CHANGES IN IRON METABOLISM IN EARLY CHLORAMPHENICOL TOXICITY [1, 2]

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The frequency of hematopoietic depression occurring as a result of chloramphenicol toxicity is not known. Generally it is assumed that the incidence is low in relation to the total amount of chloramphenicol used (1). The deleterious action of chloramphenicol has been noted in all degrees of severity from fatal aplastic anemia (2–6), to minimal marrow depression readily reversible upon removal of the drug (7).

The clinical and laboratory signs of frank marrow hypoplasia with anemia, leukopenia and thrombocytopenia, attributable to chloramphenicol toxicity, are readily apparent. This advanced stage of toxicity bears a high mortality rate, and therefore early detection before irreversible changes transpire would be highly desirable.

This report is concerned with the early detection of erythropoietic depression due to chloramphenicol, by means of ferrokinetic techniques. Studies of iron metabolism included measurement of the rate of disappearance of tracer amounts of radioactive iron (Fe^{59}) from the plasma, determination of the subsequent appearance of radioactivity in the circulating erythrocytes and changes in the plasma iron content and plasma iron binding capacity. The results indicate that erythropoietic depression due to chloramphenicol has a higher incidence than aplastic anemia, and that such depression can be detected by ferrokinetic changes before abnormalities of the peripheral blood counts appear. Likewise, erythropoietic depression is detectable when there are no demonstrable changes in leukocytes, thrombocytes or bone marrow cellularity. Although it is a reasonable hypothesis that if chloramphenicol therapy were to be continued this depression limited to red cell production might be a sign of impending aplastic anemia, such an assumption remains unproven.

METHODS AND MATERIAL

Fifteen patients receiving chloramphenicol were studied. These included 9 females and 6 males, with ages ranging from 19 to 85 years; 11 patients were colored and 4 were white. Disease states for which chloramphenicol was administered included: infected traumatic and surgical wounds; meningitis; appendiceal abcess; urinary tract infections with and without uremia; pneumonitis; and infected leg ulcer. The dosage of chloramphenicol varied from 0.75 to 4 grams per day and duration of therapy ranged from 3 to 27 days (Tables I and II). In the majority of instances the patients were selected at random, but several cases were studied because of apparent hematopoietic changes.

The rate of removal of radioactive iron from the plasma and its subsequent incorporation into the erythrocytes was determined according to the method of Huff and associates (8). Ten microcuries (5 to 10 micrograms) of radioactive iron (Fe^{59}) [5] were incubated under sterile conditions with 20 milliliters of the subjects' heparinized plasma for 20 or 30 minutes and then administered intravenously. Blood samples were drawn at 15 to 30 minute intervals for the subsequent 2 to 4 hours and the radioactivity in the plasma counted in a well-type scintillation counter with a thallium activated NaI crystal.[6] Plasma counts of radioactivity were 10 to 20 times background. Subsequently, radioactivity appearing in the circulating erythrocytes was determined daily by counting whole anticoagulated blood which had been frozen and thawed in order to lyse the red cells and maintain a homogeneous mixture. External scintillation counting was performed over the liver, spleen, sacrum, heart and ribs in four patients after Fe^{59} administration, using the methods of Huff and associates (9).

In all the graphs, tables and discussion referring to the

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[5] Fe^{59} was supplied by Abbott Laboratories in the form of ferrous citrate, 100 microcuries per milliliter and 48 micrograms per milliliter.

[6] NRD Instrument Co., Model cs-600.

TABLE I

Patients receiving chloramphenicol who did not manifest erythropoietic depression

Age, color, sex	Disease process	Bacterial organism	Chloramphenicol dosage	Duration of therapy
			Gm./day	*days*
1. 51 WF	Chronic pyelonephritis	*Coli-aerogenes*	1	15
2. 62 CF	Acute purulent meningitis	unknown	3	19
3. 53 CF	Appendiceal abscess	*Coli-aerogenes*	2	3
4. 36 CM	Infected traumatic wounds	*Staphylococcus aureus*	1	11
5. 20 WF	Chronic pyelonephritis	*Proteus vulgaris*	2	10
6. 19 CF	Fever of undetermined origin	unknown	2	10
7. 41 CF	Infected surgical wound	*Staphylococcus albus*	2	6
8. 83 CM	Pneumonia	unknown	1	12
9. 23 CF	Infected surgical wound	unknown	0.75	34
10. 60 CM	Lung abscess	*Proteus vulgaris*	2	27

patients with erythropoietic depression the individual case numbers refer to the same patient. In cases numbered 1, 3, 5, chloramphenicol was discontinued at the time of the first Fe^{59} study. Drug therapy was discontinued in case number 2, five days prior to the original Fe^{59} study. In case number 4, chloramphenicol administration was continued for 11 days after the initial radioactive studies, at which time the patient was discharged from the hospital and lost to further follow. She was restudied as soon as located two and one-half months later.

Measurements of plasma iron and plasma iron binding capacity were determined according to the method of Schade, Oyama, Reinhart and Miller (10) coincident with each radioactive iron study and additionally as indicated.

Sternal marrow aspirations were performed with a 16 gauge Osgood needle on each patient showing abnormalities of ferrokinetics. A nucleated cell count was performed and fixed sections were prepared from each marrow aspirate to aid in estimation of marrow cellularity.

Erythrocyte and leukocyte counts were performed in the standard manner and determination of packed cell volume was by the method of Wintrobe (1). Reticulocytes were counted by the wet method using brilliant cresol blue as stain. Platelets were ennumerated by a variant of the method of Dameshek (11), using 2 per cent sodium citrate as diluent.

RESULTS

In 10 of the 15 patients receiving chloramphenicol, ferrokinetic studies were either normal or compatible with the underlying disease process which was acute or chronic infection. The time elapsed for one-half of the Fe^{59} to disappear from the plasma (half time) varied from 22 to 98 minutes with appearance of significant amounts of radioactivity in the erythrocytes within 48 hours. The plasma iron values ranged from 16 to 100 micrograms per 100 millilters with saturation of the iron binding capacity varying from 5.5 per cent to 39 per cent (Table III). External scintillation counting performed in three patients revealed that as the Fe^{59} disappeared from the plasma major amounts of radioactivity were detected over the sacrum with lesser amounts counted over the liver and spleen.

The remaining five patients showed evidence of depression of erythrocyte production attributable to chloramphenicol. These five patients with erythropoietic depression received chloramphenicol in doses ranging from 1 to 4 grams per day for pe-

TABLE II

Patients receiving chloramphenicol who did manifest erythropoietic depression

Age, color, sex	Disease process	Bacterial organism	Chloramphenicol dosage	Duration of therapy
			Gm./day	*days*
1. 75 WM	Cystitis with bacteremia	*Proteus vulgaris*	4	11
2. 51 WM	Septicemia	*Escherichia coli*	3	20
3. 30 CF	Acute glomerulonephritis	*Coli-aerogenes*	2	7
4. 32 CF	Varicose leg ulcer	*Staphylococcus aureus*	2	15
5. 85 CM	Urinary infection with uremia	*Coli-aerogenes*	1	21

TABLE III

Ferrokinetic studies in patients receiving chloramphenicol without erythropoietic depression

	½ time of disappearance of Fe^{59} from plasma during therapy	Appearance of significant* amounts of Fe^{59} in red blood cells	Plasma Fe	Saturation of iron binding globulin
	min.	*hrs.*	*μg./100 ml.*	%
1.	37		77	38
2.	78		100	39
3.	85	48	16	5.5
4.	22		71	
5.	60	48	47	17.5
6.	98	48	89	31
7.	43	24	41	25
8.	27	24	26	16.5
9.	36	24		
10.	35	48	33.5	18.5

* Counts in the whole blood were at least 2 times background to be considered significant.

riods of 7 to 21 days. None had received chloramphenicol previously. Studies with Fe^{59} in these five patients showed a marked delay in the disappearance of radioactive iron from the plasma in four cases, with one-half times of disappearance ranging from 130 minutes to over 240 minutes (Table IV). The half time in the fifth patient was 98 minutes which falls in the range of normal (normal half times vary from 50 to 100 minutes in this laboratory), but significant amounts of radioactivity failed to appear in the red cells dur-

TABLE IV

Ferrokinetic studies during and following chloramphenicol administration in patients exhibiting toxicity

	½ time* of Fe^{59} disappearance from the plasma during therapy	½ time of Fe^{59} disappearance from the plasma after therapy was discontinued	Delay in appearance of significant radioactivity in the red cells
	min.	*min.*	*days*
1.† ‡	240	24	8
2.§	130	42	8
3.	260	50	9
4.‖ ¶	98	70	17
5.	145	85	11

* Normal ½ time is 50 to 100 minutes.

† In cases 1, 3 and 5, chloramphenicol was discontinued the day of the original Fe^{59} study.

‡ In cases 1, 2, 3 and 5 the second Fe^{59} study was performed when radioactivity first appeared in the erythrocytes.

§ In case 2 chloramphenicol therapy was discontinued five days prior to the original Fe^{59} study.

‖ Case number 4 received chloramphenicol for 11 days following the original Fe^{59} study.

¶ Case number 4 was restudied two and one-half months after the original Fe^{59} study.

TABLE V

External scintillation counting over various organs and radioactivity in the blood in chloramphenicol toxicity in case number 1*

Time following administration of Fe^{59}	Sacrum	Sternum	Liver	Blood
	cpm/μc.	*cpm/μc.*	*cpm/μc.*	*cpm/ml.*
15 min.	174	330	342	850
1 day	180	306	488	110
2 days	142	260	516	0
4 days	144	246	582	0
5 days	144	198	624	0
7 days	168	186	588	0

* Counts are expressed per microcurie of administered Fe^{59}.

ing the additional 11 days of chloramphenicol administration, confirming the presence of erythropoietic depression. In all five cases the disappearance of radioactivity from the plasma formed a

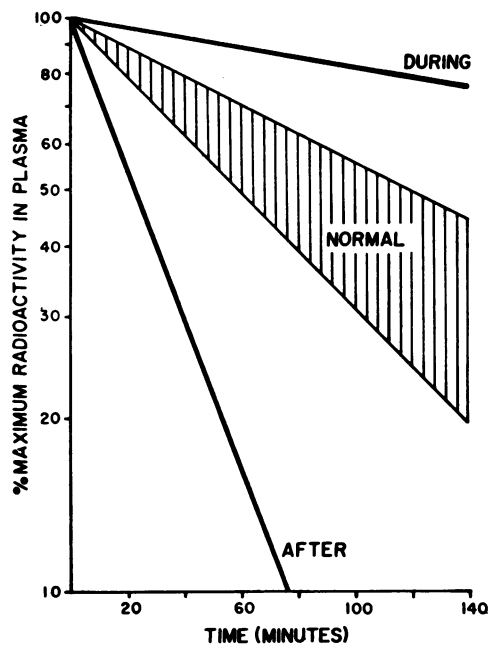

FIG. 1. THE EFFECT OF CHLORAMPHENICOL TOXICITY ON PLASMA DISAPPEARANCE OF Fe^{59}

The curves of Fe^{59} disappearance from the plasma in Patient number 1 with chloramphenicol toxicity are plotted with the logarithm of per cent maximum radioactivity on the vertical axis and time in minutes on the horizontal axis. The hatched area represents the range of normal curves of disappearance. The curve labeled "during" shows the markedly delayed disappearance at the time that chloramphenicol toxicity was detected. The rapid disappearance labeled "after" expresses the findings of a repeat study eight days after chloramphenicol was discontinued.

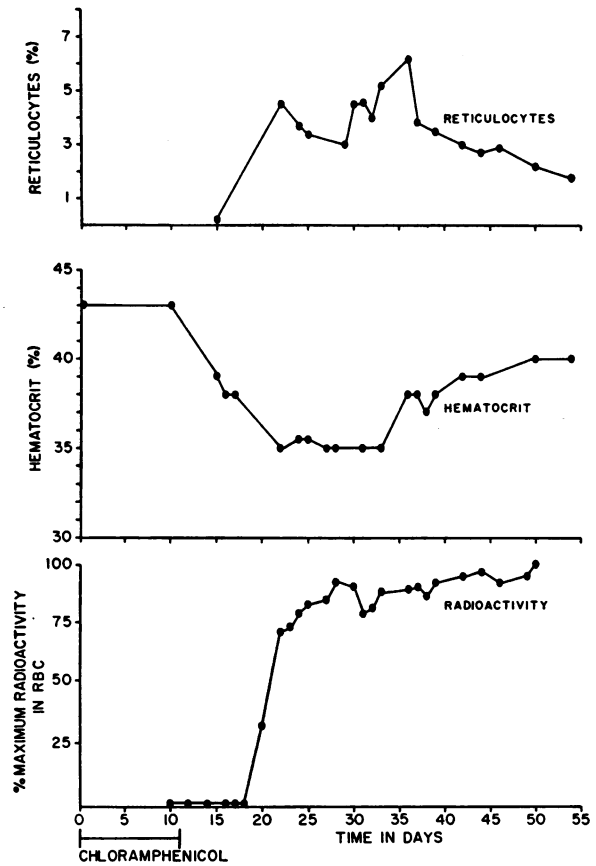

FIG. 2. EFFECT OF CHLORAMPHENICOL ON HEMATOCRIT, RETICULOCYTES AND APPEARANCE OF Fe^{59} IN RED CELLS

The data on Patient number 1 with chloramphenicol toxicity is shown on three graphs. Following administration of Fe^{59} no radioactivity appeared in the red cells for nine days. Maximum radioactivity appearing in the whole blood was 1,797 counts per minute per milliliter. Hematocrit was normal when toxicity was first detected, but subsequently dropped. A reticulocytosis was noted coincident with the appearance of radioactivity in the erythrocytes.

TABLE VI

Changes in hematocrit with chloramphenicol toxicity

Case	Hematocrit when toxicity discovered	Lowest hematocrit during toxicity	Hematocrit after recovery
no.	%	%	%
1	43	35	40
2	35	28	36†
3	20	20*	37
4	37	30.5	42
5	30	32*	34†

* Hematocrit maintained with blood transfusions.
† Continuing infection.

TABLE VII

*Changes in plasma iron and saturation of the iron binding globulin in chloramphenicol toxicity**

	Plasma iron* during therapy	Plasma iron after therapy was discontinued	Saturation of IBG† during therapy	Saturation of IBG after therapy was discontinued
	μg./100 ml.	*μg./100 ml.*	%	%
1.	222	30	86	11
2.	171	77	91	35
3.	71	26	53	12
4.	197	98	82	33
5.	161	49	100	17

* Normal plasma iron is 95 ∓ 25/100 ml. Normal saturation of the iron binding globulin is 28 per cent ∓ 2.
† IBG stands for iron binding globulin.

single exponential curve. External scintillation counting performed in one patient revealed that abnormally large amounts of radioactivity were to be found in the liver with lesser amounts counted over the sternum and sacrum (Table V).

Eight or more days after chloramphenicol ther-

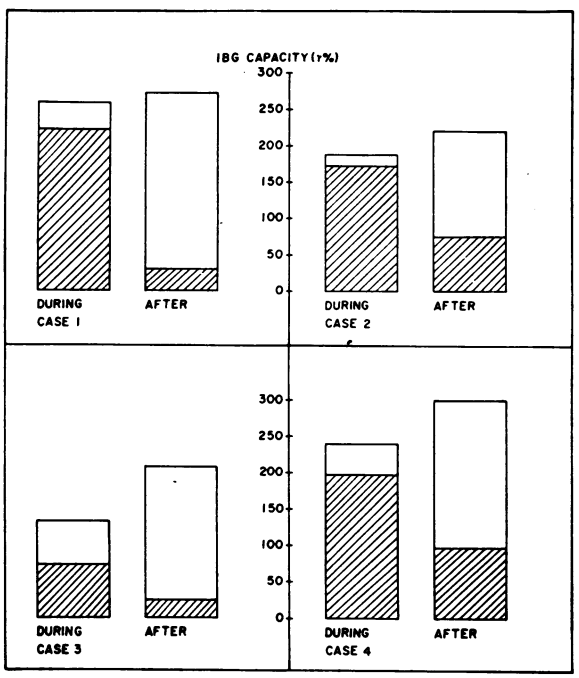

FIG. 3. SATURATION OF IRON BINDING GLOBULIN IN CHLORAMPHENICOL TOXICITY

The total height of each bar depicts the total iron binding capacity of the plasma, expressed in micrograms per 100 milliliters. The hatched portion in each case represents the plasma iron in the same units. The bar labeled "during" shows the findings during chloramphenicol toxicity, while that labeled "after" represents the measurements when definite recovery was noted.

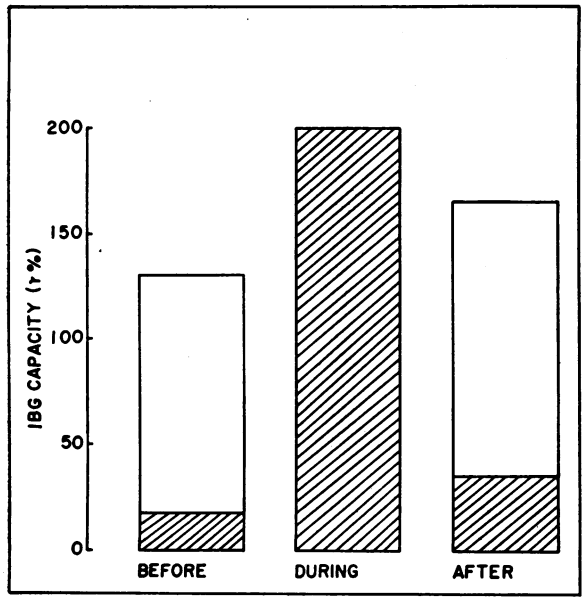

FIG. 4. CHANGES IN IRON BINDING GLOBULIN IN CHLORAMPHENICOL TOXICITY

The plasma iron data in Patient number 5 with chloramphenicol toxicity is depicted in the same manner as in Figure 3. From left to right the three bars show the plasma iron and iron binding capacity before chloramphenicol therapy, during toxicity, and after signs of recovery appeared.

apy was discontinued, repeat studies of plasma disappearance of Fe^{59} revealed half times of 24 to 84 minutes, which values either fall in the range of normal, or are shortened as is commonly seen in infection (Figure 1). For this same period of eight or more days no significant amounts of radioactivity appeared in the circulating erythrocytes. Normally significant amounts of radioactivity will appear in the circulating erythrocytes within 24 to 48 hours, and 85 per cent or more of the administered radioactivity will be incorporated into the red cells within 7 to 10 days. Characteristically the incorporation of Fe^{59} into the erythrocytes will be hastened in acute or chronic infection (12).

One of the five patients with chloramphenicol toxicity had hypoplasia of the bone marrow with anemia, leukopenia and thrombocytopenia; however, the other four cases had no leukopenia or thrombocytopenia, and bone marrow aspiration and section revealed normal or increased cellularity. Two patients were not anemic when depressed red cell production was detected, but manifested a subsequent fall in hematocrit during the period of toxicity and nonproduction (Figure 2). Two patients had azotemia and marked anemia, and further fall in hematocrit during the period of toxicity was obscured by blood transfusions. In all five patients there was recovery of hematocrit towards normal after therapy was discontinued (Table VI).

Determinations of the plasma iron pattern in the five patients with erythropoietic depression correlated with the alterations observed with Fe^{59}. Thus, there was an elevation of the plasma iron in four cases with values of 161.5 to 222 micrograms per 100 millilters. However, in all five cases the iron binding globulin was abnormally highly saturated, the percentage saturation ranging from 53.3 per cent to 100 per cent (Table VII, Figures 3 and 4). Normal values of plasma iron in this laboratory are 95 ∓ 25 micrograms per 100 milliliters, with a saturation of iron binding globulin of 28 per cent ∓ 2.[7]

DISCUSSION

The abnormalities of iron metabolism in the five patients exhibiting chloramphenicol toxicity were those of complete suppression of erythrocyte production. These were: an elevation of the plasma iron with an abnormally high saturation of the plasma iron binding globulin (13); a delay in the disappearance of radioactive iron from the plasma (14–16), with external scintillation counting in the one patient so tested revealing abnormally large amounts of radioactivity deposited in the liver rather than in the organs normally containing bone marrow (9); failure of the radioactivity to appear in the circulating red corpuscles (15) for a period of eight or more days; and a decrease in the volume of packed red cells.

These abnormalities of iron metabolism cannot be attributed to infection alone. Bacterial infection without chloramphenicol toxicity characteristically gives the following findings: a lowered plasma iron with decreased saturation of the iron binding globulin (13); a rapid disappearance of radioactivity from the plasma with external counting indicating that the Fe^{59} appears in the bone marrow as it is cleared from the plasma; and a prompt appearance of radioactivity in the erythrocytes (12). Infection alone, without chloram-

[7] Normal values were established in 20 healthy medical students studied in the morning, fasting state.

phenicol administration, has not been observed to produce a pronounced depression of red cell production, even in cases of overwhelming and fatal bacteremia.

Measurements of the disappearance of Fe^{59} from the plasma coupled with knowledge of the plasma iron and plasma volume may be utilized to calculate the plasma iron turnover (8). The resultant value is usually indicative of the rate of erythropoiesis. However, in aplastic anemia and other states associated with nonproduction of erythrocytes, verified by the failure of radioactivity to appear in the circulating erythrocytes, the plasma iron turnover may be normal or elevated (14, 16). It has been suggested that under these circumstances the plasma iron turnover cannot reflect the rate of effective erythropoiesis, but rather is indicative of the exchange of iron between the plasma and the iron stores (14). In the absence of any correlation between the plasma iron turnover and erythropoiesis, such as occurs in chloramphenicol toxicity, any calculation of plasma iron turnover will be misleading rather than informative. Accordingly, the ferrokinetic data is presented here in terms of the times in which one-half of the administered radioactivity disappeared from the plasma. With the exception of hemachromatosis, a marked prolongation of the one-half time of Fe^{59} disappearance is characteristic only of states in which erythropoiesis is suppressed. The failure of radioactivity to appear in the red cells is confirmatory and provides proof of decreased erythrocyte production, in those instances of delayed disappearance of Fe^{59} from the plasma.

Some agents such as certain antimetabolites, alkylating agents and gamma radiation have been shown invariably to cause hematopoietic depression and bone marrow aplasia if administered in adequate quantities; while others such as the 5-substituted hydantoins, the straight chain analogs of the hydantoins, and the 2,4, dione ring structures will cause only occasional and unpredictable marrow depression or aplasia. Chloramphenicol is to be found in this latter group of agents whose toxic action has been labeled "drug idiosyncrasy" (1). Previously recognition of hematopoietic depression due to chloramphenicol has depended on observing depression of leukocyte and thrombocyte counts in the peripheral blood accompanied by the morphologic changes of hypoplasia or aplasia of the bone marrow. It has been established that bone marrow function is not necessarily correlated with bone marrow morphology (1). Disparity between an actively cellular marrow and a severe depression of erythrocyte production was a prominent feature in four of the five cases exhibiting chloramphenicol toxicity, in whom there was no apparent disturbance in marrow cellularity, leukocytes or thrombocytes. In these four cases it was only by means of Fe^{59} studies and determination of the changes in the plasma iron pattern that the failure of erythrocyte production could be verified. Particularly in two of these patients (Numbers 1 and 4), in whom the degree of anemia was mild and chloramphenicol was discontinued because the infection was eradicated, there would have been no reason to suspect chloramphenicol toxicity since the bone marrows were highly cellular and leukocyte and platelet counts were normal.

It remains unknown whether these four patients would have acquired hypoplasia of the bone marrow had chloramphenicol therapy been continued, or whether a period of reversible erythropoietic depression may be an isolated toxic manifestation and be unrelated to depression of leukocytes and platelets. However, in the absence of data to the contrary it is a reasonable hypothesis that the reversible depression of red cell production is a manifestation of the same toxic action of chloramphenicol that can produce irreversible and often fatal aplasia of the bone marrow. If the latter concept is true then factors of duration of therapy, dosage of chloramphenicol, individual patient sensitivity or other unknown factors may be incriminated. In any event it is reasonable to assume that early detection of chloramphenicol toxicity may prevent more serious consequences.

In regard to early detection of erythropoietic depression due to chloramphenicol it is noteworthy that changes in plasma iron pattern correlated with the abnormalities of ferrokinetics. In each case, at the time the Fe^{59} studies demonstrated supression of erythrocyte production, the plasma iron binding globulin was abnormally highly saturated with iron, with a return to lower levels of saturation when recovery occurred. The high degree of saturation of the iron binding globulin did not occur in the other patients with infection except when an obvious hemolytic episode was present. It would appear that determination of

the plasma iron and plasma iron binding capacity provides a simple means of detecting early toxicity in patients receiving chloramphenicol and that these determinations would be useful in screening other agents with potentially toxic effects on the bone marrow.

SUMMARY AND CONCLUSIONS

1. Fifteen patients receiving chloramphenicol were studied utilizing ferrokinetic methods and determinations of the plasma iron pattern. Five of these patients exhibited severe erythropoietic depression attributable to chloramphenicol; however, this does not represent a true incidence.

2. One of the five patients with erythropoietic depression had leukopenia, thrombocytopenia and hypoplasia of the bone marrow; however, the other four cases had no discernible abnormality of leukocytes or thrombocytes, and bone marrows showed normal or increased cellularity.

3. In two cases with chloramphenicol toxicity, abnormalities of iron metabolism were observed to precede the onset of anemia.

4. Abnormalities of ferrokinetics were: a delay in the disappearance of Fe^{59} from the plasma; appearance of radioactivity in the reticulo-endothelial system rather than in the bone marrow; and failure of radioactivity to appear in the circulating erythrocytes for eight or more days.

5. Coincident with the observation of abnormalities of ferrokinetics, the plasma iron binding globulin was noted to be abnormally highly saturated with iron. With recovery from toxicity the saturation decreased.

6. The abnormalities of iron metabolism noted in chloramphenicol toxicity are readily discernable from the changes seen in infection alone.

7. Determination of the plasma iron pattern provides a simple means of detecting erythropoietic depression. Such early detection may well be instrumental in preventing the more serious consequences of aplastic anemia due to chloramphenicol or other drugs with potentially toxic effects on the bone marrow.

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