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Department of the Army
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EFFECT OF TOTAL BODY RADIATION ON THE ERYTHROPOIETIC SYSTEM IN HUMANS

PROGRESS REPORT FOR PERIOD ENDING
31 August 1955

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PROGRESS REPORT

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The earliest appraisal of the medical problem attending nuclear explosions focused on the new element of radiation injury, which was added to the familiar thermal and blast effects. The actual danger and the relative importance were unknown and the first defensive reaction was to ask "what is the lethal dose?" It is evident now that this was not a thoughtfully phrased question but it was asked at all levels with an urgency that demanded a simple and immediate estimate.

At that time a clinical investigation was in progress to determine the relative therapeutic effectiveness of certain chemotherapeutic agents and total body radiation in patients with disseminated cancer. It was recognized that maximum effectiveness would be obtained at close to tolerance doses for such drugs as nitrogen mustard or for radiation. An early estimate of the tolerance dose for total body radiation was based on this study and was reported in "Comparative effects of total body radiation, nitrogen mustard, and triethylene melamine on the hematopoietic system of terminal cancer patients." R. K. Loeffler, V. P. Collins, and G. A. Hyman. Science 118:161-163, August 1953. (Reprint #1).

Since that time this department has been occupied in a continuing program directed toward a better understanding of the role of total body radiation in therapy, the systemic response of the patient, and particularly the effect upon iron metabolism. Thirty-three patients have received total body radiation ranging from 50r in a single exposure up to 550r in ten treatments over a period of 15 days. This

group includes 23 patients who have received 200r in a single exposure. The meaning of exposure to radiation and radiation dose will be discussed later in this report. For the moment it is necessary only to point out that unless otherwise specified, measurement of radiation in roentgens is in terms of skin dose which includes backscatter at the surface of the patient nearest to the source of radiation. Some of the information is reported in "The Therapeutic Use of Single Doses of Total Body Radiation" which will appear in the American Journal of Roentgenology in the spring of 1956. (Reprint #2).

The data derived from these studies has been summarized by patient in Table I, by groups of identical factors in Table II, and in a more condensed fashion based on the approximate integral dose in time in the final summary, Table III.

On the basis of this experience, and recognizing that most patients were treated for advanced cancer who had received previous irradiation or chemotherapy, these opinions are expressed in answer to questions that have been considered important in estimating radiation hazards.

1. Considering the total civilian population, the minimal lethal dose for whole body radiation may be as low as 300r for aged or infirm individuals. For healthy young adults who have not suffered other injury, the minimal lethal dose is placed at 400r. The LD_{50} for healthy adults is of the order of 550 to 600r. It is unlikely that a significant number of persons would survive 800r. These figures apply to radiation measured at the surface of the individual with backscatter, delivered to one side from a distance of 4 meters, in a single exposure of approximately one hour.

2. The most constant change observed following total body radiation was a drop in the platelet count. This occurred in practically all patients receiving 100r or more as measured on skin and delivered from one side. The change in platelet count was first noted at five days to several weeks following radiation.

3. Change in the radioiron turnover test was the next most constant response to radiation and it was the most sensitive response in terms of time. For those patients who showed prolongation of the disappearance half time of radioactive iron from circulating blood, the alteration in the iron turnover was evident within 24 hours of exposure to radiation.

4. The white count was the least constant and least sensitive index of radiation effect, excluding of course those patients with leukemia where marked and early alteration might be produced by small amounts of radiation.

5. The symptoms of radiation sickness have not been a prominent feature at the dose level used in this study. Of those patients receiving 200r skin dose from an anterior port in a single treatment, only seven of 23 admitted to any symptoms at all. Of these 7, nausea and anorexia were the most frequent with only two patients vomiting. In none was the degree of sickness totally incapacitating. A number of patients studied had nausea and vomiting associated with their disease before treatment was undertaken.

In the studies described here the exposure to radiation has been measured in r at the surface of the patient's body, including backscatter. This method was chosen because the conditions of calibration using a phantom can duplicate the conditions of treatment with actual measurement being made on the skin surface of the patient during treatment. Other

methods of measuring exposure are in use. Sometimes roentgens are measured with an ionization chamber in air at a given distance from the source of radiation and the patient is later adjusted into position at the same distance from the treatment source. Another method is to measure the exposure of roentgens in air at a fixed distance from the radiation source and then center the patient to the point of measurement so that this coincides with the center of the patient's body. In still another method, the measurement of exposure of radiation is given only in terms of energy absorbed, although the determination ("guestimation") of absorbed energy or integral dose must be based upon actual measurements of ionization in air, on the skin and in the depth of the patient or in tissue density material. The differences in the method of measuring the exposure to radiation are of no particular importance providing that adequate description is supplied concerning the quality of the radiation, the geometry of the field, and the time pattern of exposure together with the methods of instrumentation. Much confusion has been introduced into the problem of dose and the relation of measured amounts of irradiation to biologic effects, due to the circumstances under which the bulk of animal experimentation has been carried out. A discussion of measurement of radiation at the Radiologic Research Society in Cleveland in June of 1954 indicated that at best, conditions of irradiation might be constant in one laboratory but physical and biclogical factors were not adequately controlled or described to permit comparison or confirmation of radiation effects reported from different centers.

A fundamental distinction must be made between exposure and dose. Exposure is a measurement of quantity or intensity of a physical agent that prevails in a stated place and does not take into account any re-

action to this agent or the many factors that may alter the reaction to the agent. Dose is the concept that relates measurement of a drug or a physical agent to a biologic effect. The purpose of measurement is to permit duplication of a recognized biologic effect and it entails description of all features relating to matter, space and time that will permit such duplication. The "r" is the unit of measurement for irradiation but it is not a unit of dose; if it is so considered no predictable or duplicable relationship will be found between roentgens and biologic effect.

A few years ago a survey of opinion was carried out across the country which revealed that estimates of lethal dose of radiation varied from 25r to 600r. In this report which refers to exposure to relatively intense radiation for brief periods of time, a range of 300r to 600r corresponds to the range from minimal lethal dose to LD_{50}. Such an exposure will occur only by accident. Exposure under these circumstances must be postcalculated and estimates would be considered in general agreement if they varied by no more than a factor of 2. The estimates of the range of minimal lethal dose to LD_{50} under the conditions described in this paper are believed to be accurate within 20 percent and therefore seem to provide an adequate answer to the problem so far proposed and discussed.

As knowledge and experience in the field of total body radiation have increased, it becomes evident that the question, "What is lethal dose?" is approximately equal to the question, "How long is a piece of string?" Better understanding of the conditions of probable exposure to whole body radiation presents more sharply defined questions concerning dose and radiation hazard. More accurate information will

be required to accept a responsibility for incurring risk of exposure to personnel who must occupy or carry out medical or military operations in areas of known radioactivity. The following problems are now of greater importance than estimates of minimal lethal dose or LD_{50}.

1. It is recognized that the effect of whole body radiation varies with the geometry, distance from the source of radiation, whether radiation is from one direction only or whether the body is exposed in a uniform field of radiation. It is necessary to determine whether the effect of radiation is the same for a given amount of absorbed energy (integral dose) whatever the geometry of the radiation field.

2. Information is required concerning effect of protraction or fractionation of total body exposure. This is a problem that would present itself if it were necessary to occupy an area of low level radiation. The permissible exposure would require weighing the hazard against the necessity and determining whether advantage might be gained by intermittent exposures to radiation as compared with constant exposure.

3. Where repeated exposures may be necessary, the problem of estimating recovery arises. Even though recovery is complete by all currently available tests, there is an apparent masked metabolic defect as indicated in the case of patient George Morris. This patient received two identical exposures of 200r separated by a five month interval. Prior to each treatment the patient's clinical condition, laboratory work-up, and physical findings were apparently identical. The therapeutic response to his first treatment was highly satisfactory with minimal side effects; the second treatment was accompanied by four days of malaise with vomiting, by severe depression of platelet and white count, and a more dramatic change in the iron turnover. There is a need for a method of quantita-

tively evaluating the degree of residual radiation injury even in the face of apparently complete recovery.

4. Biological differences have been claimed between the effects of megavoltage radiation and 250 Kv radiation as observed in depilation and skin erythema in animals or in the local effect of radiation upon tumors in humans. Information is still required as to whether this is a specific biologic difference in terms of systemic response to whole body radiation or whether this is directly related to integral dose or absorbed energy.

Work completed or in progress:

1. Clinical evaluation of the effect of therapy and systemic response to total body radiation. (Reprint #1 and #2).

2. Accumulation of hematologic data on patients who received total body radiation. (Tables I, II, and III).

3. Development of radioiron turnover test as a practical clinical method of estimating bone marrow function. (Reprint #3 and #4).

4. Studies on porphyrin metabolism as an index of radiation effect. (Reprint #5).

Proposed program:

1. Clinical studies on patients receiving radiation therapy in fractionated or prolonged low level exposure.

2. Comparison of effect of 2000 Kv and 250 Kv radiation.

3. Estimation of masked metabolic defect following previous radiation or chemotherapy.

4. Effect of geometry of exposure on response to radiation and correlation of exposure to absorbed dose.

5. Continued development of radioiron turnover technique and equipment for use as field test at site of radiation accidents.

TABLE I

NAME	DOSE	SYMPTOMS	Fe. T. O. (Half-times in hours)	WBC (In thousands)	PLATELETS (In thousands)	OTHER
Cerda, Joe	200r) 3 weeks 200r) between	N, A for 2 d 2V, N,A for 1 wk	Doubtful value 0.8 (7 mos)	6→3 3→.5 (at 1½ wks)	300→50 50→13 (at 2½ wks)	WBC: 1.7 (9 wks) 8. (7 mos) 8 (1 yr)
Jones, Billy	200r (anterior)	None	0.6→1.2 (1 d) 1.5 (1½ yrs)	(7→6)	Not done	Alive with 4800 WBC π TBC
Hawkins, Asta	50r (posterior)	None				
Gatlin, Ike	100r (anterior)	None				
Ponder, Sam	125r (anterior)	None	0.8→2.0 (1 d) →2.3 (2 d) →1.3 (4 d)	7→5 (2-14 days)	? value	Alive with normal TBC
Dudley, William	150r anterior	A for 1 d	0.6→2.0 (1 d) →1.3 (8 d)	180→11 (2 mos) (drop 1st day)	No change	
Collier, John	200r	N for 1 d		9→4 (at 3 wks)		
Buce, Ola	200r posterior 100r posterior (5 wks)	N, 2V (1 d) No sx.	48' ↓ 54' (1 & 36 d)	7→13 (1 d) →2 (4 wks) →5 (7 wks) (2 wks p 2nd)	150→52 (3 wks) →92 (7 wks)	
N - Nausea A - Anorexia V - Vomiting						

NAME	DOSE	SYMPTOMS	Fe. T. O. (Half-times in hours)	WBC (In thousands)	PLATELETS (In thousands)	OTHER
Frizzell, Louis	50r posterior	None	?	?	?	Considerable sx. relief. Partial recurrence of arthritis after 1½ yrs
Guillory, Octave	200r anterior + dextran	No change		6→2.4→3 start 4 days low 12 days ret. 1 m.	200→44→116 start 8 days low 3 wks ret. 1 m.	
Garza, Irene	200r anterior	V.3 N & A (5 d)	.6 ↓ 1.0 (2 d) .6 (22 d)	8→3→7 start 4 d low 4 wks ret. 3 mos.	300→84→300 start 2 wks low 4 wks ret. 3 mos.	
Toliver, Jesse	200r anterior + dextran	No change		10→2.4→3.1 start 1 d. low 8 d.	300→164 start 2 d. low at d. 9 d.	d. 9 d.
Dunham, Johnnie	200r anterior + dextran	No change		sporadic 7→4 start 17 d. low at d.	270→30 start 10 d. low at d.	bleeding tendency (liver disease) d. 21 d.
Ramos, John	200r anterior	N. A.	1°-1°17' (5 d) →21' (14 d)	8→1.6→5 start 10 d. low 5 wks. ret. 7 wks.	200→64→160 start 3 wks low 5 wks. ret. 7 wks.	d. 8 wks.

NAME	DOSE	SYMPTOMS	Fe. T. O. (Half-times in hours)	WBC (In thousands)	PLATELETS (In thousands)	OTHER
Lemons, James	200r anterior	No change	50'-1°36' No change	132→18 start 1 d. low 2 wks last count	80→44 start 1 d. low 2 wks last count	Last followup 7 wks.
Morris, Frank	200r anterior 200r anterior @ 5 mos	No change N&V for 4 d	16'-21' (1 d) 21'-3° (2 d)	No change 1st Rx 6→.5→1.3 start 1 wk low 5 wks	450→252 (2 wks) 290→30→60 start 2 wks low 5 wks	Last followup 8 mos p̄ 1st Rx
Sechelski, John	50r posterior x 6	No change		10→5 low on last count at 2 wks	250→100 low on last count at 2 wks	Last followup 2 wks.
Denson, Jewel	200r anterior	No change	21'-23' No change	No change at 5 d. Last count	150→104 at 5 d. Last count	Terminal at 5 wks.
Kahlden, Mabel	200r anterior	N.V. (severe al) 1 day	27'-43' No change	20→2.6→20 start 4 d low 5 wks ret. 2 mos.	250→44→180 start 7 d low 5 wks ret. 2 mos.	d. 6 mos.
Davis, Virgil	200r anterior	No change	58' ↓ 1°7' (1 d.)	No change in 3 days.	No change in 3 days.	d. 3 d.
Booth, David	200r anterior	No change	13'-16' No change	90-66 9 d. Last count	250-80 9 d. Last count	d. 4 wks.

NAME	DOSE	SYMPTOMS	Fe. T. O. (Half-times in hours)	WBC (In thousands)	PLATELETS (In thousands)	OTHER
Whitsey, Tommie	200r anterior	6V (3 hrs) N (Day of Rx) No other sx.		14 at Rx 8 at 3½ mos 4 at 5 mos 15 at 7 mos	300 at Rx 300 at 3½ mos 200 at 5 mos 300 at 7 mos	
Bryant, Elizabeth	200r anterior	No change	1.4° ↓ 2.7° (2 d) 3° (5 d)	6→3 start 2 d. low at d.	140→62 start 7 d. low at d.	d. 15 d.
Cole, George	100r anterior	No change	1.4 ↓ 1.7 (2 d) 2.4 (5 d)	2.3→1.2 start 12 d low 16 d	100→22 start 2 d low 3 wks	lost followup 3 wks.
Wilkins, Sylvester	200r anterior	No change	1°7' 1°22' (2 d) 1°36' (5 d)	6→3→4→.2 start 7 d low 9 d rise, then fall pre-mortem	200→100→18 start 1 d level 15 d drop pre-mortem	d. 4 wks.
Rodden, James	545r anterior in 10 Rx.	N. A. V.		8→3→4.2 start 5 d low 5 wks ret 6 mos	200→50→200 260 start 1 wk low 5 wks ret 7 wks, 6 mos	Alive and well 6 months

NAME	DOSE	SYMPTOMS	Fe. T. O. (Half-times in hours)	WBC (In thousands)	PLATELETS (In thousands)	OTHER
Booker, Earl	200r anterior	No change	15' → 43' (2 d) → 30' (5 d)	No change to 12 d	No change to 12 d	d 5 weeks
Boss, Chester	200r anterior	No change	47' → 1.9° (1 d) → 2° (3 d)	16 → 2.6 start 4 d low at d.	260 → 50 start 1 d low at d.	d. 3 weeks
Baker, Lucille	200r anterior	No change	30' → 47' (2 d) → 37' (5 d)	5 → 4 low at 5 d. last count	No change 5 d.	d 3½ mos.
Scott, Annie	120r each anterior & posterior spleen shielded	? A. (1 wk)	1° 1°8' (1 d) 1°30' (3 d)	2 → 1.2 → 3 start 2 d low 3 wks. ret. 6 wks.	No change	d. 6½ wks.
Thomas, Richard	200r posterior	No change		6 → 4 → 5 Low at 5 wks. ret. at 2 mos.	200 → 140 → 200 Low at 6 wks. ret. at 2 mos.	

TABLE II

DOSE	NO. OF PATIENTS	NO. OF SYMPTOMS	Fe. T. O. (Half-time in hours)	WBC (In thousands)	PLATELETS (In thousands)	OTHER
50 posterior	2	None	Not done	Not done	Not done	1 yr. 8 mos. followup on one. No sequelae.
100 anterior	2	None	1.4 → 2.4 (5d) One not done.	2.3 → 1.2 (16d) One not done.	100 → 22 (3 wks) One not done.	Both lost to followup.
125 anterior	1	None	0.8 → 2.3 (2d)	7 → 5 (14 days)		1 yr. 9 mos. followup. TBC normal.
150 anterior	1	Anorexia 1 day	0.6 → 2.0 (1d)	180 → 11 (2 mos) Leukemia	No change.	Alive at 4 months.
200 anterior	18	None-13 N&V-1 d 3 pts N&A-1 d 1 pt A-1 d 1 pt 1st Rx. of two N&A-2 d. N&V-1 d. None-1	N.C.-5 0.6-1.2 (1d) N.D.-4 0.6-1.0 (2d) 1.0-1.3 (5d) 1.4-3.0 (5d) 1.1-1.6 (5d) 58'-67' (1d) 15'-43' (2d) 0.8-2.0 (3d) 0.5-0.8 (2d) N.D.-1 N.C.-1 16'-21' (1d)	N.C.-5 N.D. 9 → 4 (3 wks) 6 → 2.4 (12d) 8 → 3 (4 wks) 10 → 2.4 (8d) N.C. 17 days, ↓ d. 8 → 1.6 (5 wks) 14 → 4 (5 mos) 6 → 3 (d. @ 15d) 6 → 3 (9d) 132 → 18 (14d) Leukemia 20 → 2.6 (5 wks) 90 → 66 (9d) 16 → 2.6 (3 wks) 7-2 (4 wks) 6-3 (3 wks) N.C.	N.C.-1 (7 mos) N.D.-2 200 → 44 (3 wks) 300 → 84 (4 wks) 300 → 164 (9d) died 270 → 30 (21d) died 200 → 64 (5 wks) 140 → 62 (15d) died 200 → 100 (15d) 80 → 44 (2 wks) 150 → 104 (5d) lost 250 → 44 (5 wks) N.C. 3 d. 250 → 80 (9d) lost N.C.-12 d. 260 → 50 (3 wks) N.C.-5 d. 300 → 50 (3 wks) 150 → 52 (3 wks) 450 → 250 (2 wks)	

N-Nausea A-Anorexia V-Vomiting N.C.-No Change N.D. Not Done

DOSE	NO. OF PATIENTS	NO. OF SYMPTOMS	Fe. T. O. (Half-time in hours)	WBC (In thousands)	PLATELETS (In thousands)	OTHER
200 anterior ≠ 100 (5 wks)	1	N; 2V (1d) No sx's.	48' → 54' (1d) → 54' (36d)	7 → 13 (1d) → 2 (4 wks) → 5 (7 wks)	150 → 52 (3 wks) → 92 (7 wks)	
200 anterior ≠200 (5 mos)	1	No change N&V (4 days)	16' → 21' (1d) 21' → 30' (2d)	No change 6 → .5 (5 wks)	450 → 250 (2 wks) 290 → 30 (5 wks)	Alive 8 mos. after 1st Rx.
200 anterior ≠200 (3 wks)	1	N, A-2d 2V, N, A-1 wk	Not done	6 → 3 3 → .5 (1½ wks)	300 → 50 50 → 13 (2½ wks)	WBC: 1.7 (9 wks) 8 (1 yr) 8 (7 mos) died (1 yr)
200r posterior	1	No change	Not done	6 → 4 (5 wks)	200 → 140 (6 wks)	
120r each anterior and posterior shielding spleen	1	? A	1° → 1°30' (3d)	2 → 1.2 (3 wks) → 3 (6 wks)	No change	Died (6½ wks)
50r posterior x 6	1	No change	Not done	10 → 5 (low on last count-2 wks)	250 → 100 (low on last count-2 wks)	Lost to followup (2 wks)
55r anterior x 10	1	A,N,V during 10 days	Not done	8 → 3 → 4.2 5 wks 6 mos	200 → 50 → 260 5 wks 6 mos	living and well 6 mos.
25r each x 5	2	No change	.7 → 1.3 (End Rx) No change	6 → 2.4 (still down at 1 wk) 8 → 4.5 (4 d)	200 → 150 (still down at 1 wk) 250 → 200 (4 d)	

TABLE III

SUMMARY SHEET

DOSE	SYMPTOMS	WBC	PLATELETS	Fe
50	0/2			
100	0/2	1/1	1/1	1/1
125	0/1	? 1/1		1/1
150	An. 1 d 1/1	1/1	0/1	1/1
200 including 1st Rx.	7/23	16/23 (3 with leukemia)	16/19 (1st 12 days only)	11/17
200r +100r (5 wks)	0/1	0/1	0/1	0/1
200r +200r (5 mos)	1/1	1/1	1/1	1/1
200r +200r (3 wks)	1/1	1/1	1/1	
50r x 5	0/3	3/3	3/3 (2 Slight)	1/2
55r x 10	1/1	1/1	1/1	

x/y indicates number of patients showing a change out of the number studied for the item in question.

Reprint #1

Reprinted from SCIENCE, August 7, 1953, Vol. 118, No. 3058, pages 161–163.

Comparative Effects of Total Body Radiation, Nitrogen Mustard, and Triethylene Melamine on the Hematopoietic System of Terminal Cancer Patients[1]

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Nitrogen mustard and allied agents have come to be considered as alternative agents for radiotherapy in the treatment of several forms of cancer. Comparable clinical regression of disease and similar histologic changes have led to the use of the term "radiomimetic." Where both agents are of known effectiveness, nitrogen mustard is usually preferred for generalized disease because the dose of total body radiation is believed to be limited to an ineffective level by radiation sickness and bone marrow depression. At the same time, the anticipated nausea, vomiting, and profound hematologic changes which follow vigorous chemotherapy are considered justifiable, and recovery is known to be reasonably certain. Comparably severe effects have not been produced by doses ordinarily employed in total body radiation. It seems possible that tolerance and recovery from radiation have been underestimated. A study was therefore undertaken to compare the effects of nitrogen mustard and allied agents with the effects of increasing doses of total body radiation.

All patients had advanced cancer and were considered suitable candidates for chemotherapy. One patient was given 5 mg of methylbis (2-chloroethyl)-amine (HN_2) intravenously, one was given 5 mg of triethylene melamine (TEM) orally, and two received 50 r total body radiation. The x-ray factors were two million volts peak potential, target-skin distance 360 cm, filter 4 mm of lead, half value layer 6.6 mm of lead, air dose 50 r, skin dose 55 r, depth dose at 10 cm—41 r, and depth dose at 20 cm—23 r. In a second group, two patients were given 10 mg of HN_2, two were given 10 mg of TEM, and one received 100 r total body radiation under the same conditions as the previous group. When the patient receiving 100 r total body radiation failed to show any appreciable hematologic depression as a result of this treatment, one further patient was given 150 r total body radiation.

All patients studied received the following laboratory examinations before treatment: red blood count, hemoglobin, white blood count, differential count, reticulocyte count, platelet count, gastric analysis, bone marrow aspiration biopsy, plasma protein determination, serum bilirubin determination, plasma iron concentration, plasma iron turnover rate using Fe^{59} as a tracer, and red cell uptake of injected Fe^{59}. The iron studies were carried out because of the work of Huff, Hennessy *et al.* (*1, 2*). This demonstrated an effect of total body irradiation on the turnover of plasma iron and the utilization of this iron in the

[1] Supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract No. DA-49-007-MD-302.

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TABLE 1

Patient	Disease	Treatment	RBC (millions)	Hb (grams %)	WBC (thousands)	Differential	Platelets (thousands)
W. B.	Carcinoma of lung	50 r	4.3 ā rx* N. C.	12 ā rx N. C.	6–8 ā rx N. C.	N. C.†	230–260 ā rx N. C.
J. F.	Carcinoma of colon	50 r	3.0 ā rx N. C.	9 ā rx N. C.	9–11 ā rx N. C.	N. C.	270 ā rx 90 at 2–3 wk
D. R.	Carcinoma of scalp	100 r	4.9 ā rx 4.0 at 6 wk	14 ā rx 11 at 6 wk	7–9 ā rx N. C.	N. C.	250 ā rx N. C.
R. F.	Carcinoma of sigmoid colon	150 r	3.4 ā rx N. C.	9 ā rx N. C.	7–9 ā rx 5 at 5 wk	N. C.	200–210 ā rx 100–130 at 4–5 wk
G. S.	Carcinoma of cervix uteri	5 mg HN_2	4.0 ā rx 3.0 at 16 days	9 ā rx 6 at 16 days	15 ā rx 8 at 3 wk	N. C.	300 ā rx N. C.
J. P.	Carcinoma of lip	10 mg HN_2	4.0 ā rx 3.3 at 31 days	12 ā rx 10 at 31 days	7–9 ā rx 5 at 18 days	N. C.	175–200 ā rx N. C.
M. B.	Carcinoma of vulva	10 mg HN_2	3.6 ā rx 3.1 at 23 days	10 ā rx 7 at 23 days	8–15 ā rx 7 at 23–30 days	12% lymph. ā rx 28% lymph. at 23–30 days	300–330 ā rx N. C.
J. A. H.	Carcinoma of prostate	5 mg TEM	4.2 ā rx 3.7 at 6 days	12 ā rx 10 at 6 days	15 ā rx N. C.	N. C.	210–240 ā rx N. C.
A. M.	Carcinoma of lung	10 mg TEM	4.5 ā rx 3.4 at 36 days	13 ā rx 9 at 36 days	11 ā rx 5 at 28 days	14% lymph. ā rx 5% lymph. at 7 days	170–200 ā rx N. C.
J. E. H.	Glioblastoma multiforme	10 mg TEM	5.1 ā rx N. C.	15 ā rx N. C.	9–11 ā rx N. C.	N. C.	180–210 ā rx N. C.

* ā rx before treatment.
† N. C. no significant change.

TABLE 1 (*Continued*)

Patient	Marrow aspiration	Other	Iron turnover rate (half-time in hr)	Plasma iron concentration (μg %)	Period of follow-up
W. B.	Hypoplasia ā rx* N. C.	N. C.†	1.0 ā rx 0.5 at 3 hr 1.1 at 7 days	65 ā rx 41 at 3 hr 40 at 7 days	5 wk died
J. F.	Normal ā rx N. C.	Free HCl: 55° ā rx 0° at 1 day 41° at 7 days	0.4 ā rx 0.5 at 3 hr 0.7 at 7 days	49 ā rx 43 at 3 hr 58 at 7 days	5 wk died
D. R.	Normal ā rx Hypoactive at 7 days	N. C.	2.0 ā rx 1.1 at 1 day 1.5 at 7 days	68 ā rx 112 at 7 days 89 at 51 days, 18 at 66 days	4 months living
R. F.	Normal ā rx Hypoactive at 7 days Normal at 36 days	Icterus: 2–5 ā rx 14 at 4 days 2–4 at 8 days	0.7 ā rx 0.7 at 1 day 7 days and 35 days	150 ā rx 75–85 at 1–23 days 150 at 29 days	3 months living
G. S.	Normal ā rx Greatly decreased at 21 days	N. C.	0.8 ā rx 0.8 at 1 day 3.0 at 8 days	93 ā rx 73 at 1 day, 59 at 8 days 45 at 17 days, 27 at 24 days	Deteriorating at 27 days signed out
J. P.	Normal ā rx N. C.	N. C.	1.1 ā rx 1.2 at 1 day 0.9 at 7 days 0.8 at 35 days	85 ā rx 75–100 at 1–36 days 38 at 44 days	4 months living
M. B.	Normal ā rx Increased cellularity at 15 days	N. C.	1.0 ā rx 1.1 at 1 day 0.6 at 8 days	85 ā rx 72 at 8 days 33 at 23 days, 85 at 38 days	4 months living
J. A. H.	Normal ā rx N. C.	N. C.	1.2 ā rx 1.2 at 1 day 1.2 at 7 days	123 ā rx 121 at 1 day 83 at 7 days	7 days signed out
A. M.	Normal ā rx N. C.	N. C.	0.7 ā rx 0.6 at 1 day 0.5 at 7 days 0.6 at 36 days	85 ā rx 55–65 at 4–30 days 47 at 36 days	3 months living
J. E. H.	Not done	Not done	1.1 ā rx 0.9 at 1 day 0.6 at 8 days	123 ā rx 82 at 1 day 115 at 8 days	11 days died

* ā rx before treatment.
† N. C. no significant change.

formation of red blood cells which appeared to be quantitatively related to the dose delivered.

These tests were repeated as frequently as was feasible following treatment. All patients were in a terminal state and it was not expected that the treatment would appreciably change the course of their diseases. None of the ten patients studied showed a deterioration in his condition that could be ascribed to the treatment given although several expired within a relatively short time thereafter.

Both chemotherapy and radiotherapy were given as single doses or exposures to eliminate the time factor. It is generally appreciated that higher doses are tolerated if radiation is given over a period of time (*3, 4*). The demonstration of higher tolerance levels of total body radiation may well prove of value in the management of diseases currently being treated routinely with nitrogen mustard and its derivatives.

All patients receiving nitrogen mustard or TEM developed malaise of varying severity, but none of the patients receiving radiation mentioned any changes in symptoms. One patient stated (J.F.) that he felt much better for 2 days following the treatment.

The data is summarized in Table 1. Except for the few changes mentioned specifically, there were no alterations in plasma proteins, serum bilirubin, reticulocytes, or gastric acidity.

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Reprint #2

The Therapeutic Use of Single Doses of Total Body Radiation*

by

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Radiotherapy and chemotherapy are recognized as having equivalent or complementary roles in the treatment of certain types of cancer. In general, radiation has been restricted to the treatment of relatively localized manifestations, and chemotherapy has been used when the disease is more widely disseminated. There has been limited use of total body radiation in the treatment of generalized neoplastic disease. In the Heublein technique (7,11) low intensity, continuous radiation was used to administer doses up to 450r in three weeks. Hempelmann (6), writing on the acute radiation syndrome, noted that 100r of 200 KV x-rays could be delivered to the entire body of the patient without causing symptoms of any sort. Recently, Osgood (12) has advocated a "titration technique" in the treatment of leukemia in which doses of 10r - 25r were given to the whole body at regularly spaced intervals. In a scrupulous effort to avoid radiation sickness and bone marrow depression, a conventional technique employing total body radiation for the treatment of polycythemia utilized doses of 25r repeated to a total of 300r in three weeks. From these different techniques, using varying formulae of intensity, dose, and time, it is difficult to assess the tolerance to whole body radiation.

Perhaps the most extensively employed chemotherapeutic agent has been nitrogen mustard, methyl bis (beta chlorethyl) amine (HN_2). This agent has been used in almost every form of malignant disease and in some benign conditions such as rheumatoid arthritis (2). It has become the standard form of therapy for generalized Hodgkin's disease. In accepted clinical doses, nitrogen mustard produces a sharp systemic reaction and the patient is heavily sedated before treatment to control nausea and vomiting. Hematopoietic depression is constant, and severe leukopenia and thrombocytopenia is a recognized hazard of repeated courses. These toxic side effects have not been a deterrent to common use although they exceed the severity of radiation sickness.

In 1950 Gellhorn and Collins (5) undertook a comparative study of two groups

of patients with Hodgkin's disease, one of which had received radiation and nitrogen mustard in alternating therapeutic courses, and the other radiotherapy alone. It could not be shown that the addition of nitrogen mustard to the therapy regime increased survival time but the drug was regarded as a useful adjunct to the extent that it reduced the time under treatment and the amount of radiation required. If nitrogen mustard did not increase survival time beyond that obtained with radiotherapy alone, its only purpose was to offer the convenience of a systemic approach to treatment. It seemed possible that higher doses of total body radiation might offer equal benefit with side effects of less severity. Phillips (13) had studied the application of massive doses of radiation to liver for metastatic disease. Court Brown (4) had reported observations on symptoms associated with single therapeutic doses of x-ray to large portions of the body, and to the whole body.

In a previous report the tolerance to total body radiation was compared with the effects of nitrogen mustard and triethylene melamine (10). Total body radiation in single doses up to 150r was less depressing for hematopoiesis and patients were free of malaise that was constantly associated with these drugs. These observations suggested that a therapeutic effect of total body radiation might be sought in patients having disseminated disease ordinarily treated by a variety of chemotherapeutic agents.

In the present series of patients, dose has been expressed in roentgens on skin and measurements were made with a Victoreen chamber on the surface of a prestwood phantom at treatment distance. Court Brown (3) reported his series of single exposures to radiation in terms of energy absorbed or integral dose. There was some necessity for this in that his patients received treatment to varying portions and proportions of the body, and dose in roentgens would have no direct correlation with tolerance of the individual to radiation. For purposes of duplication, dose on skin represents the simplest expression of exposure to radiation. Boden and

Cohen (1) have called attention to variations that exist in measuring dose in different institutions and it appears that no accuracy would be gained in the assumptions and calculations necessary for determining integral dose for total body radiation.

In addition to the usual blood examinations, radioactive iron tracer studies were carried out because of the work of Hennessy and Huff (8,9), indicating a quantitative relation between the turnover of plasma iron and dose of total body radiation in mice. The half time of disappearance of radioiron from the blood is normally one and one-half to two and one-half hours for humans. Prolongation of the half time indicates decreased hemoglobin formation and may be useful as an early and sensitive index of change in guiding further therapy.

Case 1. B. L. AR-8422. A male, age 73, developed generalized lymphadenopathy in November 1951 and a diagnosis of "lymphoma" was made by biopsy. Treatment was withheld because of lack of symptoms. In March 1953 the patient was admitted to the hospital because of 20 pounds weight loss, pain in left shoulder, and edema of left upper extremity. There was extensive lymphadenopathy with nodes as large as five cm. in diameter; liver extended five cm. below the costal margin. On March 23, 1953 the patient received 75r (skin dose) total body radiation anteriorly and 75r posteriorly (250 KV CP, HVL 2.3 mm. Cu., TSD. 360 cm.). In the first 48 hours the patient was anorexic but there was no nausea, vomiting or diarrhea. On the third day the patient's appetite returned and the nodes began to diminish in size. On May 12, 1953 he was discharged from the hospital and returned to work. Later in the month he received 600r tissue dose to the mediastinum in eight days on an out-patient basis. In December 1953, in another city, he underwent partial gastric resection for lesser curvature ulcer. At present he is convalescing satisfactorily.

Laboratory data during the period associated with total body radiation were as follows: Hgb. from initial 11.9 gm. to low of 7.6 gm. at five weeks; R.B.C. from 4.3 million to low of 1.7 million at five weeks; W.B.C. from initial 29,000 (mostly lymphocytes) to low of 4,000 with normal differential at eight weeks; iron turnover half time from initial 1.3 hours to 2.3 hours at two days, 4.2 hours on 23rd day, and 0.8 hours on the 53rd day. Most recent data on 2-15-54 were Hgb. 11.0 gm., R.B.C. 3.8 million, platelets 148,000, W.B.C. 10,500 with 45% lymphocytes, and iron turnover half time 0.4 hours.

Case 2. W. D. AJ-6024. A 72 year old white male was admitted for repair of inguinal hernia and was discovered to have a white cell count of 155,000. The differential was consistent with chronic myelogenous leukemia. Liver and spleen were palpably enlarged. The blood pressure was 200/90. He had lost 20 pounds in weight in the past year. On June 24, 1953 he received 150r (skin dose) total body radiation to the anterior aspect of body (250 KV, HVL 2.3 mm. Cu., TSD. 360 cm.). There was mild anorexia on the following morning but his appetite was normal by noon. There was no nausea, vomiting or malaise. Liver and spleen gradually diminished in size and were no longer palpable after two weeks.

Laboratory data during treatment and follow-up were as follows: Hgb. from initial 10.1 gm. to low of 7.5 gm. at four weeks; R.B.C. from initial 3.8 million to low of 2.9 million at four weeks; W.B.C. from initial 193,000 to low of 11,000 at three months with change of differential from a preponderance of myeloid elements to a normal distribution; platelets from initial 265,000 to a low of 225,000 at seven days; iron turnover half time from initial 0.6 hours to 2.0 hours at one day, and 1.3 hours on the eighth day.

Most recent data on February 17, 1954 are: Hgb. 11 gm., R.B.C. 3.1 million, platelets 216,000, W.B.C. 98,000 with myeloid differential, iron turnover half time 1.5 hours. Patient was asymptomatic; liver and spleen were not palpable.

Case 3. J.B.C. AX-7573. A 51 year old white male was admitted to the hospital on September 10, 1953 with a biopsy diagnosis of multiple mycloma. He had been paraplegic and incontinent of urine and feces for three weeks; he had developed a large sacral decubitus ulcer, his general condition was poor and he complained of severe dorsal and lumbar back pain. He was placed on a Foster frame and daily care was given to the decubitus ulcer which showed slow improvement. On September 15 he was given 200r (skin dose) total body radiation (250 KVP, 2.3 mm. Cu. HVL, TSD 360 mm.). There was mild nausea of one day's duration; there was no vomiting. Because there was only partial loss of tactile sensation accompanying the motor paralysis, the total body radiation was followed two weeks later by local x-ray treatment to the spine. A tissue dose of 675r was delivered in ten days to vertebral bodies from C-4 to S-1. No change was detected in neurologic findings; however, narcotic requirements for pain were considerably less than before treatment. The patient's condition gradually deteriorated and he died on October 28, 1953. No autopsy was obtained.

Laboratory studies initially were: Hgb. 10 gm., R.B.C. 2.2 million, platelets 240,000, W.B.C. 8,500 with normal differential; iron turnover half time was 0.5 hours. Following therapy Hgb. gradually dropped to 7 gm., and W.B.C. to 5,000 without change in differential at five weeks.

Case 4. O. B. Y-13361. This 58 year old white female gave a six year history of backache radiating to the legs. In April 1948 she received x-ray therapy to a destructive lesion in the body of the second lumbar vertebra with diminution of pain. In 1951 a second course of therapy was given to this area, and in January 1952 a third course. On February 23, 1952, a spinal fusion was carried out and a diagnosis of multiple myeloma was established. Further lesions developed in skull and ribs. On 1-26-53 the patient, now a paraplegic with complete destruction of the body of the third lumbar vertebra, and constant severe back pain, was transferred from another hospital for total body radiation. On November 11, 1953 with patient turned on her side in bed, she received 200r (skin dose) to back (250 KV, HVL 1.8 mm. Cu., TSD 365 cm.). There were two episodes of vomiting without nausea at four and six hours after treatment and minimal nausea with further vomiting on the first and second post-treatment days. From the third treatment day there were no further symptoms of nausea and moderate pain relief was noted. Pain began to become more severe again after five weeks. On December 16, 1953 (five weeks) the patient received another 100r (skin dose) to the back under the same conditions as previously. There were no symptoms of nausea or vomiting, and again there was some reduction in back and leg pain. Pain relief persisted for about one month, and then recurred with the same severity as before treatment. For this reason a cervical cordotomy was planned, but the patient died at operation on January 28, 1954.

The laboratory data during the treatment period showed maximal depression from the 200r dose: Hgb. from 12.5 gm. to 8.7 gm. at three weeks; R.B.C. from 4.0 million to 2.7 million at three weeks; W.B.C. from 11,000 to 2,600 at four weeks; platelets from 150,000 to 50,000 at five weeks. The differential showed a percentage increase in lymphocytes at the time of the drop of the total white cell elements, such that the absolute lymphocyte count remained approximately constant.

An autopsy was performed. Tumor that had received intensive local radiation showed histologic changes consistent with this treatment. Normal tissues that had been subjected to total body radiation showed no gross or microscopic effects of such radiation.

Case 5. J. C. AX-5284. A white male, age 35, had suffered a fracture of cervical spine in an accident three years previously and extensive lesions of multiple myeloma were discovered at that time. A year later the diagnosis was confirmed by biopsy. Except for a gradual weight loss of 45 pounds, the patient remained well, subjectively, until four months before admission. From this time he was confined to bed by severe back pain and increasing weakness. X-rays showed compression fractures of several vertebral bodies and he was transferred to the hospital on a Stricker frame. On July 22, 1953 he received 200r (skin dose) total body radiation (250 KV, HVL 2.3 mm. Cu., TSD 400 cm.). Immediately after treatment he vomited once without nausea. He ate his noon day meal two hours later and had no further reaction. Pain relief was evident the day following treatment, and by the third day appetite was improved. On August 12, 1953, a second dose of 200r, under the same conditions as the first, was administered. This time there was no nausea or vomiting and there were no new complaints. One week later he was completely relieved of back pain and was up and about the ward voluntarily. His only complaint was of pain in feet and ankles. There were no x-ray changes evident in the bones of this area and the pain apparently was due to ambulation after five months in bed. He received five transfusions of 500 cc. each during September and was then discharged home. He has received no further treatment and now talks hopefully of returning to work.

Laboratory data during this period was as follows: Hgb. from initial 8.3 gms. to low of 6.0 gms. at five weeks, with transfusion at that time; R.B.C. from 3.8 million to 1.7 million at five weeks; W.B.C. from 6,000 to low of 550 at five weeks; platelets from initial 295,000 to low of 13,000 at six weeks; iron turnover half time from 0.5 hours before treatment without change following the first treatment, to 1.0 hours one day after the second treatment.

On February 17, 1954 laboratory data was: Hgb. 11 gm., R.B.C. 3.2 million, platelets 210,000, W.B.C. 8,250 with 80% neutrophils, 11% lymphocytes and 9% eosinophils. Iron turnover half time was 0.8 hours.

For both radiotherapy and chemotherapy, the ultimate effects are mediated by intracellular chemical changes. For this reason the two may be compared on terms of tolerance of the individual, response of the disease, and factors governing their role in application.

Tolerance to radiation does not lend itself to ready measurement. In Court Brown's (4) series of 50 patients, he was able to define three phases of radiation sickness: 1) the latent period extending from the time of treatment to the onset of symptoms; 2) a period of acute disturbance lasting from one and one-half to four hours after the onset of symptoms; 3) recovery period extending as long as four or five days. The length of the latent period was inversely proportional to the period of acute symptoms and was used as an index of severity of reaction. In the patients presented here, a pattern of symptoms was not apparent; nausea and vomiting were inconstant and the patients' condition before treatment was such as to mask any minor degree of weakness or lethargy. From a symptomatic point of view, the reaction to single doses up to 200r was less than would be anticipated with conventional doses of nitrogen mustard and the hematopoietic depression was acceptable as compared with this agent.

The response of the patients' disease can only be considered as indicative that total body radiation in single doses up to 200r has therapeutic possibilities. The tolerated dose is beyond that necessary or desirable for leukemia but is in the effective range for lymphosarcoma and multiple myeloma. The patient who received 200r on two occasions in three weeks had a clinical response that could be described as impressive.

No agent currently in use for treatment of generalized cancer is offered with intent to cure. Even increased survival as a result of therapy is difficult to confirm. Under these circumstances the principal benefit of treatment is early relief of signs and symptoms and this benefit may be mitigated by the severity of side effects of treatment, prolongation of treatment or delayed response to treatment.

In all these respects single doses of total body radiation has some advantage.

There is an additional advantage in the matter of dose. Prescription of radiation dose is unique in its precision. It is possible under controlled conditions to deliver a desired amount to any tissue or to calculate what had been delivered to any site. To relate dose and effect, it is desirable to determine dose at the site where effect is produced, so that a given result can be repeated or modified. Prescription of dose of pharmaceutical agents is usually on the basis of body weight, administered intravenously, orally, or intramuscularly. In the processes of absorption, dilution and transportation, the amount of the agent finally producing an intracellular chemical effect must be subject to wide and unpredictable variations. If the clinically effective dose closely approaches the tolerance dose, it is desirable to have knowledge and control of the amount of concentration of an agent, at the cellular level where its effect is exerted.

Summary

Total body radiation in a single exposure up to 200r of 250 KV x-rays may be administered safely to patients with generalized cancer. Considered as one form of systemic therapy rather than as an alternative or competitive method of treatment, total body radiation in doses of this order is a useful addition to the management of advanced cancer.

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Reprint #3

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Radioiron Citrate as Tracer to Determine Disappearance Rate of Plasma Iron in Normal Subjects.* (21612)

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Huff, Hennessy, Elmlinger and others working with Lawrence have reported studies using radioiron as a tracer to study the turnover of iron in animals, and in patients with a variety of diseases(1-5). Other groups have used this test to follow disorders of iron turnover associated with disease conditions (6-7). Generally, the tracer administered consists of globulin bound radioiron prepared from the patient's serum or using purified globulin (IV-7 fraction, Cohn)(8). The tracer is given intravenously and its rate of disappearance determined from multiple assays of plasma radioactivity. The course of iron from plasma to liver, spleen and bone marrow, can be obtained from repeated external countings over representative sites. As radioiron is incorporated in hemoglobin of newly formed red cells, it reappears in circulating blood. The percentage of tracer iron which appears in red cells is quantitatively determined at intervals of several days. The reports of Lawrence's group and others indicate the value of this test on a routine clinical basis, as an aid in diagnosis of hematopoietic disorders, as well as an index of the effect of therapy since changes in the functional status of the hematopoietic system can thus be suspected in a matter of hours, and estimated in a few days. Preparation of radioiron bound to IV-7 globulin, suitable for use in patients, has been a tedious procedure and difficult to reproduce. We have been able to prepare consistently dependable protein bound tracer only by incubating patient's plasma with radioferric chloride, buffered to pH of approximately 6 with sodium citrate, prepared just before injection(1). This requires the withdrawal of a large blood sample, centrifuging, removal of plasma, incubation with iron preparation, and reinjection, all of which must be done under sterile conditions. To obviate this time consuming procedure for each turnover, attempts were made to prepare a stock solution using either dried pooled plasma or purified IV-7 fraction obtained from Cohn's laboratory in Boston or from Cutter Laboratories in Berkeley. While several of these preparations were satisfactory, others failed to demonstrate adequate iron protein binding and gave rapid disappearance of tracer iron consistent with disappearance of injected ionized iron. In addition, there is always the problem of sterile preparation and continuous refrigeration. When stock solution is prepared from dried pooled plasma, infectious hepatitis is a serious hazard even though the likelihood is remote. Since the IV-7 plasma fraction binds the iron in undialysable form so readily *in vitro* with fresh plasma, the transfer *in vivo* may well be sufficiently rapid to permit injection of iron preparation directly to obtain a disappearance curve similar to that followed by bound material. Furthermore, since bringing ionic iron (as $FeCl_3$ in acid solution) to a pH of 6 will result in precipitation of $Fe(OH)_3$ the iron preparation must consist of iron citrate. This report gives experimental data on rate of disappearance of intravenously administered ferric citrate in patients without primary hematopoietic disorders.

Procedure. A stock solution of radioferric citrate is prepared in quantity, and transferred to sterile withdrawal bottles. The stock solution is prepared by adding $Fe^{59}Cl_3$ in HCl, with specific activity of 2000-5000 mc/g (as obtained from Oak Ridge) to 0.1% (w/v) Na citrate in isotonic saline. The solution is adjusted to a pH of approximately 6 using indicator paper, then transferred to 60

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ml serum bottles, and sterilized by autoclaving at 120°C for 15 minutes. The resultant solution is prepared so that initially 10 ml of solution will contain between 3 and 5 μc of Fe^{59}. Ten ml of blood are drawn into a heparinized syringe for determination of hemoglobin, Wintrobe hematocrit, red blood cell count, and a baseline determination of normally occurring radioactivity in blood, chiefly radiopotassium. Through the same needle, ferric citrate solution is administered containing approximately 4 μc of Fe^{59}, and containing from 1-5 μg iron depending on age of preparation. Five minutes after injection, another 10 ml sample of blood is withdrawn from a different vein. Two ml of this whole blood is used for determination of radioactivity, and the remainder centrifuged to obtain 2 ml of plasma for analysis of radioactivity and of iron concentration. A modification of the method of Burch *et al.*(9) is used for plasma iron concentration, normal values in this laboratory ranging from 70 to 120 μg %. At 5 minutes, and every half-hour thereafter for 4 hours, 2 ml of whole blood are withdrawn for determination of radioactivity. Radioactivity assays are determined using a well-type sodium iodide crystal with a DuMont Photomultiplier tube, operated along a plateau from 1350-1500 volts. This gives a background of approximately 250 cts/min, and a detection efficiency for Fe^{59} of about 30%. The disappearance curve is plotted on semilogarithmic paper, and plasma volume estimated from extrapolated zero-time dilution factor(1).

Experimental. Six patients with a variety of disease conditions not related directly to the hematopoietic system were chosen, on the basis of their availability for 8 hours without undergoing other procedures. Two disappearance rates were determined for each subject, first, using direct injection of ferric citrate in the morning, and second, ferric citrate incubated with plasma in the afternoon. Forty-five ml of blood were withdrawn initially for preparation of plasma, then radioferric citrate was injected along with 5 ml of Evans blue dye. Samples were taken at 5 minutes and every half hour thereafter for 4 hours. During this time an equal amount of ferric citrate

TABLE I. Disappearance Rates and Plasma Volumes Using Ferric Citrate or when Incubated with Plasma.

Patient	Disease	Age, yr	Ht	Wt	Hgb	Wintrobe Hct	Evans blue plasma vol	Direct citrate: Half time	Direct citrate: Pl vol	Direct citrate: Isotope Hct	Direct citrate: Iron conc., μg %	Incubated: Half time	Incubated: Pl vol	Incubated: Iron conc., μg %
D.S.	Diabetes	18	5' 2"	89	10.0	38	2200	37'	1740	34	32	37'	1790	40
R.M.	Carcinoma cervix	45	5' 6"	134	10.0	38	3700	1°23'	3200	39	74	1°20'	3120	68
M.M.	Amebiasis	44	5' 3"	116	14.1	45	2200	1°24'	2100	42	84	1°24"	2100	92
O.C.	Bartholin's cyst	28	4'11"	125	12.3	35	2200	38'	1700	32	84	38'	2060	52
D.C.	Nephrosis	28	5' 7"	139	11.3	36	2700	42'	2300	38	60	42'	2100	36
H.S.	Stab wound	24	6' 1"	172	13.3	39	3300	1°16'	3100	35	56	1° 7'	2960	60

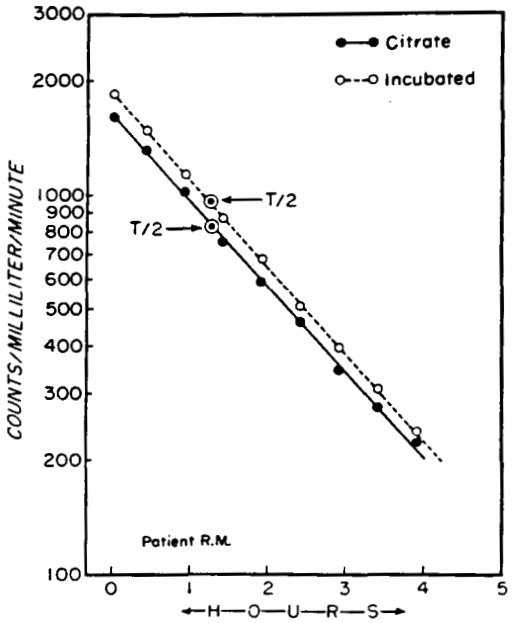

FIG. 1.

was added to the separated plasma, and incubated at room temperature. Four hours after injection of ferric citrate, the incubated plasma was injected, and again blood samples taken at 5 minutes and at half hourly intervals for 4 hours. The results, demonstrating similarity of behavior of the two forms of iron, are presented in Table I. Urine samples were collected on the first three subjects, and contained less than 0.5% of injected radioiron administered as citrate, with no excretion of radioiron following injection of incubated plasma. Urine of fourth subject was collected at one minute intervals through ureteral catheters during the first hour of ferric citrate injection. All radioisotope excretion occurred during the first 2 minutes. These studies are being continued on patients having a wide variety of disease conditions, and utilizing other iron preparations. It may be questioned whether the iron citrate technic would be valid for use with patients having a very high serum iron as in hemochromatosis or hemosiderosis. Assuming that there is an absolute amount of iron binding protein available, a serum iron level of 300 μg % may approach saturation. Then the tracer dose of iron citrate could not be incorporated with protein bound iron. Data of Gitlow(10,11) indicate that saturation in excess of 95% will rarely be encountered. Five % unsaturation of a binding capacity of 300 μg % would represent an ability of protein to chelate an additional 150 μg/l of plasma. The tracer dose of 1-5 μg should not be excessive even in these clinical conditions, though we have not yet had a patient of this category for study. To date, the citrate disappearances have been identical to those of the globulin-bound iron. These studies will be submitted as a separate report when the general applicability has been better established.

Conclusions. Radioferric citrate, administered intravenously to patients without primary disorder of hematopoiesis, disappears from the plasma at a rate similar to that of the disappearance of globulin-bound radioiron. It is probable that the iron is rapidly incorporated into the iron binding globulin of the plasma (IV-7 fraction, Cohn), and the disappearance rate obtained following the injection of ferric citrate is actually that of the disappearance of protein bound iron. The use of ferric citrate as a tracer for iron turnover studies is a simplification which permits

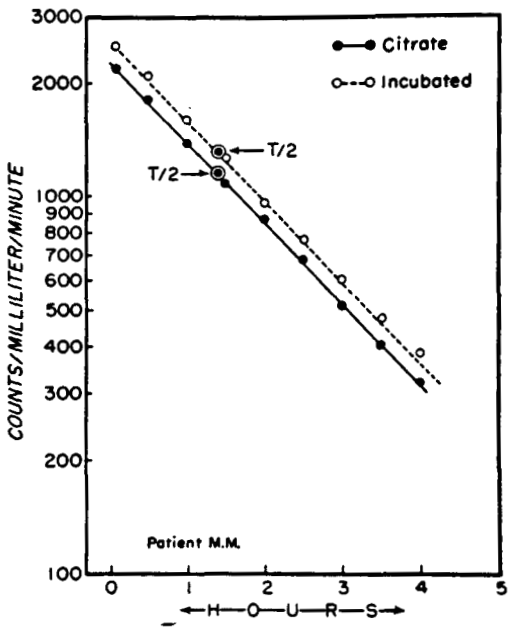

FIG. 2.

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Reprint #4

RADIOIRON TRACER STUDIES: CLINICAL APPLICATIONS IN HEMATOLOGY

Scientific Exhibit

American Medical Association

by

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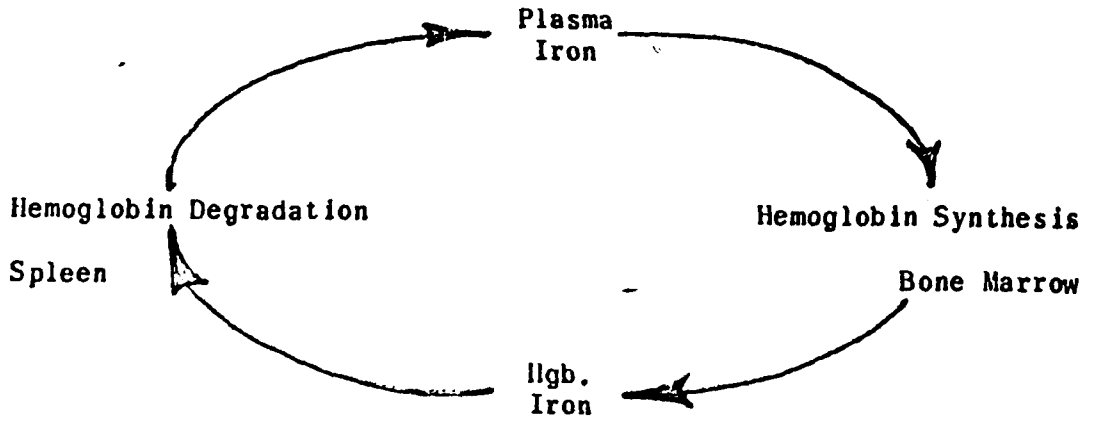
An adult man has 3.5 - 4.5 gm. of iron in his body. From 60 to 70% of this iron is in the red cells as hemoglobin iron, less than 1% in the circulating plasma as plasma iron attached to a protein, Transferrin; and the rest is in other forms of body irons (storage, myoglobin, resp. enzymes).

Although the plasma iron represents less than 1% of the total body stores, it is conveniently available for uniform sampling. It is in labile equilibrium with iron involved in the hemoglobin cycle and in other forms throughout the body.

The hemoglobin cycle consists of three main steps:

1. Synthesis of hemoglobin from iron available in bone marrow.
2. Degradation to available Fe of hemoglobin liberated by breakdown of aged or defective red cells in spleen or other sites.
3. Transport of iron as plasma iron from sites of breakdown and storage to bone Marrow.

The Hemoglobin Cycle



The average life span of normal red cells is generally accepted as 120 days. This means that 100% of red cells are replaced every 120 days, or 0.83% are destroyed and replaced daily.

A 70 kg. man has:

(1) a blood volume of 5000 cc., with

(2) 5×10^6 red cells per mm^3

(3) 12 gm. of Hgb. per 100 cc. of blood, and

(4) 3.34 mg. of iron per gm. of Hgb.

The orders of magnitude for the various components in this hemoglobin cycle may be estimated in the following terms:

Number of red cells destroyed and replaced = 2.1×10^{11} red cells per day
or
2.4×10^6 red cells per second

Calculation:

(0.83×10^{-3})	x	$(5 \times 10^6 \times 10^3)$	x	(5×10^3)
Fraction of red cells destroyed or replaced		No. of red cells per cc. of blood		Total blood volume

<u>Amount of hemoglobin degraded and synthesized</u> = 6.2 gm. of Hgb. per day

Calculation:

(0.83×10^{-2})	x	(15×10^{-2})	x	(5×10^3)
Fraction of hemoglobin degraded and synthesized		Gm. of hemoglobin per 100 cc. of blood		Total blood volume

<u>Amount of iron required for (1) and (2)</u> = 21 mg. of Fe per day

Calculation:

6.2	x	3.34
Gm. of hemoglobin synthesized per day		Mg. of Fe per gm. of hemoglobin

Since 21 mgm. of iron is the daily requirement for hemoglobin production, and since the total iron in circulating plasma is relatively constant at approximately 3 mg., then iron must enter the plasma (from the spleen and other sources) at the same rate at which it leaves (to be incorporated in hemoglobin in newly formed red cells or to enter storage depots).

As mentioned above, the plasma iron pool is in equilibrium with all compartments of body iron, the rate of iron leaving the plasma must therefore represent a composite rate of all pathways involved. However, since the rate of iron leaving for storage is comparatively slow, that for excretion and other purposes even slower, it may be assumed, with few exceptions, that the rate of iron leaving the plasma is by and large a reflection of the functional status of hemopoiesis. Exceptions are pernicious anemia and Mediterranean anemia.

A more direct and more accurate index of hemopoietic activity is the rate of incorporation of iron in the new formed red cells.

Under most hematological conditions, the rate of plasma iron disappearance and the rate of incorporation of iron in red cells are directly related. One exception is seen in untreated pernicious anemia where a rapid plasma iron disappearance is associated with sluggish iron uptake, the latter is presumably evidence of the so-called maturation arrest.

It is obvious that the determination of these rates adds the dimension of time and brings the concept of dynamics to the understanding of the physiology of iron and hemoglobin metabolism in health and disease.

Both of these rates can be measured by the use of Fe^{59} as a tracer. It is the purpose of the exhibit to demonstrate the usefulness of tracer iron turnover study in the diagnosis and prognosis of various hematologic conditions.

The equipment necessary is a well-type scintillation counter and scaler. This can be of a dual purpose design so that the same facilities can be used for the determination of radioiodine uptake in thyroid studies. It is predicted that the radioiron turnover test will become as useful in the study of anemia as radioiodine in the study of thyroid disease.

Normal and Abnormal Values Encountered
with Radioiron Tracer Studies

Hematological Disorders	$(Fe)_p$	Fe^{59} T.O. Half Time	Uptake of Fe^{59} by RBC
	ug%	Hour	%
Normal	70-120	1-2	Up to 90% in 1 wk. Then plateau
Anemias			
Iron deficiency	0-50	0.3-0.5	Approaching 100% in 3-4 days Then plateau
Chronic blood loss	0-50	0.3-0.5	Approaching 100% in 3-4 days Then steady fall off
Hemolytic	15-200	0.3-0.5	Approaching 100% in 3-4 days Then plateau
Chronic infections Malignant tumors Leukemias Liver diseases Uremia	40-60	0.5-1	
Aplastic and Myelophthisic	150-200	2-4	Very low and slowly rising
Anemias			
Addisonian untreated	150-200	0.3-0.5	Slow rise over many weeks, no plateau
remission			
early	40-70	0.3-1	approaching normal
late	70-120		
Mediterranean	150-200	0.3-0.5	Slow rise over many weeks, no plateau
Polycythemia Vera			
untreated	150-200	0.3-0.5	approaching 100%
remission	70-120	1-2	normal
repeated phlebotomy	40-70	0.3-0.5	approaching 100%
Hemochromatosis	200-250		

TECHNIQUE

1. Withdraw 10 cc. of blood into a heparinized syringe for whole blood and plasma samples (1.0 - 2.0 cc.) for the determination of natural radioactivity.

2. Inject intravenously 5.0 - 10.0 cc. of Fe^{59} citrate solution containing 4 microcuries of Fe^{59}. Avoid venostasis.

3. Obtain heparinized blood samples (5 - 10 cc.) at 5, 15, 30, 60, and 120 minutes after injection. Avoid venostasis. Pipette 1.0 - 2.0 cc. aliquot of whole blood from the 15 minute sample, and 1.0 - 2.0 cc. of plasma from from all samples into counting tubes.

4. Count all samples from steps 1 and 3.

5. Plot plasma activity (log scale) against time (linear scale) on semi-log paper.

6. Extrapolate to obtain zero time plasma activity. Determine disappearance or turnover half time ($T\frac{1}{2}$) of plasma Fe^{59}.

7. Calculate plasma volume: $PV = \frac{\text{Total counts injected}}{\text{Zero time counts/cc. plasma}}$

8. Calculate hematocrit from activity of 15 minute samples:
$Hct = 1 - \text{Plasmacrit} = 1 - \frac{\text{Counts/cc. whole blood}}{\text{Counts/cc. Plasma}}$

9. Calculate zero time whole blood activity by proportionality:
Zero time counts/cc. Whole Blood = Zero time counts/cc. Plasma x Pct.

10. Calculate blood volume:
$B.V. = \frac{\text{Total counts injected}}{\text{Zero time counts/cc. whole blood}}$

11. Pool plasma samples, determine iron concentration on duplicate aliquots.

12. Obtain blood samples, (5 cc., heparinized), at 3, 7, and 14 days. Count 1.0 - 2.0 cc. aliquot.

13. Determine approximate per cent red cell incorporation of Fe^{59}:
$\% \text{ uptake} = \frac{\text{Count/cc. follow-up blood sample x 100}}{\text{Zero time count/cc. whole blood}}$

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Chromatographic Separation and Determination of Porphyrin Methyl Esters

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By means of horizontal paper chromatography with filter paper placed between glass plates, the methyl esters of uroporphyrin I, coproporphyrins I and III, and protoporphyrin IX were separated within 1 hour. Elution of individual bands from the paper chromatogram followed by fluorometric analyses permitted quantitative estimations of each porphyrin ester.

DURING an investigation of various methods for the separation and quantitative determination of porphyrin isomers, difficulties were experienced in separating for analyses sufficient amounts of various porphyrins from biological fluids. In this laboratory, repeated clear-cut separations of porphyrin methyl esters from biological fluids with the paper chromatographic methods of Chu, Green, and Chu (*2*) and the modifications of this method by Bogorad and Granick (*1*) was found to be difficult because of interference by impurities. The resultant bands of the porphyrin esters, from either ascending or descending chromatograms, were somewhat irregular and overlapping and at best insufficiently separated for quantitative recoveries.

Using horizontal paper chromatography with a modified solvent system, a rapid method for the complete separation of methyl esters of uroporphyrin I, coproporphyrin I, coproporphyrin III, and protoporphyrin IX has been developed which permits quantitative estimation of each of these porphyrin esters.

EQUIPMENT AND MATERIALS

Two glass plates, 13 inches square, cut from 0.25-inch commercial plate glass. Any other suitable size may be used. One glass square is marked at the exact center with a wax pencil, and a hole 0.25 inch in diameter is drilled through. This is done by forming a ridge of putty around the center mark so that water can stand over this area. Using a short length of 0.25-inch brass tubing in an electric drill press and adding abrasive powder intermittently, a hole is drilled halfway through on one side of the plate, then completed by drilling from the opposite side.

Two barrels from hypodermic syringes, 10 or 20 ml., each of which is fitted with a piece of thin-walled (5-mm. outside diameter) gum rubber tubing over the entire length of the syringe tip. Each syringe is packed with Whatman cellulose powder to a depth of about 2 cm. Before use, each packed syringe is rinsed with the particular solvent to be used for chromatography, and the rate of solvent flow is adjusted to approximately 1 drop per 2 seconds by tamping the cellulose packing with a glass rod.

Six lead bricks, weighing about 25 pounds each (Nuclear Instrument and Chemical Corp., Chicago, Ill.).

Filter paper sheets, Whatman No. 3MM, cut in squares (13 × 13 inches) the same size as the glass plates. At the center of each paper the location for the sample is made by drawing a circle 1 inch in diameter. This circle is divided into arcs by two to six radial lines allowing as many as six samples to be chromatographed simultaneously.

Developing Solvents. SOLVENT A. Three volumes of petroleum ether (boiling point 30° to 60° C.) and 1 volume of chloroform.

SOLVENT B. Twenty volumes of heptane, 1 volume of ethylene dichloride, and 1.5 volumes of *tert*-butyl alcohol.

AUXILIARY SOLVENT. Equal volumes of petroleum ether and chloroform.

Methyl esters of coproporphyrins I and III and of uroporphyrin were furnished by Samuel Schwartz. Paper chromatographic analysis indicated that the esters were single components with only traces of impurities. The methyl ester of protoporphyrin IX was prepared from crude protoporphyrin (H. M. Chemical Co., Ltd., Santa Monica, Calif.) and purified by means of paper chromatography.

Ultraviolet lamp, 360 mμ, Hanovia Type 1C103 (Hanovia Chemical and Mfg. Co., Newark, N. J.).

Fluorometer, Lumetron Model 402-EF, used with a primary filter of Corning glass 5543 (365 mμ) and secondary filter of Cor-

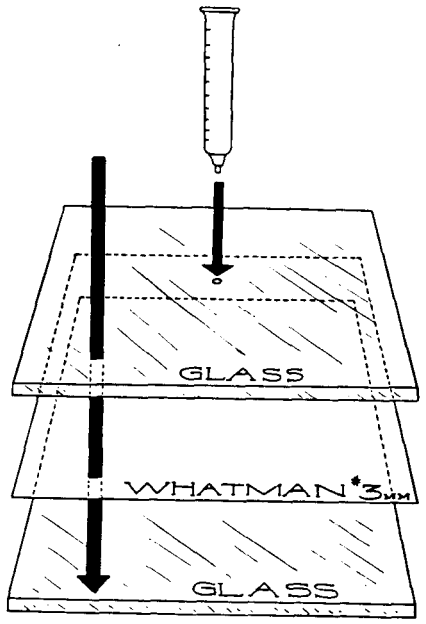

Figure 1. Schematic representation apparatus used in horizontal paper chromatography

ning glass No. 2418 (600 mμ) (Photovolt Corp., New York, N. Y.).

Calibrated micropipets, Levy type (Microchemical Specialties Co., Berkeley, Calif.).

PROCEDURE

Porphyrins are isolated from biological fluids by an ether–ethyl acetate extraction (*4*), by calcium phosphate precipitation (*5*), or by lead salt precipitation (*3*). The mixture of porphyrins is esterified by dissolving the crude extract in 3 to 4 volumes of methanol saturated with hydrogen chloride gas, and the solution is allowed to stand overnight at room temperature in the dark (*1, 5*). Five volumes of water are added to the methanol–hydrochloric acid and the porphyrins are extracted from the diluted solution into chloroform. The chloroform phase is separated, washed with 10% sodium acetate solution, then with water, decanted into a dry beaker, and concentrated in a vacuum desiccator to a volume of 0.5 to 2.0 ml.

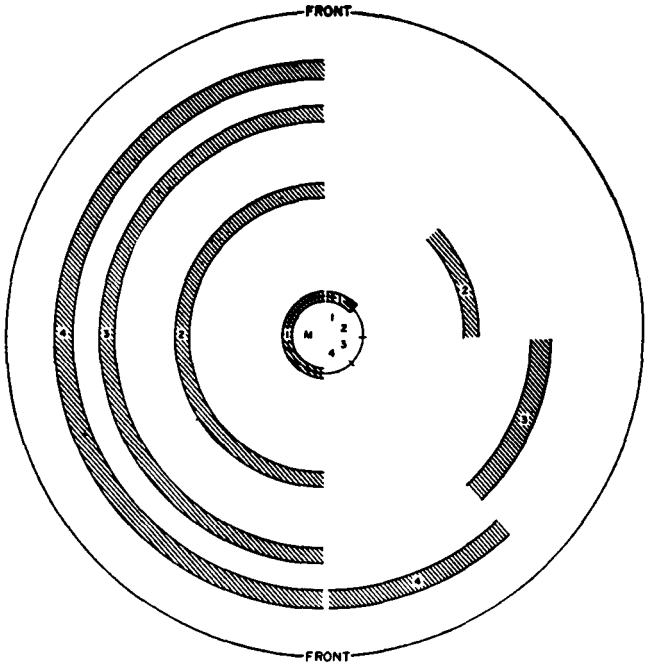

Figure 2. Chromatographic separation of methyl esters of uroporphyrin I (1), coproporphyrin I (2), coproporphyrin III (3), and protoporphyrin IX (4)

Position of individual porphyrin esters is shown to the right; on left is separation from mixture (*M*)

Depending on the concentration of porphyrin esters in the final chloroform solution, 50 to 200 μl. of this solution is transferred by means of a calibrated micropipet to the marked position on the filter paper. The sample is carefully placed on the sample line to form a narrow curved band. As many as six samples can be transferred to one sheet of paper in this manner. When dry, the paper is placed between two glass plates with the center of the paper in line with the opening in the upper plate, and the syringe is then securely fitted into the opening. Figure 1 illustrates the position of the relevant parts. Six lead bricks are placed on the top of the glass plates close to the syringe so that there is a uniform pressure on the paper.

About 2 ml. of solvent A is transferred to the syringe and the chromatogram is allowed to develop until the solvent front is approximately half way across the paper. The filter paper is then removed and allowed to dry in air. The paper is again placed between the glass plates, a new packed syringe is fitted into the opening, and the plates are weighted with the lead bricks. About 8 ml. of solvent B are transferred to the syringe and the chromatogram is allowed to develop until the solvent reaches the edge of the paper. The filter paper is then removed, air dried, and examined under the ultraviolet lamp so that the position of each band can be marked. Figure 2 illustrates the relative position of the porphyrin methyl ester after chromatography by the described procedure. The R_f values are as follows:

Uroporphyrin I octamethyl ester	0
Coproporphyrin I tetramethyl ester	0.53
Coproporphyrin III tetramethyl ester	0.62
Protoporphyrin IX dimethyl ester	0.83

Using solvent B only, the methyl esters of protoporphyrin and coproporphyrins I and III are separated. Figure 3 illustrates a chromatogram developed with solvent B. This solvent does not separate the methyl esters of coproporphyrin I and uroporphyrin I, since both have an R_f of zero.

If porphyrin extracts contain large amounts of impurities, such as are normally found in extracts from urine, it is necessary to use the auxiliary solvent as the first developing solvent to separate the porphyrin esters from the impurities. If 2 ml. of auxiliary solvent are transferred to the syringe, the porphyrin esters will move about 1 inch from the origin along with the solvent front. After the paper is dry, use of solvents A and B, respectively, as described previously, will result in a complete separation of the porphyrin methyl esters into distinct bands, whereas the impurities will remain at the origin. Figure 4 illustrates such a chromatogram of porphyrin esters from a urine to which uroporphyrin I, coproporphyrins I and III, and protoporphyrin IX were added before extraction, esterification, and chromatography. The R_f values are as follows:

Uroporphyrin I octamethyl ester	0.13
Coproporphyrin I tetramethyl ester	0.55
Coproporphyrin III tetramethyl ester	0.70
Protoporphyrin IX dimethyl ester	0.91

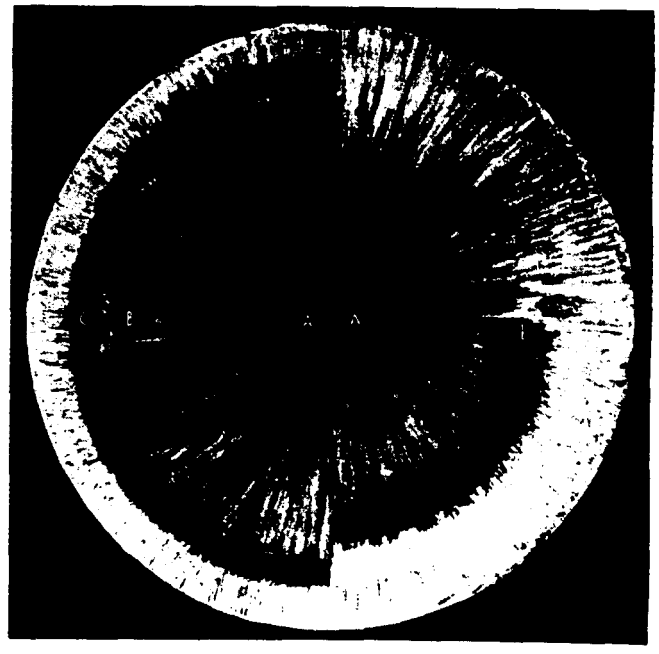

Figure 3. Drawing of actual chromatogram, made under ultraviolet illumination of methyl esters of coproporphyrin I (*A*), coproporphyrin III (*B*), and protoporphyrin IX (C) developed with solvent B

For quantitative determination of each porphyrin ester after chromatography, each band is cut from the paper, the paper shredded, and placed in test tubes (180 × 15 mm). After addition of 15 ml. of 1.5*N* hydrochloric acid, the test tubes are placed in the dark for 30 minutes or longer with occasional shaking. Subsequently, the acid solution is decanted into clean test tubes for fluorometric reading. Standard curves were established with methyl esters of protoporphyrin IX, coproporphyrin III, and uroporphyrin I, as shown in Figure 5. Two filters were used, a monochromatic primary filter with a maximum transmittance at 365 mμ, and a secondary filter transmitting above 600 mμ.

Table I. Recovery of Porphyrin Methyl Esters from Known Mixtures

Sample	Protoporphyrin IX Present, γ	Protoporphyrin IX Recovered γ	Protoporphyrin IX Recovered %	Coproporphyrin III Present, γ	Coproporphyrin III Recovered γ	Coproporphyrin III Recovered %	Uroporphyrin I Present, γ	Uroporphyrin I Recovered γ	Uroporphyrin I Recovered %
1	30	26	87	30	27	90	28	27	97
2	30	25	84	30	27	90	28	27	97
3	30	26	87	30	27	90	28	27	97
4				30	28	93	28	27	97
Average			86			91			97

RESULTS

Tests of the above quantitative chromatographic procedure with known mixtures of pure methyl esters of protoporphyrin IX, coproporphyrin III, and uroporphyrin I gave recoveries of 86, 91, and 97%, respectively, as shown in Table I.

It is apparent from the above data that with increased R_f values, lesser amounts of the porphyrin methyl esters are recovered. This is probably due to tailing along the paper during chromatography, which becomes more significant with components that travel the greatest distance from the origin.

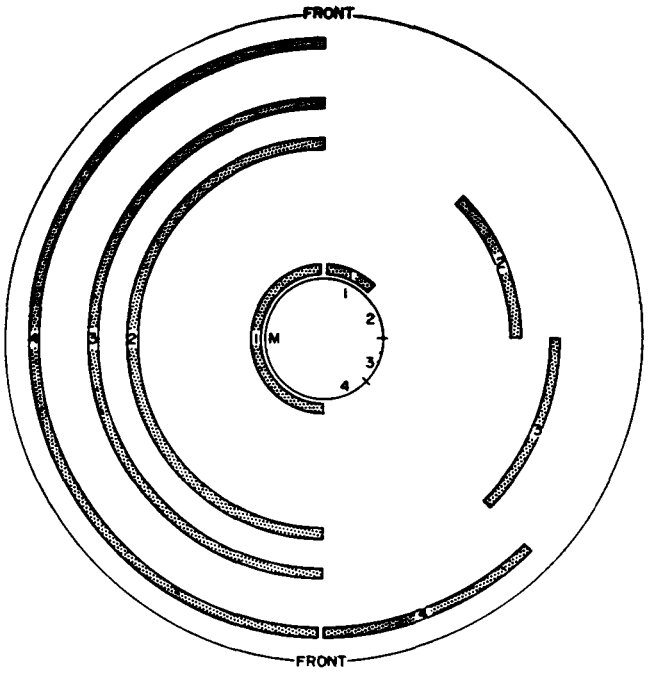

Figure 4. Diagram of chromatogram, made under ultraviolet illumination, of porphyrin methyl esters from urine

Numbers designate same components as in Figure 2

Recovery experiments were made with coproporphyrin added to different urine samples. Table II shows the results of these experiments. The average recovery of coproporphyrin in these experiments was 96% with a range of ± 3%.

In general this procedure for separation and determination of porphyrins showed an accuracy within ± 3% and a sensitivity of approximately 0.1 γ.

The entire process was carried out under ordinary diffuse room lighting. Care was taken to protect the materials from direct sunlight, but no special effort was made to protect them from room light during the time required for chromatography and drying. No losses were observed under diffuse light (3 to 4 hours) although exposure to sunlight caused appreciable loss.

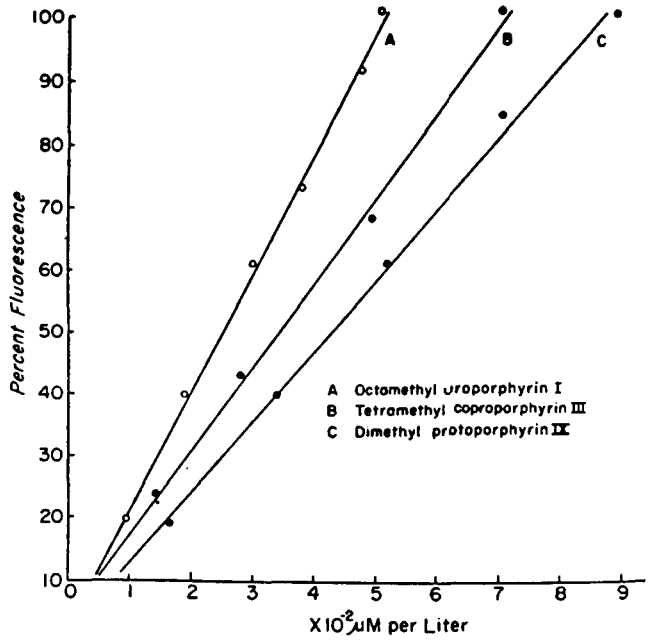

Figure 5. Fluorescence-concentration relation of porphyrin methyl esters

After development of the chromatograms and drying, they were placed in a drawer and covered with filter paper, as recovery experiments indicated a loss of pigment or fading within 24 hours if left in room light. Those placed in desk drawers, however, gave the same recovery as long as 3 days after completion of the chromatogram.

Table II. Recovery of Coproporphyrin III from Urine

Sample	Original Concentration, γ	Added, γ	Total Concentration, γ	Recovered γ	Recovered %
1	157	30	187	180	96
2	189	30	219	216	99
3	2.5	28	31.5	29	95
4	13	28	41	38	93
			Average		96

ACKNOWLEDGMENT

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