-day stability of counter background. To obtain counting rates with low statistical error, a minimum ^{59}Fe whole body activity of 0.21 uCi is required which gives a net whole body count rate equal to the counter background and a standard deviation of ± 1 per cent for a standard 10-minute traverse.

5. Discussion

Several investigators have obtained improved whole-body counting results with several different isotopes using the combined counts of the photopeak and scatter region (Gibbs, Hodges and Lushbaugh 1966; Warner and Oliver 1966; and Dudley and van Hain 1968),

and it has been suggested (Dudley and van Hain 1968) that the majority of gamma rays which are emitted in the body with energies exceeding 1.0 MeV ultimately emerge from the surface although their energy may be reduced and their angular distribution altered by Compton collisions. Although this suggests that both photopeak and Compton events should be used, much more constant counting rates can be obtained when only Compton scatter counts are used. Sargent and Plycase ( ) have also shown that the use of only the Compton scatter region provides a count rate more independent of distribution than that using photopeak counts. Measurements on phantoms reported here indicate that the total body content of ^{59}Fe can be determined in any size person within a maximum error of ± 2 per cent when using the Compton scatter energy band. The application of this Compton scatter technique counting to gamma ray energies that are different from those of ^{59}Fe have not been investigated. The data shown in table 1 indicate that the method will work with gamma ray energies as low as 0.66 MeV, but a slightly different detector arrangement may be required for different energies.

The counter employed here is simple to use and requires rather unsophisticated instrumentation because of the broad band of photon energies used. All whole body counts can be compared directly to that of a standard which is measured with identical counting efficiency when placed in a Delrin phantom. A multi-channel analyzer was used in these studies but a single-channel analyzer and scaler will adequately perform the counting. Slight instrumental changes

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affect standard and subject comparably so that the relationship between the two is unchanged. Counter materials are relatively inexpensive and easily assembled. Like other types of shadow shield counters it can be easily disassembled and built up in a different location or can be mounted in a truck to service several locations.

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1190384

TABLE 1

COMPARISON OF COUNT RATE IN COMPTON SCATTER REGION WITH THAT IN PHOTOPEAK REGION FOR A POINT SOURCE OF ^{137}Cs IN CYLINDRICAL WATER PHANTOM

Source Position	Compton Scatter (counts/min)	Photopeak (counts/min)
Center of phantom	67,662	30,919
From center: 1"	68,170	31,943
2"	68,357	34,343
3"	69,231	38,207
4"	69,453	44,572
5"	67,216	56,508
Average	68,348	39,398
Range	+ 1.6 % - 1.6 %	+ 43 % - 22 %

FIGURE CAPTIONS

FIGURE 1. Water Phantom Used to Determine Optimum Crystal Position

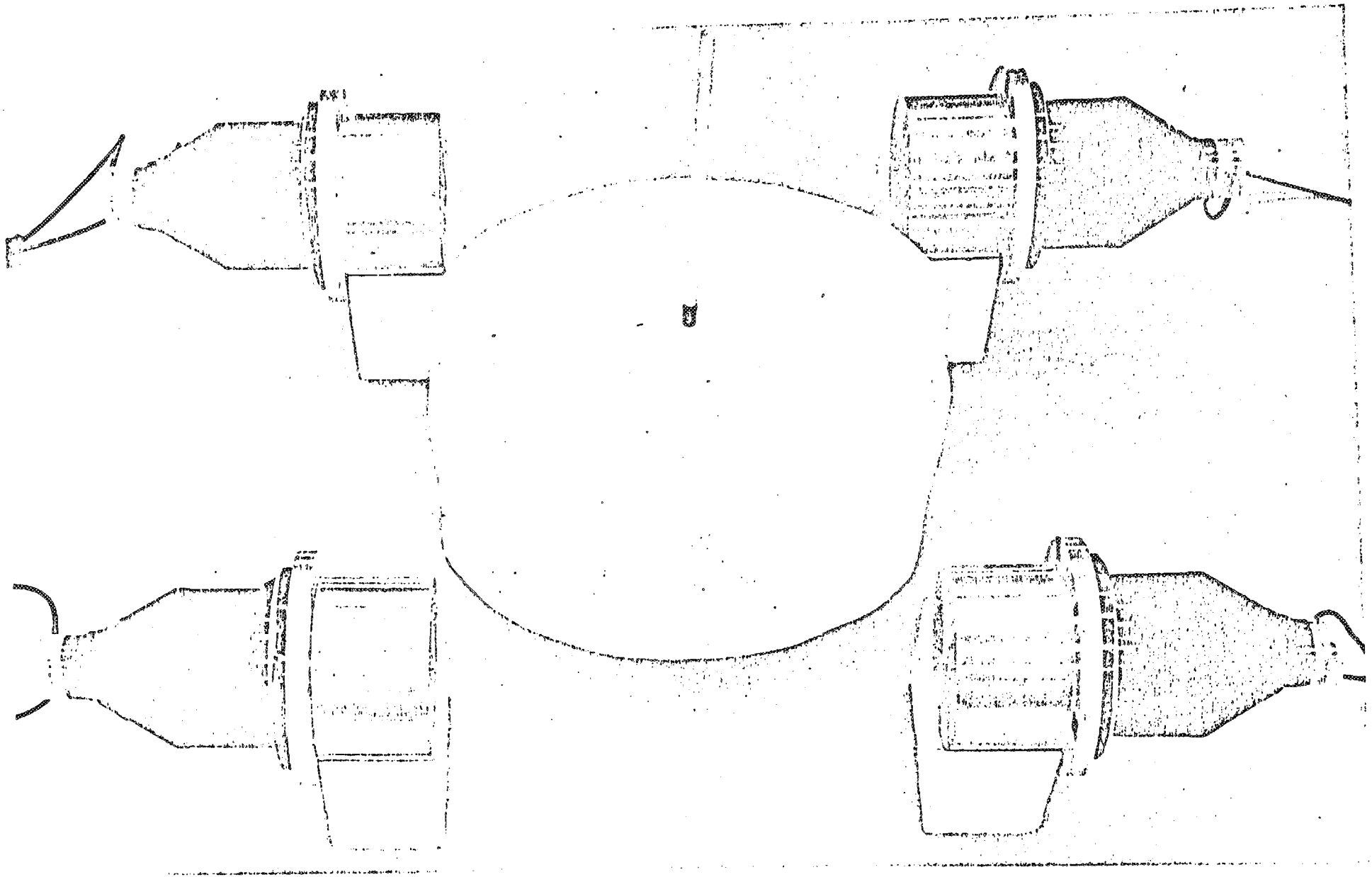
FIGURE 2. ^{59}Fe Whole-Body Counter Demonstrating the Use of Delrin Plastic Placed Around the Subject to Obtain Uniform Thickness

FIGURE 3. Relative Count Rates Obtained with Different Locations of a Point Source of ^{59}Fe in a Vertical Cross Section of Delrin Plastic Pellets

FIGURE 4. Gamma Ray Spectrum of ^{59}Fe From Two Different Conditions of Absorption by Tissue Equivalent Material

FIGURE 5. Gamma Ray Spectrum of a Whole Body Count in a Subject Containing 1 uCi of ^{59}Fe

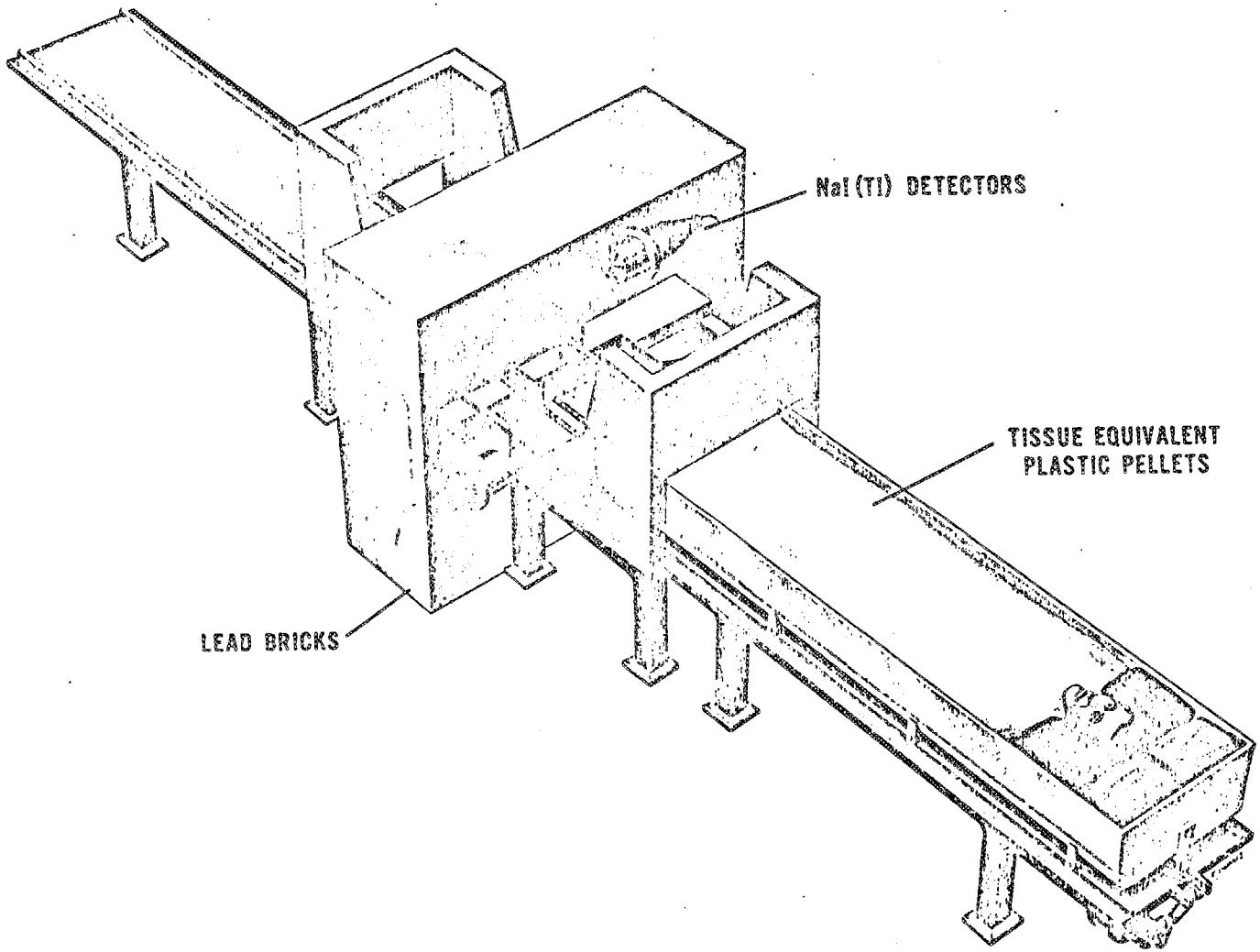
1190386



1190387

FIGURE 2

PRECISION Fe^{59} WHOLE BODY COUNTER



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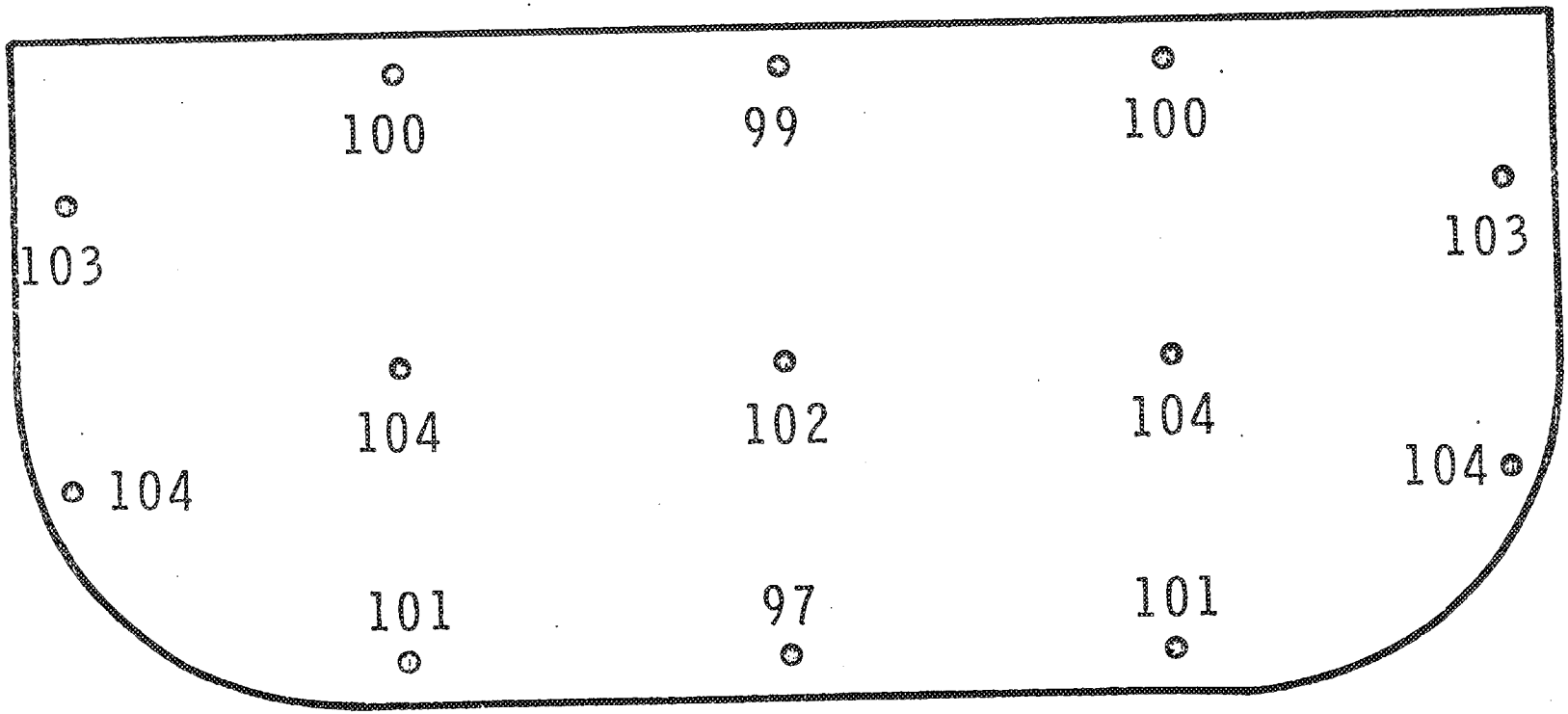
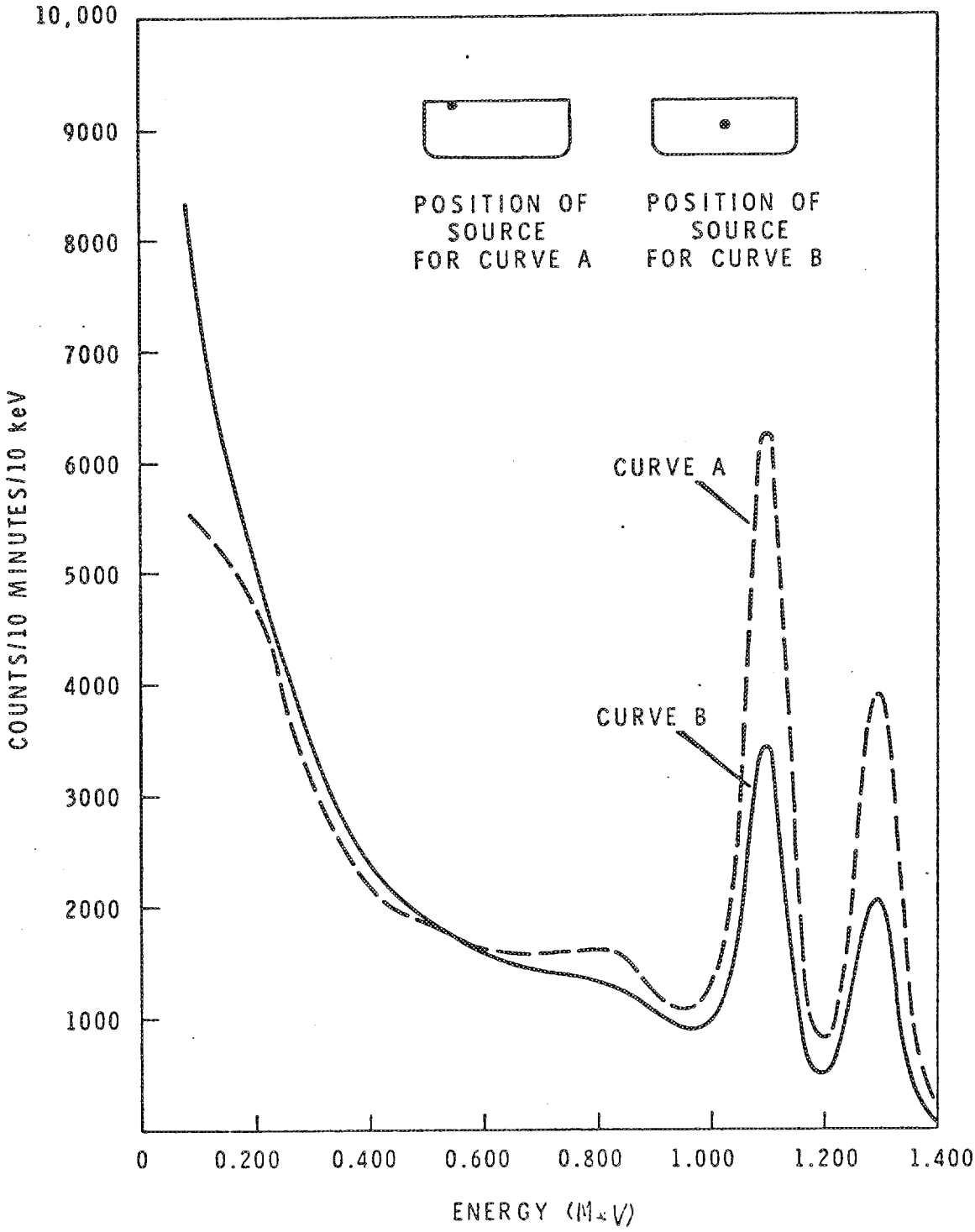
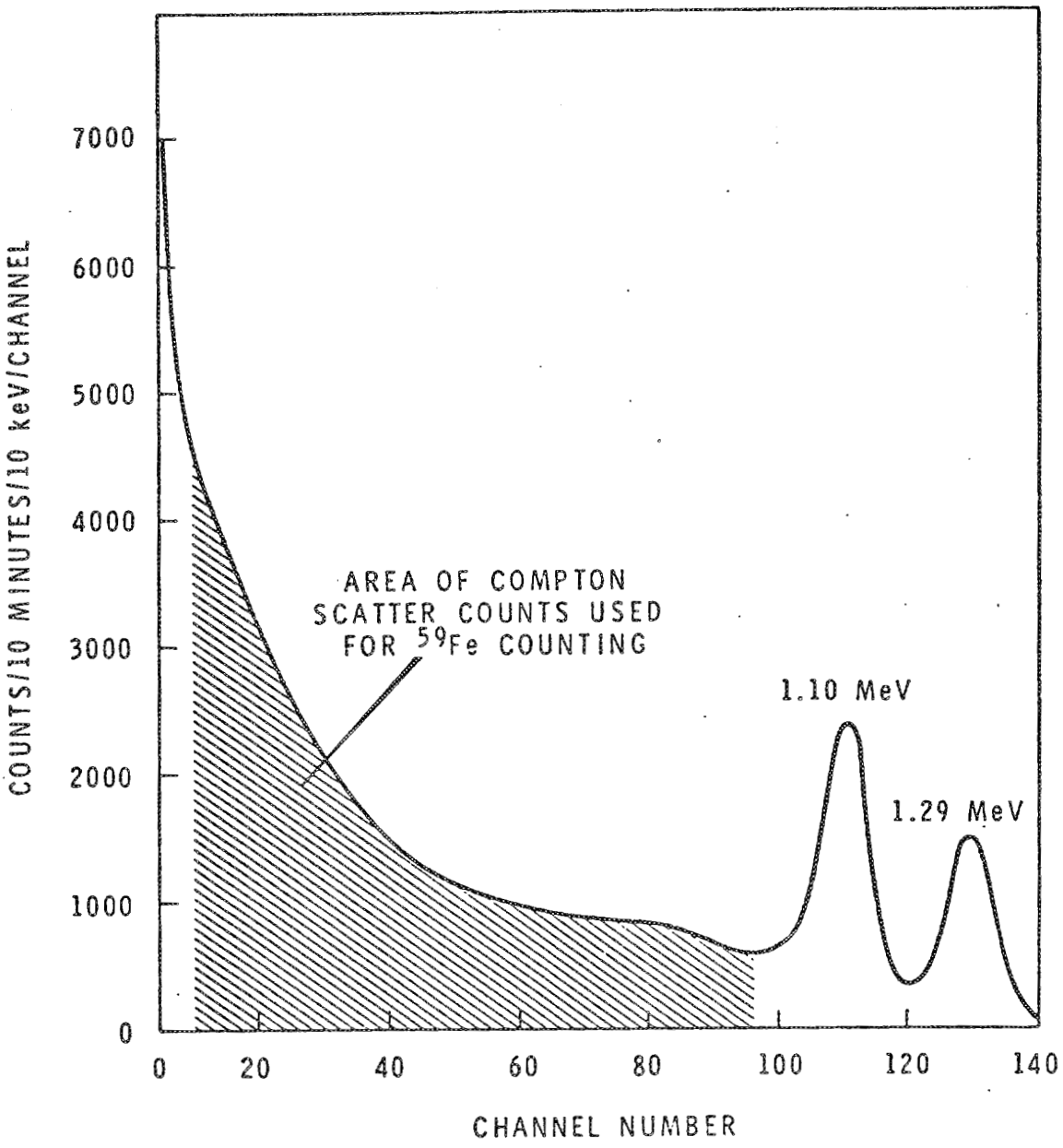


1190389


10,000
9000
8000
7000
6000
5000
4000
3000
2000
1000
COUNTS/10 MINUTES/10 keV
POSITION OF SOURCE FOR CURVE A
POSITION OF SOURCE FOR CURVE B
CURVE A
CURVE B
0
0.200
0.400
0.600
0.800
1.000
1.200
1.400
ENERGY (MeV)

1190390


COUNTS/10 MINUTES/10 keV/CHANNEL
7000
6000
5000
4000
3000
2000
1000
0
AREA OF COMPTON
SCATTER COUNTS USED
FOR ^{59}Fe COUNTING
1.10 MeV
1.29 MeV
0
20
40
60
80
100
120
140
CHANNEL NUMBER

1190391

RLC-2048-

THE MEASUREMENT OF IRON ABSORPTION BY WHOLE-BODY COUNTING

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1190392

Summary

A method of whole-body counting which employs longitudinal scan geometry, a unique arrangement of multiple NaI(Tl) detectors and measurement of photons in the region of Compton or scattered energy has been evaluated. Radioactivity has been quantitated from a point source embedded in plastic pellets (Delrin), from isotope localized in the gastrointestinal tract of the subject, and during movement of in vivo radioiron into and out of the bone marrow. Counting levels in all instances have been shown to vary by no more than ± 2 per cent regardless of the location and distribution of radioiron. In addition, measurements have been carried out over a two-week period after oral or intravenous administration to demonstrate the day-to-day reproducibility of counting and to establish the quantitative validity of measuring radioiron absorption and losses by this technique. These studies provide evidence of the suitability of this technique for measurement of iron absorption.

1190393

1. Introduction

The predominant control of iron balance in man by its intestinal assimilation has placed considerable clinical importance on accurate measurements of iron absorption. Earlier studies involving methods of clinical iron balance impose considerable technical difficulties and were not sufficiently quantitative to measure the very limited iron exchange between man and his environment. Radioisotope techniques have been more useful. Initially, radioiron balance studies were carried out in which absorption was calculated from the difference between radioiron administered and that appearing in stools. Absorption was also estimated from the amount of radioiron appearing in newly formed red cells. Despite extensive use, both of these isotope methods have disadvantages. The technique of radioiron balance has the major drawback that subjects must be admitted to a special metabolic ward if complete collections are to be ensured. Measurements of red cell radioactivity are simpler to perform, but are valid only if the incorporation of radioiron by the erythroid marrow is known to be normal. A compensation for variable utilization is achieved in the double isotope method (Saylor and Finch 1953) in which a second isotope of iron is given intravenously at the time of the oral dose. The greater precision of the dual tracer method is, nevertheless, achieved at the expense of more difficult methods of dose administration and isotope counting. Furthermore, it may be invalid in patients with saturated transferrin due to the direct hepatic uptake of absorbed iron (Wheby and Umpierre 1964).

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The theoretical advantages of a method by which retained whole-body activity could be measured directly has led to much interest in whole-body counting during the past decade. A major shortcoming of this method, however, has been the effect on counting efficiency of the body distribution of radioactivity. The present report deals with a technique for measuring absolute levels of whole-body ^{59}Fe which is unaffected by differences in the tissue location of activity or the physical size of the subject.

2. Methods

The subjects of the study were healthy male and female subjects between the ages of 25 and 40 years. All studies were performed with 1 μc dose of ^{59}Fe (specific activity, 13 mc/mg). Oral test doses were given after an overnight fast in 50 ml of water containing 3 mg of elemental iron as ferrous sulphate and 2 M of ascorbic acid/M of iron. For parenteral administration, carrier-free ^{59}Fe was mixed with a 50:1 molar excess of sodium citrate and bound before injection to 5 ml fresh plasma.

A description of the whole-body counter and the theoretical considerations which led to its design are outlined in the accompanying report (Palmer, Cook and Pailthorp, in preparation). Briefly, the technique involves transporting the patient in a mobile aluminum carrier through a detecting field of an array of 4 NaI(Tl) detectors shielded with lead bricks by the "shadow" principle. The combined count from all detectors is accumulated during a single traverse of

1190395

the carrier which begins and ends beyond the major viewing area. The Compton scatter region of photon energy was counted while higher photopeak energies usually employed in scintillation counting were excluded. A small plastic pellet marketed commerically as Delrin (dePont Chemical Company) and having a bulk density comparable to that of the human body was employed to permit direct comparison of in vivo activity with that of a standard counted as a point source.

The protocol for measuring body ^{59}Fe activity was similar for all studies. A counting standard of ^{59}Fe was employed to eliminate the effects of decay and day-to-day changes in counting sensitivity. To reduce the counting time, the standard was placed in a fixed and reproducible position between the detectors and counted for one minute. Other measurements were made with a 10-minute traverse time. Prior to administration of the dose, traverse counts were obtained to determine the counter in patient background. The difference between these values which were unaffected by filling the carrier with Delrin, was added to the counter background measured at the time of all subsequent in vivo counts. A traverse count of the test dose was made by placing the vessel or syringe containing the dose to be administered in a plastic sack and burying it centrally in the carrier filled with Delrin. The volume of the latter was adjusted to the same thickness as the trunk of the subject. Appropriate corrections for

administered activity were made by subtracting residual counts of the equipment used to administer the test dose. After emptying the carrier of Delrin, the subject was positioned in bed before receiving the dose in order that the first in vivo measurement could be started immediately.

A second technique which was evaluated for measuring absolute levels of whole-body ^{59}Fe involved filling the mobile carrier to a constant level with Delrin when counting both the test dose and the subject. The subject was totally immersed in the pellets except for the face. The objective here was to make subjects of widely differing sizes appear alike in length and thickness by using a constant volume of tissue or tissue-equivalent material for all measurements. All in vivo measurements of whole-body ^{59}Fe activity presented in the results, however, were obtained without addition of Delrin to the carrier unless otherwise stated.

3. Results

The sensitivity of the present technique to differences in counting geometry was first evaluated by giving subjects an oral dose and then an intravenous dose of ^{59}Fe. Whole-body counts were obtained immediately after the oral dose and again at 3 and 7 hours after administration. The intravenous injection was then given. The results are expressed in table 1 as a per cent of the dose measured as a point source prior to administration. The maximum disparity among post-oral whole-body

counts was 1.4 per cent, between oral and intravenous counts 1.2 per cent, and between either of these and the activity of the dose measured prior to administration 1.7 per cent.

The next study was performed to assess the difference in counting efficiency of ^{59}Fe circulating in blood throughout the body and that localized in the erythroid marrow of the skeleton. Subjects were counted immediately following an intravenous dose of transferrin-bound ^{59}Fe and at two-day intervals over the subsequent 12 days. The results are shown in figure 1 as a per cent of the preadministration count. Deviations from the theoretical 100 per cent value were minimal for the initial count when the radioactivity was present in plasma, for counts during the first week when the ^{59}Fe was localized to the medullary cavity of the skeleton and for counts in the latter part of the study when approximately 80 per cent of the radioiron was in circulating red cells.

Statistical evaluation of the above data suggests that the present method is suitable for measurements of radioiron loss from the body (table 2). Although the average change in activity over the 12-day interval was less than 1 per cent of the administered dose, significant correlations were obtained in all three subjects for body activity plotted against time in days. Regression analysis gave an average value of 100.2 per cent for the zero time intercept and a mean rate of body ^{59}Fe loss of 0.08 per cent per day during the two-week period.

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A more direct assessment of the method as applied to measurements of iron absorption took the form of a recovery study in which fecal collections were obtained for a period of 14 days following an oral dose of radioiron. Excreted fecal activity was measured by the same method described for counting the test doses prior to administration. The results of whole-body ^{59}Fe and excreted fecal activity are summarized in table 3. The maximum discrepancy between the test dose activity and the sum of whole-body and fecal ^{59}Fe activities was 3.4 per cent over the range in iron absorption of 16 to 66 per cent.

The day-to-day variations in whole-body ^{59}Fe measurements with the present method was roughly ± 1 per cent as shown by the standard error of the estimate for repeated measurements following intravenous radioiron (table 2). For counts obtained in the period immediately following orally administered ^{59}Fe which have fluctuated widely in previous reports, a SD of ± 1.01 per cent was calculated from 22 measurements in the eight hours following oral ^{59}Fe. Included with these data are measurements over a 72-hour period in a subject given 1 μc of ^{59}Fe in a sealed polyethylene tube to prevent absorption by the intestinal mucosa.

There appeared to be little advantage in filling the mobile carrier to a constant level with Delrin when performing in vivo measurements. With respect to reproducibility, in two of the three subjects given intravenous radioiron, the correlation of Delrin "packed" whole-body counts with time was not significant. For measurements in the early post-oral period, a slight improvement in

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reproducibility was observed over measurements without packing; the SD based on 26 whole-body counts performed with Delrin was ± 0.93 per cent. In terms of the accuracy of determining absolute levels of whole-body ^{59}Fe, measurements obtained with and without Delrin added to the mobile cot were in most instances remarkably similar. The use of Delrin is nevertheless advised when measuring absolute levels of whole-body Fe^{59} and this approach becomes particularly important when studying subjects with a wide range of body size as for example when pediatric patients are included. In the context of iron absorption measurements, however, its use can be circumvented by obtaining a whole-body count immediately following administration of the oral dose to establish the ratio of the test dose counts measured before and after administration.

4. Discussion

In previous reports of iron absorption measured by whole-body counting, absorption has been determined by measuring body ^{59}Fe activity in the early postadministration period to establish the "100 per cent value" and again 10 to 14 days later after all unabsorbed activity has been excreted in the feces. The sensitivity of whole-body counter to differences in counting geometry have posed a perplexing problem. In the three-to-four hour period following oral administration of ^{59}Fe, for example, the changes in counting geometry as the adsorbed activity is transferred from the gut to other body tissues has been accompanied by differences in whole-body counts of 10 to 30 per cent (van Hoek and Conrad 1961; Price, Cohn, Wasserman, Reizenstein and Cronkite 1962; Lindell, Strandberg and Reizenstein 1964). Similarly,

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the counting efficiency after oral and intravenous doses of ^{59}Fe to the same subject has been shown in a previous work to range as high as 25 per cent for 4 liquid counters and 35 per cent for single crystal counters (Pollack, Balcerzak and Crosby, 1966). These variations are to be compared with the SD of ± 1 per cent obtained in the present study for in vivo measurements in the two-hour period following orally administered ^{59}Fe and with a maximum dispartiy between oral and intravenous doses of 1.2 per cent.

In an attempt to avoid the errors due to varying counting efficiency, a more complex standardization technique has been described for previous methods in which a second intravenous dose of ^{59}Fe is given at the beginning or end of the study to determine the counting efficiency of whole blood radioactivity (Sargeant 1962; Pollack, et al. 1967). Apart from sacrificing the inherent simplicity of whole-body counting, the intravenous calibration method may be unreliable when the adsorbed radioiron does not appear in circulating red cells. In the patients with ineffective erythropoiesis, for example, most of the absorbed ^{59}Fe may remain in the erythroid marrow where body counts are influenced by the greater shielding of skeletal tissue; this difference in counting efficiency between whole blood and erythroid marrow ^{59}Fe has ranged as high as 10 to 16 per cent (Melmoss, Belcher, Binopoulos and Constantinides 1962; Sargeant 1962) as shown by the difference in whole-body counts two and ten days after an intravenous dose of ^{59}Fe. In the present study, no discernible effect was apparent as an intravenous dose of ^{59}Fe was removed and subsequently returned to the

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circulation. Despite this changing geometry, it was possible to detect a decrease of less than 1 per cent in whole-body activity over the 12-day period of observation. The magnitude of this fall appears consistent with isotopes which have been obtained by other than whole-body counting (Green, Charlton, Seftel, Bothwell, Mayet, Adams, Finch and Layrisse 1968).

The present system for whole-body counting was patterned after that described by Warner and Oliver (Warner and Oliver 1966; Callender, Witts, Warner and Oliver 1966) who were the first to incorporate principles of shadow shielding and scanning bed geometry in designing a counter for clinical measurements of iron absorption. It is difficult to state what advantage was gained within the present counting system by using a more specific arrangement of NaI crystals and measuring Compton scatter to the exclusion of photopeak energy. Apart from the statement made by the workers that the maximum variation in whole-body counts during the early post-oral administration period did not exceed ± 3 per cent, no direct evaluation by in vivo study was made to determine the magnitude of geometry errors. A small source of ^{59}Fe placed in a water phantom showed a somewhat larger variation of 96 to 110 per cent as compared with the present counter (Palmer, Cook, Pailthorp and Finch, in preparation) although it is difficult to extrapolate this data to the variation which might be seen in iron absorption measurements. It is unlikely, however, that any major difference exists between the two counters in relation to the sensitivity of the in vivo distribution of radioactivity. One

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advantage which is afforded by the present method is the ability to dispense with an early postadministration count and to measure whole-body ^{59}Fe in absolute terms by surrounding the subject with Delrin plastic pellets. In this way, iron absorption can be determined from a whole-body background count of the patient and a measurement two weeks later after unabsorbed radioactivity has been excreted.

1190403

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1190404

BLE 1

COMPARISON OF WHOLE-BODY COUNTS FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION OF RADIOIRON IN THE SAME SUBJECT

SUBJECT	WHOLE BODY ACTIVITY (% of dose)					
	ORAL				INTRAVENOUS	ORAL MINUS INTRAVENOUS
	Zero Time	3 Hours	7 Hours	Mean		
1	99.9	100.1	100.3	100.1	99.2	+ 0.9
2	101.8	102.2	100.3	101.4	101.7	- 0.3
3	101.7	100.3	100.3	100.8	99.6	+ 1.2
MEAN	101.1	100.9	100.3	100.8	100.2	+ 0.6

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TABLE 2

STATISTICAL ANALYSIS OF WHOLE-BODY Fe^{59} ACTIVITY MEASURED OVER A TWO-WEEK PERIOD FOLLOWING INTRAVENOUS ADMINISTRATION

SUBJECT	CORRELATION COEFFICIENT	SLOPE (%/day)	0 TIME INTERCEPT (%)	STANDARD ERROR OF ESTIMATE (%)
1	0.647 (p<.02)	-0.088	100.5	1.03
2	0.707 (p<.01)	-0.090	101.1	0.93
3	0.686 (p<.02)	-0.063	100.3	0.98
MEAN		-0.080	100.6	0.98

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TABLE 3

MEASUREMENT OF RETAINED WHOLE-BODY ACTIVITY AND EXCRETED FECAL ACTIVITY FOLLOWING ORALLY ADMINISTERED Fe^{59}

SUBJECT 1				SUBJECT 2				SUBJECT 3			
	ACTIVITY (% of Dose)				ACTIVITY (% of Dose)				ACTIVITY (% of Dose)		
Day	WBC*	Fecal**	Total	Day	WBC	Fecal	Total	Day	WBC	Fecal	Total
2	65.8	33.7	99.5	1	89.9	9.6	99.5	2	84.9	12.0	96.9
3	46.8	53.0	99.8	2	88.3	9.6	97.5	3	73.9	24.3	98.2
5	29.0	71.1	100.1	4	35.7	65.7	101.4	4	68.2	30.6	98.8
7	24.7	74.1	98.8	6	24.2	74.7	98.9	6	68.4	32.1	100.5
12	24.9	75.0	99.9	11	20.4	79.2	99.6	8	64.1	32.5	96.6
14	25.0	75.0	100.0	13	17.6	82.1	99.7	13	66.4	32.5	98.9
				15	16.3	83.0	99.3	14	66.2	32.5	98.7

* Whole-body activity expressed as a percent of the dose counted prior to administration.
** Excreted fecal activity measured in mobile bed of whole-body counter filled with Delrin plastic pellots.

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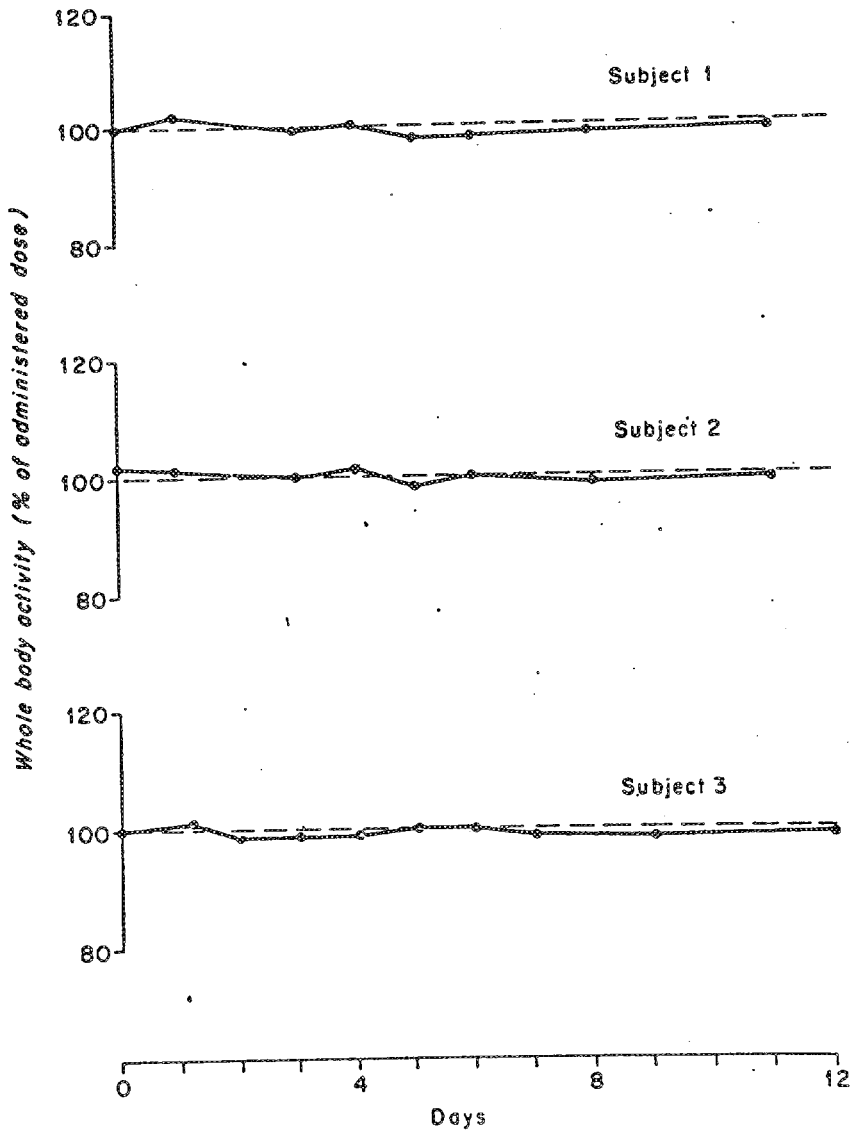
Whole body activity (% of administered dose)
120
100
80
Subject 1
Subject 2
Subject 3
0
4
8
12
Days

1190408

D. PILOT STUDIES OF ACUTE EFFECTS OF HIGH ALTITUDE ON IRON ABSORPTION.

Description of Study. Dual radioiron absorption measurements were performed in four healthy medical students to determine the acute effect of high altitude exposure on iron absorption. All subjects were given 5 mg of elemental iron as ferrous sulfate to which had been added ascorbic acid at a 2:1 M ratio. After an overnight fast, a dose of Fe^{55}-tagged iron was given to all subjects simultaneously at sea level in Lima and later the same morning, the subjects began their ascent to high altitude, arriving in Morococha in the late afternoon. Two subjects (SA and SB) were given a second dose of Fe^{59}-tagged iron on the following morning (16 hours after arriving at high altitude) while the remaining subjects (SC and SD) received their second dose 40 hours after arriving at high altitude. Blood was obtained on the morning of the first test for hematocrits, reticulocyte counts and peripheral blood smears and further measurement of these parameters was performed 8 days later. Serum iron and total iron-binding capacity determinations were performed on each subject on the morning of the first test and on the subsequent two mornings at high altitude (fasting specimens).

Blood was obtained for triplicate measurements of Fe^{55} and Fe^{59} activity on 10 ml portions of blood on day 8 and day 9 of the test in the first and last two subjects respectively. Approximately one month later,

blood was again obtained from these subjects in Lima and forwarded by air to Seattle for repeat assays of blood activity.

Results are summarized on the accompanying table with the exception of reticulocyte counts and peripheral blood smears which have not yet been processed.

Interpretation. As anticipated for this pilot study, no definite conclusions can be drawn from the results. However the fact that absorption was doubled in two subjects perhaps suggests that with a larger group, a definite early effect of high altitude exposure may be demonstrated on iron absorption.

The failure to demonstrate a more definite increase in iron absorption in the present study might be explained by a physiologic mechanism. If we accept the fact that a significant proportion of iron entering the mucosal cell is never transported into the circulation but is lost with sluffing of the mucosa, it is conceivable that the transport of these subjects to high altitude on the same day of the initial test dose (given at sea level) had an effect on its absorption. Thus if hypoxia acts by enhancing the transfer of mucosal cell iron into the body rather than from the lumen into the cell, it is possible that this process acted to increase the absorption from the initial dose given in Lima. Some clue that this may have been the case might be obtained from the mean level of absorption seen in these subjects. Here we are faced with the wide difference in mean values for this dose level reported in different countries. Thus Kuhn observed in 15 male subjects

a mean of 7.1% whereas under identical conditions, 18 male subjects from Caracas who had no evidence of iron deficiency as determined by the hematocrit and transferrin saturation, mean absorption was 25.0%. These differences are borne out by means in England of 25, 30 and 25% compared with means of 7 and 12% in the United States. Although no evidence of iron deficiency could be detected in the present group of Peru subjects, mean absorption from both test doses lay at the upper range of means which have been reported for normal subjects in other countries. Although the number of subjects is perhaps too limited to warrant further study, it would be feasible to administer to these same four subjects a third set of doses tagged with either isotope to determine their basal level of absorption at a more remote time from any high altitude exposure.

Dr. Cook 4-30-69
Summary of Results--Peru Study

	SUBJECTS			
	SA	SB	SC	SD
Subject Initial	AB	EU	RS	SF
Age	21	23	21	21
Hct				
Day 0	42.4	45.6	48.6	46.7
Day 8	44.5	46.7	46.3	47.8
Serum Iron				
Sea Level	114	102	92	111
High Altitude--16 hrs.	61	81	100	104
High Altitude--40 hrs.	92	79	116	127
TIBC				
Sea Level	317	314	337	366
High Altitude--16 hrs.	335	341	342	369
High Altitude--40 hrs.	323	330	379	337
Transferrin Saturation				
Sea Level	35.9	32.5	27.4	30.3
High Altitude--16 hrs.	18.2	23.8	29.2	28.0
High Altitude--40 hrs.	28.3	23.9	30.8	37.5
Estimated Total Blood Vol.	5533	4422	5157	4851
Mean Activity (net cpm/ 10 ml WB)				
Fe^{55}	315	1000	343	798
Fe^{59}	126	206	115	132
Iron Absorption*				
Sea Level (Fe^{55})	12.4 (12.3)	31.4 (31.8)	12.6 (11.7)	27.5 (25.7)
High Altitude (Fe^{59})				
16 hours	24.7 (24.0)	32.8 (29.8)		
40 hours			21.3 (20.3)	23.0 (22.4)

SUMMARY

Mean absorption at sea level--20.9
Mean absorption at high altitude--25.4
Mean ratio sea level : high altitude--0.761**
Students t--1.10 (ns)

* Bracketed values were obtained on samples drawn on day 8 and 9 of the test while unbracketed values were obtained on samples drawn one month after administration.
** Calculated on logarithmic scale and retransformed.

1190412

C. ACUTE EFFECTS OF HIGH ALTITUDE ON THE PLASMA IRON TURNOVER (T Group).

A return to sea level was made on January 28 for the purpose of obtaining baseline PIT determinations on four male subjects from the city of Lima. The following day, these subjects were transported to high altitude where repeat PIT determinations were obtained immediately and two, four and six hours after their arrival at Morococha.

Both PIT determinations in each subject were performed by the identical protocol except for the dose of injected activity which was 2.5 μc of citrated Fe^{59} in 2 ml of normal saline for the baseline studies in Lima and 5 μc of Fe^{59} for repeat determinations at high altitude. Blood was obtained immediately prior to injection of each dose for background measurements, duplicate macrohematocrits, and reticulocyte and peripheral blood smears. Two separate blood samples were obtained at 0 and 10 minutes of each study for serum iron determinations. Blood was drawn at 10, 20, 30, 45, 60 and 90 minutes following injection of each dose from which 2 ml of plasma was pipetted directly into counting vials. The four subjects on which these studies were performed were used by Dr. Lenfant and Torrance for baseline studies in Lima on January 16 - 19, for high altitude studies from January 29 - February 2 and for a final four days of study in Lima from February 2 - February 6.

D. IRON ABSORPTION STUDIES (S Group).

Radioiron absorption tests were performed on January 25 in Lima on four medical students who were transported on the afternoon of the same day to high altitude. Repeat test doses were administered 16 hours (SA and SB)

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and 40 hours (SC and SD) after their arrival at Morococha. All test doses were administered in the early morning following an overnight fast (January 25, 26 and 27) and the latter continued for a further two hours following each test dose.

All test doses were prepared in an identical fashion except for the radio-iron label. For tests at sea level, the latter was 50 μc of Fe^{55} for each subject while high altitude measurements were performed with 7.5 μc of Fe^{59}. Each dose contained 5 mg of elemental iron as ferrous sulfate with sufficient added ascorbic acid to give a 2:1 M ratio of ascorbate:iron. The test doses were pipetted from a single solution prepared each morning immediately prior to administration. The same volume employed for each test was pipetted into a 500 ml volumetric flask containing 150 mg of EDTA in a 100 ml volume of water and after making to volume, quadruplicate unmeasured volumes were frozen for later preparation of the standard.

Immediately prior to administration of each test dose, blood was obtained for serum iron, hematocrit and reticulocyte and peripheral blood smears. Sufficient additional blood was placed in EDTA vials for duplicate background measurements on 10 ml of whole blood.

On the morning of February 4, sufficient blood was obtained from each subject to allow triplicate measurements of Fe^{55} and Fe^{59} activity on 10 ml portions of whole blood. This gave an interval from the time of administration of the final test dose of 8 days in two subjects and 9 days in the remaining two subjects. Duplicate macrohematocrits were performed at the time of obtaining these samples and the height and weight of each subject was recorded in order to estimate blood volume.

Firm arrangements were made with each of the volunteers to return on February 11 when further blood will be drawn by Dr. Faura for repeat measurements of Fe^{59} and Fe^{55} activity. Tubes appropriately marked and all additional equipment required to obtain these samples were left with Dr. Faura and a carton suitable for transport of these samples to Seattle was also prepared.

SUMMARY OF ASSAYS TO BE PERFORMED IN SEATTLE

MEASUREMENT	STUDY				TOTAL
	A	B	C	D	
PB/retic smears*	56	41	8	4	109
Serum Fe/IBC/EID (single determination only)	72	45	32	12	162
Digestion/plating for Fe^{55} activity	36			38	74
Tubes for Gamma Counting					295
Cr^{51} +/- Fe^{59} activity	72				(72)
Fe^{59} activity only	38	129	56		(223)

* Duplicate paired PB smears and triplicate paired reticulocyte smears.

1190415

Dr. Cook 7-11-69
Peru A Study

SUMMARY OF HEMATOCRIT DETERMINATIONS

		ORAL IRON		IMFERON	
		RA	RB	RC	RD
Sea Level Baseline*		41.0	40.8	44.5	43.4
Day at High Altitude	1	44.6	44.0	47.0	47.0
	2	45.4	45.0	50.4	48.1
	3	46.4	44.6	51.0	47.6
	4	45.8	44.0	49.8	50.6
	5	47.4	45.6	51.6	49.5
	6	46.7	45.5	52.3	48.9
	7	49.5	47.1	56.5	49.3
	8	51.3	47.3	57.3	49.3
	9	50.4	48.2	57.4	49.5
	10	52.0	49.2	54.0	52.5
(start of second ferrokinetics)	11	51.3	49.3	55.8	53.0
(finish of second ferrokinetics)	12	50.8	47.7	56.3	50.5
Repeat at Sea Level* (2+ hours after returning)		45.4	44.9	49.9	44.5

* Mean of three duplicate determinations.

1190416

C. EARLY CHANGES IN THE PIT WITH HIGH ALTITUDE EXPOSURE.

Comments. The present measurements indicate a clear effect of hypoxia on erythropoiesis as measured by the plasma iron turnover in all subjects. This increase in turnover was independent in three of the four subjects of changes in the plasma iron level. Although the increase was least in the subject studied six hours after arrival at high altitude, the results in this subject may be suspect. Approximately one hour prior to beginning the turnover studies, the subject underwent a series of nitrogen rebreathing tests with which he encountered considerable difficulty. This same subject developed acute mountain sickness and for the following 48 - 72 hours, had anorexia, severe headache and recurrent vomiting and required continuous bedrest. Whether either the nitrogen rebreathing studies or the subsequent illness which this subject developed was related to the less marked increase in the plasma iron turnover is uncertain. In the first three subjects, no clear differences were seen in the magnitude of the increase in PIT.

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	TA*	TB**	TC#	TD##
STUDIES AT SEA LEVEL				
Mean Hematocrit	46.5	51.0	49.2	53.6
Plasma Iron Values (a) zero time	49	141	115	78
(b) 10 minutes	51	138	111	78
mean	50	139	113	78
TIBC	290	331	310	295
Fe^{59} Clearance ($T_{1/2}$ in min.)	60.5	112.6	98.0	56.1
PIT (mg/100 ml/blood/day)	0.442	0.607	0.622	0.649

STUDIES AT HIGH ALTITUDE				
Time of Study (time of radioiron injection after arrival at high altitude)	Immediate	2 hrs.	4 hrs.	6 hrs.
Mean Hematocrit	45.0	50.0	44.0	46.5
Plasma Iron Values (a) zero time	142	154	99	82
(b) 10 minutes	141	158	109	75
mean	142	156	104	78
TIBC	286	349	318	300
Fe^{59} Clearance ($T_{1/2}$ in min.)	107.5	88	78	57
PIT (mg/100 ml/blood/day)	0.725	0.884	0.986	0.736
CHANGE IN PIT				
Absolute	0.283	0.277	0.489	0.087
Relative	1.64	1.46	1.49	1.13

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RÉSUMÉ OF PERU STUDIES--January, 1969

A. ERYTHROPOIESIS (R Group)

Four healthy male residents of Lima, 21 - 33 years of age, were transported to high altitude for a period of 12 days to determine the maximal erythropoiesis which could be achieved in the face of adequate iron supply. Baseline measurements at sea level were performed in Lima on January 17 and 18 and consisted of simultaneous dual isotope measurements of PIT and plasma volume with Fe^{55} and of red cell mass with Cr^{51}. The subjects were transported to high altitude (Morococha) on January 19 where blood samples were obtained daily for serum iron, duplicate macrohematocrits, reticulocyte and peripheral blood smears. Repeat determinations of PIT were performed with Fe^{59} on the day prior to return to sea level (January 30) and a repeat Cr^{51} red cell mass determination performed in Lima on February 1 within 24 hours of return to sea level.

Two schedules of iron therapy were employed. Two subjects (RA and RB) were given oral iron therapy which consisted of six daily doses of 100 mg of elemental iron as ferrous gluconate and 500 mg ascorbic acid beginning on the day prior to their ascent to high altitude and continuing until 18 hours prior to repeat ferrokinetic measurements on January 30. No gastrointestinal symptoms were noted by the subjects. The remaining two subjects (RC and RD) were given 1.5 g of dextran iron intravenously (30 minute infusion in 250 ml 5% G/W) on the day prior to their ascent to high altitude (January 18). The discoloration

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of the plasma due to circulating Imferon could no longer be detected by visual inspection by the fifth day at high altitude (January 24) and on the same day, a further 1.0 g of dextran iron was given in the same manner as the first infusion. The only reactions to therapy noted were minor arthralgias complained of by one subject on the morning following the second intravenous dose. The circulating Imferon could no longer be detected by visual inspection by day 10 at high altitude--the day prior to repeat ferrokinetic determinations.

A detailed description of isotopic determinations is as follows. Baseline measurements in Lima began with the removal of 20 ml of whole blood into 5 ml of sterile ACD solution in a Unitag bag*. After centrifugation, 5 ml of plasma was drawn under sterile conditions into a syringe containing 20 μc of citrated Fe^{55}. The red cells were suspended in the remaining plasma and tagged by the addition of 15 μc of Cr^{51} in 1 ml normal saline. Cells were incubated at room temperature for 30 minutes with mixing at 3-5 minute intervals. The labeled cells were then washed with three successive 15 ml volumes of normal saline. For the final centrifugation, the bag was spun in the inverted position and the tagged cells drawn directly into a 20 ml syringe containing 5 ml of the Fe^{55} labeled plasma and the same volume of normal saline. The syringe was weighed with a Mettler electronic balance before and after removing one tenth of the total volume for preparation of the standard. The latter was placed directly into a 500 ml volumetric flask containing 100 mg of disodium EDTA in 150 ml of water.

*Abbott Laboratories.

1190420

Injection was made by means of a Coleman adaptor and sampling was performed in the opposite arm through an indwelling scalp vein needle. Sampling was as follows: 1) Duplicate 10 ml volumes of whole blood at 0 and 10 minutes for serum iron determinations; 2) Twelve ml of blood obtained at 0, 15 and 25 minutes in EDTA and used for triplicate macrohematocrits and duplicate 4 ml volumes pipetted directly into counting tubes for measurement of Cr^{51} activity; 3) Ten ml of whole blood obtained at 0, 5, 15, 25, 40, 60, and 100 minutes (exact time recorded for each subject separately) placed into an EDTA tube. After centrifugation, an unmeasured quantity of plasma was frozen in plastic vials for subsequent determination of Fe^{55} activity.

Repeat PIT determination was made on day 10 at high altitude. For the two subjects receiving oral iron therapy, 2.5 μc of citrated Fe^{59} was given in 2 ml of normal saline while in the iron dextran treated subjects, the same dose of activity was first tagged to 3 ml of fresh plasma. Duplicate samples for serum iron determination were obtained at 0 and 10 minutes while 2 ml of plasma obtained at 0, 10, 20, 30, 45, 60 and 90 minutes following injection were pipetted directly into counting tubes for measurement of Fe^{59} activity. In the two subjects who had received iron dextran treatment, additional plasma was obtained at the time of injection for direct isotopic UIBC determination with $MgCO_3$. The latter involved triplicate determinations in which 1 ml of plasma was added within 15 minutes of obtaining the blood to 2 ml of $Fe^{59}Cl_3$ containing 250 μc elemental iron per 100 ml. After incubation at room

temperature for 15 minutes, 200 mg of light $MgCO_3$ was added and the tubes repeatedly mixed for a further 15 minutes. Two ml of the supernatant obtained after two successive centrifugations was pipetted directly into counting tubes together with the same volume of an appropriate dilution of the saturating radioiron solution. Aliquots of the latter were also stored for later iron determinations.

Records were kept of all blood removed for sampling purposes from each subject throughout the period of study. A total of 150 ml of whole blood was removed from each subject during the first 10 days at high altitude and a further 90 ml removed for the purpose of ferrokinetic measurements on day 11.

Repeat Cr^{51} red cell mass determinations were performed simultaneously on all subjects within 24 hours of their return to sea level (February 1). The method was as previously described with the omission of radioiron tagging of the plasma.

Isotopic determinations were in general considered to be technically sound. The only apparent technical error occurred during the infusion of Cr^{51}-tagged cells in subject RD during the second red cell mass determination. Despite the use of a Coleman adaptor, the first ml of the infusion was given interstitially. The infusion was stopped and after a two hour waiting period which was felt to be well beyond the period of reabsorption of the interstitial activity, the bulk of the tagged cells was given after preparation of a new counting standard and repeat measurements of syringe weight.

1190422

B. FERROKINETIC REFLUX DETERMINATIONS (Subject M, FA, FB).

Percentage feedback of intravenous radioiron was measured in three subjects by determining the plasma Fe^{59} disappearance curve over a 13 day period. The first study was started on the morning after arrival at high altitude (January 20) in a native of Morococha (Subject M) and the remaining two studies were performed in subjects (FA and FB) from Lima, 42 and 66 hours after their arrival at high altitude. The total period of observation was 13 days in each of the subjects with the final samples in subjects FA and FB being obtained after return to Lima. Each subject was given 30 μc of citrated Fe^{59} which had been tagged to 5 ml of fresh plasma. The lack of a suitable balance at high altitude precluded quantitative measurements of injected activity. The baseline measurements of serum iron, duplicate macrohematocrits and reticulocyte and peripheral blood smears were obtained in the two subjects from Lima prior to their ascent and in all three subjects immediately prior to injection of the Fe^{59} activity. A total of 30, 29 and 31 post-injection samples were obtained in subjects M, FA and FB respectively at progressively increasing intervals following injection. For samples obtained during the first 24 hours of study, 2 ml of plasma was pipetted directly into the counting tubes while 4 ml of plasma was processed for all remaining samples. After the first 36 hours of study, additional blood was drawn in EDTA from which duplicate macrohematocrits were obtained and from which 2 ml of whole blood was pipetted directly into counting tubes. Two separate samples of blood were

obtained at 0 and 5 minutes of the study for serum iron determinations and thereafter, single samples were obtained 6 and 9 hours and at daily intervals throughout the period of study.

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Peru A Study

	ORAL IRON		IMFERON	
	RA	RB	RC	RD
BASELINE STUDIES				
Hematocrit	41.4	42.0	45.4	43.9
Plasma Iron	73.2	101.7	92.6	92.2
Fe^{59} T 1/2	63.5	93.0	74.5	85.1
PIT	.723	.680	.735	.656
UIBC	283	209	199	265
TIBC	357	311	292	357
% Saturation	20.5	32.7	31.8	25.8
Cr^{51} RBCM	1605	1490	1980	1760
CONCLUDING STUDIES				
Hematocrit*	51.3	49.3	55.8	53.0
Hematocrit**	45.4	44.9	49.9	44.5
Plasma Iron*	62.4	74.1	94.2	118.0
Fe^{59} T 1/2	37.0	35.6	35.8	42.9
PIT	0.966	1.235	1.308	1.439
UIBC	374	284	195	223
TIBC	436	363	289	341
% Saturation	14.3	21.8	32.6	34.7
Cr^{51} RBCM	1760	1657	2133	1892
CHANGES				
Ratio of PIT Value	1.336	1.816	1.780	2.194
Increase in Cr^{51} RBCM#	295	307	293	272

* At time of ferrokinetic study.
** At time of Cr^{51} measurements.
# 140 ml of packed red cells is added to the difference in Cr^{51} red cell masses which represents the volume of packed cells removed during sampling.

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Appendix 1

Effect of Altitude on Erythropoiesis

By Jose Faura, Jose Ramos, Cesar Reynafarje, Eugenia English, Per Finne and Clement A. Finch

ERYTHROPOIESIS is stimulated by ascent to high altitude and suppressed after return to sea level. The resulting changes in the erythron have been previously described.[1,2] The present study was undertaken to characterize the role of erythropoietin in mediating this marrow response.

Materials and Methods

Volunteer subjects participating in this study were inhabitants of Lima and Morococha. *Group I* was composed of 7 healthy males between the ages of 18 and 30 living in the sea-level city of Lima, where baseline studies were performed. The group was then transported by car over a 4 hour period to Morococha (4530 meters above sea level). They remained there for 7 days, confined to living quarters in the building where further measurements were made. All these subjects experienced some disability for the first 48 hours at high altitude and subjects B, C, and E were sufficiently symptomatic to be confined to bed for the first day.

Group II was composed of male inhabitants of Morococha between the ages of 20 and 39. They were first studied at high altitude and then transported to Lima where the observations were continued over the following week. One individual (L) suffered contusions from a car accident on the second day in Lima.

The procedure for collecting, processing and assaying urinary erythropoietin has been described elsewhere.[3] All urine specimens were refrigerated at once after voiding at 4 C. A 25 per cent aliquot of the preceding day's collection was dialyzed within 24 hours, concentrated to less than 100 ml., and frozen. At the time of altitude change 8 hour collections were processed. All aliquots were further concentrated in Seattle to 25 ml. and assayed in polycythemic protein-starved female, Swiss-Webster mice, 11 to 12 weeks old. The assay procedure was identical to that previously reported except that urinary concentrate was injected twice daily for only 2 days. Results were calibrated against a known erythropoietin standard* and the activity was expressed as units/24 hours.

Plasma iron turnover (PIT) measurements were made between 9 and 11 a.m. in each subject. ^{59}Fe citrate (2 to 5 μc. and less than 1 μg. of iron) was injected intravenously and 4 samples were taken beginning at 10 minutes after injection and at intervals of 15 to 30 minutes depending on the anticipated rate of disappearance of radioiron. Activity was measured in a liquid scintillation vial counter to an accuracy of less than $\pm$ 2 per cent.

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No reprints will be available for this article.—The authors.

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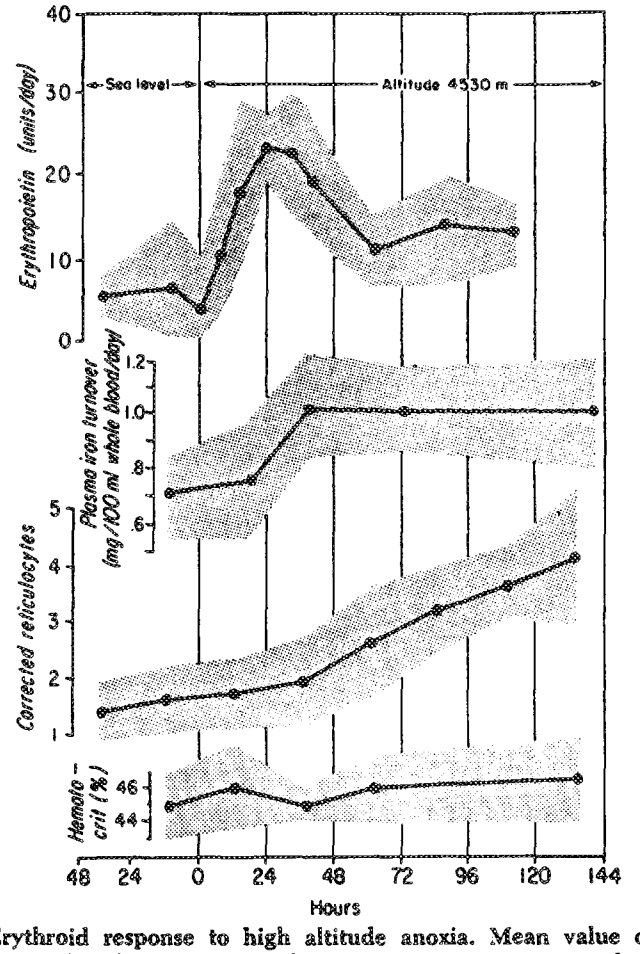

Fig. 1.—Erythroid response to high altitude anoxia. Mean value of the seven subjects is shown by the solid line; shaded area indicates 1 standard deviation. Urinary erythropoietin levels increased within 12 hours of high altitude exposure and became maximal at 24 hours. Plasma iron turnover increased maximally within 24 hours of the increase in erythropoietin and showed no further increase over the following 4 days. Reticulocytes increased threefold, but half of this increase would appear to be related to the prolongation in maturation time. Hematocrit values remained constant over the 7 day period.

PIT was calculated by the following formula:

$$\frac{\text{Plasma iron } (\mu\text{g. } \%) \times 100 - \text{Hct} \times 0.9}{\text{T } 1/2 \text{ (min.)} \times 100}$$

On a different date for each subject, ^{55}Fe citrate (15 μc.) was injected intravenously so that the marrow transit time as well as plasma iron turnover could be determined. Samples were then drawn every 12 hours for 4 days and at the end of 2 weeks for digestion, electroplating and differential counting.[4] The marrow transit time was calculated as the interval after injection required for 50 per cent of the 14 day red cell radioactivity to appear in circulation.

All samples for erythropoietin, isotope and iron determinations were shipped in the frozen state from Lima to Seattle where the analyses were carried out. Plasma iron was measured by the method of Bothwell and Mallet,[5] iron binding capacity by a modification of the method of Morgan[6] and by the immunodiffusion technic.[7] Reticulocyte smears

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Table 1.—Ferrokinetic Measurements of Group I

Subject	Time (hrs.)	Plasma iron (μg.%)	Iron disappearance (T 1/2-min.)	Plasma iron turnover*
A.	Basal	113	65	1.02
	14	91	69	0.72
	36	64	39	1.00
	60	49	33	0.88
	132	44	30	0.85
B.	Basal	80	79	0.60
	14	42	45	0.55
	36	76	48	0.95
	60	58	40	0.87
	132	42	40	0.64
C.	Basal	64	53	0.76
	14	43	31	0.86
	36	79	34	1.37
	60	54	26	1.24
	132	42	25	1.01
D.	Basal	79	58	0.80
	14	103	60	1.05
	36	87	51	1.00
	60	63	37	1.01
	132	75	33	1.31
E.	Basal	83	74	0.66
	14	95	78	0.70
	36	161	78	1.22
	60	59	38	0.87
	132	55	33	0.93
F.	Basal	53	55	0.54
	14	141	85	0.96
	36	139	77	1.07
	60	174	92	1.14
	132	79	40	1.09
G.	Basal	114	110	0.63
	14	72	90	0.47
	36	107	80	0.81
	60	131	67	1.07
	132	98	52	1.14
Average	Basal	84	71	0.715
	14	84	65	0.917
	36	102	58	1.06
	60	84	48	1.01
	132	62	36	0.995

* mg./100 ml. whole blood/day.

were coded and 4000 cells counted blind. Hemoglobin was determined by the cyanmethemoglobin method and the packed cell volume by the Wintrobe technic.

Results

I. *Ascent to High Altitude*

The effect of altitude on erythropoiesis in Group I is summarized in Figure 1 and Table 1. Over a 7 day period at high altitude, the mean hematocrit changed little despite the removal of approximately 400 ml. of blood. Reticulocytes showed an increase from a mean value of 1.5 to 4.2 per cent with

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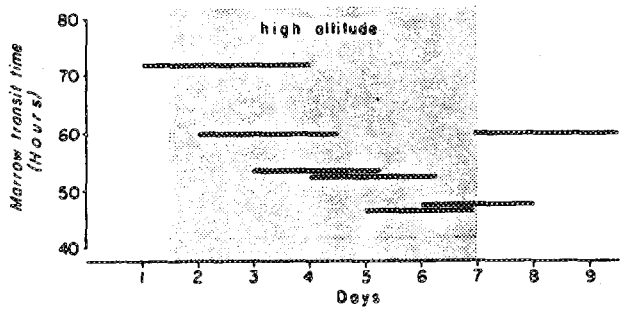

Fig. 2.—Marrow transit time of subjects exposed to high altitude. The ordinate indicates the duration of marrow transit of radioiron (time between injection and appearance of 50 per cent of activity in the red cell mass). The horizontal line also indicates by its beginning the time when radioiron was injected and by its end the time when 50 per cent of the red cell activity had appeared in circulation. Each measurement is in a different subject. The mean value for normal subjects is 90 hours.

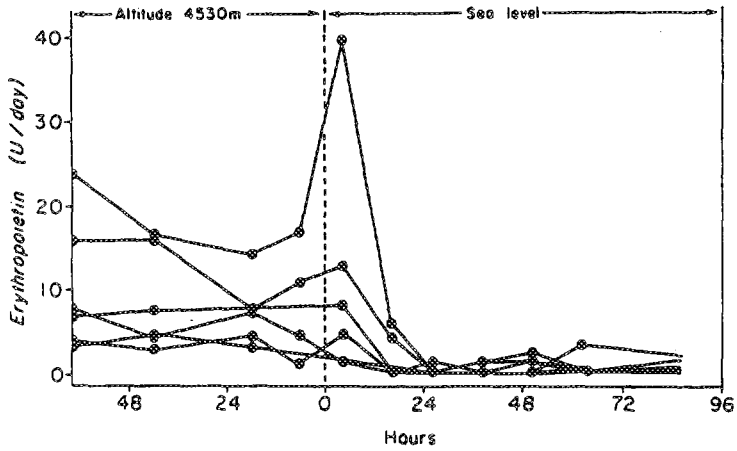

Fig. 3.—Urinary erythropoietin response to low altitude. High altitude dwellers were moved to sea level. Original erythropoietin levels were not significantly elevated in 4 of the 6 subjects. In the two individuals with elevated levels, there was an unexplained increase at or just before the descent. In all subjects erythropoietin decreased rapidly after descent.

the greatest increase occurring during the second and third day after exposure to anoxia. The PIT increased abruptly from 0.76 at 12 hours of high altitude exposure to 1.06 at 36 hours; thereafter it fell slightly. Mean erythropoietin output did not change during the first 6 hours at high altitude from the baseline of about 7 units per day, but by the second day, it had risen to 20 units per day. Over the subsequent 3 days, it fell to a level of between 10 and 15 units per day. Marrow transit time (Fig. 2) shortened progressively when subjects were at high altitude. The shortening occurred whether the iron was injected before ascent (subject A) or immediately before descent (subject C).

II. *Descent to Low Altitude*

Hematologic values of the second group were more heterogeneous than the

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Table 2.—Hematologic Measurements of Group II

Subject	Time (hrs.)	Hematocrit (%)	Corrected reticulocyte (%)	Plasma iron (μg.%)	Iron disappearance (T 1/2-min.)	Plasma iron turnover *	Erythropoietin (units in preceding 24 hr.)
H.	Basal	54	1.9	68	48	0.72	7
	16	53	1.5	55	38	0.75	2
	40	53	1.4	58	45	0.67	< 1
	64	51	2.0	83	56	0.79	< 1
	88	48	2.3	94	78	0.68	< 1
I.	Basal	72	2.2	40	14	0.94	18
	16	72	1.9	88	18	1.70	27
	40	70	2.2	56	24	0.86	3
	64	70	1.5	138	50	1.05	2
	88	67	1.6	212	101	0.82	< 1
J.	Basal	54	3.6	89	41	1.03	7
	16	57	3.0	92	38	1.18	9
	40	55	3.2	160	38	2.13	< 1
	64	55	2.3	73	41	0.93	< 1
	88	48	1.7	128	77	0.94	< 1
K.†	Basal	64	2.8	128	50	0.99	4
	16	64	2.8	101	48	0.89	< 1
	40	61	1.8	144	48	1.35	1
	64	61	1.7	127			< 1
	88	56	2.2	128	62	1.02	< 1
L.	Basal	73	3.0	64	16	1.30	13
	16	71	3.2	118	15	2.84	21
	40	74	2.6	197	37	1.78	2
	64	74	3.0	157	41	1.38	< 1
	88	70	2.3	349	99	1.30	< 1
M.	Basal	60	2.8	218	78	1.14	4
	16	60	2.8	97	59	0.76	3
	40	59	2.8	113	83	0.64	< 1
	64	59	2.3	130	88	0.70	1
	88	55	2.4	234	145	0.81	1
Average	Basal	63	2.7	101	41	1.05	9
	16	63	2.5	92	36	1.11	11
	40	62	2.3	121	46	1.17	1
	64	62	2.1	118	55	0.98	< 1
	88	58	2.1	191	94	0.98	< 1

* mg./100 ml. whole blood/day.

† This subject traumatized on the first day at sea level.

first. Two subjects had basal hematocrits of 72 and 73 per cent and elevated erythropoietin levels. The other four subjects had initial hematocrits between 54 and 64 and erythropoietin levels similar to those of Group I. The effect of moving the inhabitants of Morococha to sea level is shown in Figure 3 and Table 2. During the two days at high altitude and four days at sea level, approximately 450 ml. of blood were removed and their mean hematocrit fell from 63 to 58 per cent. Reticulocytes decreased from 2.6 to 2.1 per cent. The mean PIT of 1 mg./100 ml. of whole blood per day at high altitude actually rose during the first 42 hours at low altitude and thereafter decreased. The rise was largely attributable to subjects I, J, and L who showed increases in erythropoietin on the preceding day. During the following three days PIT

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showed a minimal fall. Erythropoietin levels fell very rapidly after descent to sea level and were undetectable within 12 hours.

There was an increase in iron binding capacity as measured by both charcoal and immunologic methods from a mean value of 361 to 392 in Group I and a decrease in Group II from 376 to 359 μg. per cent.

Discussion

Previous studies by Huff et al.[1] and Reynafarje et al.[2] have defined the general response of the erythroid marrow to changes in altitude. The measurements of myeloid:erythroid ratios, reticulocytes, and erythroid iron turnover indicate an augmentation of blood production to approximately twice normal when sea level dwellers moved to high altitudes, whereas erythropoiesis decreased to about one-third normal when high altitude dwellers move to sea level.

This study was focused on the erythropoietin output and its immediate effect on erythropoiesis with such altitude changes. Upon exposure to altitude anoxia, there was a lag period of about 6 hours, followed by a marked increase in erythropoietin output over the next 24 hours and then a decline to twice basal levels. These results are consistent with previous data on erythropoietin response in animals, which have been shown to have a peak response at 8 to 18 hours, and a fall to normal after 48 to 72 hours.[8-14] The more limited studies in man also indicate demonstrable erythropoietin activity in plasma only during the first 3 days at increased altitude.[15-17] The reason for the initial exaggerated response and secondary fall is not clear. The slight increment in marrow activity makes the speculation of marrow utilization less likely. A more likely possibility is the reduction in anoxia by another mechanism such as the shift in the oxygen dissociation curve which occurred during the first 2 days.[18]

In subjects in Group II, who were transported from high to low altitude, response occurred immediately, so that erythropoietin was not measurable within 12 hours. This rate of disappearance is more rapid than would be expected from some clearance studies in man.[19] The conspicuous rise of erythropoietin observed in two subjects at the time of descent and the increase in plasma iron turnover in all subjects during the first 2 days at low altitude is unexplained; it is possible that the increased oxygen supply actually reduced blood flow and led to tissue anoxia.[20]

Information concerning the mode of action of erythropoietin on the erythroid marrow is provided by the ferrokinetic measurements. Radioiron taken up by the erythroid marrow is known to be incorporated within minutes into hemoglobin.[21,22] Thus, radioiron uptake by the marrow is assumed to represent the hemoglobin synthesizing capacity of that organ. In the stimulated erythropoiesis of high altitude, the amount of iron going to the erythroid marrow and appearing in circulating red cells increases from about 80 per cent at sea level to 90 per cent or more.[2] There is then an increase in both the proportion of iron turnover concerned with erythropoiesis and the absolute amount. This increase in PIT occurred in the Group I subjects between 12 and 36 hours after altitude exposure or within 24 hours of the erythropoietin

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response. The same time relationship was observed in the two subjects of Group II who were most polycythemic: a temporary increase in erythropoietin at the time of descent was followed by an increase in PIT. Such an effect on marrow iron uptake is too rapid to be related to stem cell proliferation; the maximum effect of stem cell differentiation and proliferation of normoblast progeny requires several days.[28] The rapid increase in PIT must then be taken as evidence of a direct action of erythropoietin on hemoglobin synthesis by the existing erythroid cells. This is in agreement with in vitro studies which have indicated an enhancement of hemoglobin synthesis within 24 hours through the action of erythropoietin on DNA-dependent RNA synthesis.[24-28]

Another effect of erythropoietin is to shorten the marrow transit time of radioiron.[29] The time relationships of this effect are illustrated in Figure 2. When radioiron was administered at sea level and the subject later moved to a high altitude, transit time was shortened from the normal of 90 hours to 84 hours, suggesting a slight effect on marrow cells labeled with iron before anoxic stimulation. The shortening of marrow transit time also persisted in the subject injected at high altitude but moved 8 hours later to sea level, suggesting that the action of erythropoietin on marrow transit time did not require continued erythropoietin stimulation.

The removal of the erythropoietin stimulus in Group II did not interfere with completion of maturation of those cells already synthesizing hemoglobin. PIT and reticulocytes remained near normal levels over the following 3 days. This response resembles the wave of erythropoiesis in the polycythemic animal given a single injection of erythropoietin, where cells converted to the stage of hemoglobin synthesis by the injection continue to mature despite the absence of further erythropoietin.[30]

This short study permits only an approximation of the rate of erythropoiesis which followed erythropoietin stimulation. However, it appears from the increase of reticulocytes to 1.6 times normal* and the plasma iron turnover to 1.4 times normal that production was only moderately increased and that the iron going to the marrow may actually have decreased after the second day at high altitude. The red cell balance cannot be calculated in the absence of blood volume measurements; however, considering the decrease in plasma volume which usually occurs,[31] the stable hematocrit with removal of 400 ml. of blood over a week also suggests a production rate of less than 2 times normal. Any of these estimates falls far short of the production rate expected in hemolytic anemia. It has been suggested that the reason for this difference may be the limitation of iron supply to the bone marrow in nonhemolytic states.[32] In the present study (Group I) the decrease in mean plasma iron to 62 μg. per cent and the fall in mean clearance time of radioiron from 71 to 31 minutes are consistent with a restricted iron supply.

Summary

Measurements were made to characterize the relationship between erythropoietin output and erythropoiesis in two groups of subjects, one moved from

*The absolute increase of three times normal in reticulocytes should be reduced to 1.6 by a factor of 47/90 to correct for the longer maturation time of "shift" reticulocytes.[32]

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a sea level habitat to high altitude, and the second moved from a high altitude habitat to sea level. In the first group, there was a latent period of 6 hours followed by a rapid increase in erythropoietin, and a secondary fall to a level of approximately twice normal. The increased erythropoietin stimulus was also reflected in a shortened marrow radioiron transit time. In the second group, there was an initial unexplained rise, after which erythropoietin fell within 8 hours to undetectable amounts.

Elevated erythropoietin was associated in Group I with an increased iron uptake within 24 hours of the stimulus, suggesting a direct action of erythropoietin on hemoglobin synthesis by the existing marrow population. Limitation in erythropoiesis to a rate of less than twice normal was tentatively explained by a restricted iron supply. In the second group, marrow activity continued for 3 days despite a marked fall in erythropoietin, indicating that cells in the maturation phase completed their normal development.

SUMMARIO IN INTERLINGUA

Esseva effectuate mesurationes pro characterisar le relation inter le rendimento de erythropoietina e le erythropoiese in duo gruppos de subjectos, i.e., un gruppo que habeva essite transferite ab domicilios al nivello del mar ad un ambiente de grande altitude e un secunde gruppo que habeva essite transferite ab domicilios in grande altitudes ad un ambiente al nivello del mar. In le prime gruppo, un periodo latente de 6 horas esseva notate, sequite de un rapide augmento in le erythropoietina e un declino secundari a un nivello de approximativemente duo vices le norma. Le augentate stimulo erythropoietinic esseva etiam reflectite in un reducite tempore de transito medullari de sero radioactive. In le secunde gruppo, un inexplicate augmento initial esseva notate, sequite de un declino de erythropoietina intra 8 horas ad nivellos non plus detegibile.

Elevate nivellos de erythropoietina esseva associate in Gruppo I con us augmentate acceptation de sero intra 24 horas post le stimulo, suggestionante un effecto directe de erythropoietina super le synthese de hemoglobina per le existente population medullari. Le reduction del erythropoiese a un intensitate de minus que duo vices le norma esseva tentativemente explicate como effecto de un restringite provision de ferro. In le secunde gruppo le activitate medullari continuava durante 3 dies in despecto de un marcate declino in erythropoietina, indicante que cellulas in le phase de maturation completava lor disveloppamento normal.

ACKNOWLEDGMENT

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