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STUDIES OF THE HEMOLYTIC EFFECT OF RADIATION

By

Samuel Schwartz, Elaine J. Katz, Lillie Mae Porter,
Leon O. Jacobson and Cecil James Watson

July 10, 1946

[redacted]

Report Received: May 28, 1947
Figures Received: June 13, 1947
Issued: June 24, 1947

This investigation was aided by a grant from the Rockefeller Foundation.

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ABSTRACT

STUDIES OF THE HEMOLYTIC EFFECT OF RADIATION

By

Samuel Schwartz, Elaine J. Katz, Lillie Mae Porter,
Leon E. Jacobson, and Cecil James Watson*

Evidence is presented indicating that both acute and chronic radiation anemia are due in part to a hemolytic reaction, and not solely to an inhibition of erythropoietic activity as has been assumed by most investigators.

The hemolytic concept is based on the following evidence taken from a survey of the literature as well as from our own experimental studies. (1) The red cell count in human and animal subjects may fall at a rate that is faster than can be accounted for by assuming even 100 per cent inhibition of erythropoiesis. (2) The red cell count may decline at a time when the reticulocyte count is actually elevated. (3) Histological evidence of increased red cell destruction has been demonstrated repeatedly. (4) The bone marrow of humans that have anemia due to chronic radium poisoning is typically hyperplastic, not aplastic. (5) Increased bile pigment excretion has been shown to occur following administration of large doses of P^{32} or total body X ray.

Excessive irradiation appears to destroy both mature and immature cells in increased amounts. More studies of human subjects are required to evaluate the importance of this hemolytic factor. Special attention in these studies should be given to bile pigment excretion and reticulocyte measurements.

* This investigation was aided by a grant from the Rockefeller Foundation for medical research.

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STUDIES OF THE HEMOLYTIC EFFECT OF RADIATION

by

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Leon O. Jacobson, and Cecil James Watson*

1. Introduction

Despite considerable study of the hematological effects of radiation there is still little fundamental information available on the nature of radiation anemia, especially as regards the relative importance of hemolytic and regenerative factors. This has been due chiefly to the fact that the investigative tools used heretofore have been limited largely to routine studies of the peripheral blood. This has tended to yield a relatively static picture that is merely the end result of a number of factors such as the rate of production, release from the bone marrow, and life span of the red blood cells. Studies correlating bone marrow and peripheral blood findings are few, and biochemical investigations of red cell destruction have, to our knowledge, not been described. Even reticulocyte measurements have been reported in only rare instances.

In a review of the literature up to 1943, Cantril et al[1] pointed out that most investigators believed that the anemias appearing both in humans and in experimental animals following administration of either external or internal radiation are due to suppression of the erythropoietic system, or to abnormalities of regeneration. During the preceding eighteen

* This study was aided in part by a grant from the Rockefeller Foundation for Medical Research

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years, Martland[2-7] repeatedly spoke of exhaustion of the bone marrow due to chronic radium poisoning (despite his own consistent evidence to the contrary, as noted below) and more recently Reinhard et al[8] in an excellent study and review of the therapeutic uses of P^{32} in diseases of the hemopoietic system stated that "Radioactive phosphorus exerts its therapeutic effect, as does x-radiation, by slowing the rate of erythrocytogenesis. Since, however, mention is made neither of reticulocyte counts or bone marrow studies to indicate the "rate of erythrocytogenesis", nor of bile pigment excretion studies to indicate the rate of red cell destruction, this statement must rest on incomplete evidence.

While it is obvious that suppression of erythropoiesis occurs following administration of certain types and doses of radiation and may result in extreme aplasia of the erythroid elements in the bone marrow[9], it appears equally obvious that radiation anemia cannot be due to inhibition of erythropoiesis alone; a hemolytic component* must play a variable and often crucial role in the development of both acute and chronic radiation anemia. Evidence for this assumption is threefold, viz., histological, mathematical and biochemical, as will appear in the following.

1.1 Histological Studies

(a) Bone Marrow Histology in Chronic Radium Poison-

* In the present report the term "hemolysis" is used to indicate all types of red cell destruction, regardless of their nature.

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ing. Studies of red cell metabolism probably have been most important in humans with chronic radium poisoning since anemia has been the most consistent clinical feature of this disease, as well as the usual cause of death. Because other radiation emitters, now available, may be expected to act similarly, and because the confusion regarding radiation anemia is so well exemplified by the literature on radium poisoning, this subject will be dealt with here in greatest detail. The reports by Martland, especially, will be considered since his studies have been most extensive and, in general, most valuable.

Martland has described histological changes in the bone marrow of six individuals in the dial painting industry who died of chronic radium poisoning. In the first case (2) he found that, "There was entire replacement of the fatty marrow of the femur by actively regenerating tissue, a marrow hyperplasia of the megaloblastic type."* He also observed that, "The spleen showed....diffuse fibrosis and slight increase in hemosiderin pigment." Before death peripheral blood study in this patient showed a hemoglobin value of 20 per cent and red blood count of 1,064,000.

In the second case (3) Martland reported that, "Necropsy showed an intense replacement of normal adult fatty marrow by a red regenerating bone marrow. Histologically, the marrow was characterized by an enormous number of nucleated red cells, normoblasts and megaloblasts which showed a re-

* It is not clear whether these cells were true megaloblasts, or merely large cells.

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generation of the megaloblastic type." A hemoglobin value of 20 per cent and a red blood cell count of 964,000 were previously reported before the death of the patient[2].

The third case, reported by Reiter and Martland[4], also showed a bright red marrow and "... a marked erythroblastic regeneration. The marrow consists almost entirely of groups of closely packed erythrocytes in which can be found many normoblasts and a few megaloblasts.... Considerable hemosiderin pigment is present."

In his fourth autopsy report [4] Martland described a "regenerative leucopenic anemia of the megaloblastic type." Similar findings were also described in his final report[7] in which Martland noted that, "The general architecture, structure and landmarks were entirely obscured by the extreme hyperplasia, with a packing of immature and primitive cells."

Martland's evidence, therefore, consistently indicated heightened erythropoietic activity of the bone marrow in fatal radium poisoning in humans. Despite this evidence, however, he concluded after his first case that, "There is a stage of stimulation followed later by sudden exhaustion of the erythroblastic centers with production of a rapid fatal anemia." Of his second case (before the patient's death) he said, "It is due to exhaustion of the blood forming centers...." and even after necropsy examination showed the "....intense replacement.... by a red regenerating bone

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marrow". He said of the irradiation that it, "...may go on for years before the centers are finally exhausted". This hypothetical "exhaustion" of the blood forming centers was also mentioned in the discussion of his later cases.

Martland was aware of this contradiction and pointed out in his 1929 review of the subject[6] that, "The occurrence of this apparently hyperplastic marrow was puzzling since, heretofore in the report of anemias due to undue exposure to x-ray and gamma radiation from radioactive substances they have usually been described as distinctly aplastic in type. As in practically none of these cases was necropsy performed or study of the bone marrow made, this conception is purely a clinical one and may not always be substantiated by the facts as we know them."

Martland repeatedly points out that the anemia is not a hemolytic one since the icterus index is normal and hemosiderin deposits are not increased as much as in pernicious anemia. Obviously, however, these criteria are insufficient for ruling out the presence of a chronic hemolytic anemia, since the latter may be associated with a normal icterus index if liver function is adequate[10], and since mention of some increase in splenic and marrow hemosiderin is made in a few of the detailed reports, as noted above.

A similar finding of a hyperplastic bone marrow in a human with chronic radium poisoning has been reported by Gettler and Norris[11]. On the other hand, we are not

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familiar with any clear cut evidence of generalized bone marrow exhaustion in chronic radium poisoning in humans. Flynn's negative report of one case[12] states only that, "The bone marrow presented the same general condition as is found in benzol poisoning." This statement is ambiguous since the bone marrow in benzol poisoning may be either hypo- or hyperplastic[13]. Neither is the extent of the post-mortem examination indicated by this author. It may be concluded therefore that the typical bone marrow in this condition is not exhausted; it is actually hyperactive, as one would expect it to be, for example, in cases of chronic hemolytic anemia. It is also very similar to the hyperplastic marrow described in dogs with experimental anemia due to bleeding[14].

There are few published studies on the hematological and histological findings in radium poisoning in experimental animals. Even where adequate studies have been made, it should be pointed out that the doses administered were vastly greater than those found in cases of human radium poisoning. This is especially important since aplasia of the bone marrow is commonly associated with acutely lethal doses of both external and internal radiation, whereas normal erythropoiesis or hyperplasia is associated with chronic low doses of radiation such as were found in the human patients[9].

Thomas and Bruner[15] gave a total of 40 to 60 micrograms of radium chloride subcutaneously to four female rats

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over a period of 117 to 191 days. Post-mortem examination showed that, "In all cases, approximately the middle two-thirds of the shafts of the long bones contained a hyperplastic marrow in which the fat cells were completely replaced by the red marrow. Also, in all cases, an aplastic marrow was found in the extremities of all the long bones, in the vertebrae, and mandibles, and the illii of the injected rats." The authors point out that, "The greater concentration of radium in the ends of the bone accounts for the earlier destruction of the marrow at these points."

Sabin and co-workers[16] studied the effects of both radium and mesothorium in rabbits. In one of the rabbits that developed an acute anemia these authors noted that, "The signs were of a peripheral destruction of red cells; marked fragmentation of these cells, as seen in the supravital preparations and the extreme increase in iron-containing pigment in spleen and bone marrow seen in sections." Scattered areas of aplasia were noted in the bone marrow.

Dunlap et al[17] fed a total of 100 micrograms of radium chloride to 13 rats over a 20 day period. Only one animal developed a severe anemia. Extensive hypoplasia of the bone marrow was found in all animals. Abundant hemosiderin deposits were found in both spleen and bone marrow.

(b) <u>Erythrophagocytosis Following Acute Radiation Exposure</u>. Heinicke in 1905[18] pointed out the occurrence of red cell destruction in rabbit bone marrow about 48 hours after acute x-ray exposure. Increased pigmentation in the

spleen was also described. Other investigators[17, 19, 20] have described similar phenomena. This problem is dealt with in considerable detail elsewhere in these volumes[21]. It has also been reviewed, along with the general problem of the hemotological effects of radiation, by Selling and Osgood[22].

1.2 The Speed of Development of Acute Radiation Anemia. While there is no unanimity regarding the life span of the red blood cells, most investigations have indicated that red cells remain in circulation for an average of 100, plus or minus 30 days[23-26]. Assuming this figure to be correct, it is obvious that complete cessation of erythropoiesis alone would result in about one per cent fall in red blood count (or hemoglobin) per day. Reports from various laboratories indicate that during the period of most rapid development of anemia the rate of red count (or hemoglobin) fall may be much greater than this[20, 27, 28, 29].

1.3 Biochemical Evidence of Increased Red Cell Destruction. We are not familiar with any quantitative studies of bile pigment excretion in irradiated subjects that might be used as a measure of the rate of red cell destruction.

In a study of the effect of irradiation on cats, Wright and Bulman [30] noted that "... with fatal doses, a marked anemia usually develops several days before death. That this may be due to hemolysis is suggested by the greenish tinge of the plasma which frequently gives a positive Fouchet reaction and a positive indirect van den Bergh for bilirubin."

Harold and Meiszner[31] quantitated total urine pigment according to Heilmeyer's method and, like Heilmeyer[32], found increased pigment excretion following local irradation of five female subjects with uterine tumors. This was interpreted as being due to a hemolytic reaction. The quantitative interpretation of this data, however, is uncertain.

In May, 1945, we reported preliminary studies of fecal urobilinogen excretion in four dogs given 200 to 300 r total body x ray[33]. These studies, as well as previous studies on excretion of urinary bilirubin in irradiated dogs[34] indicated the presence of a hemolytic reaction following irradiation. These studies, though somewhat expanded in the present report are still to be considered only exploratory in nature. As such, they require further investigation, especially in human subjects

2. Materials and Methods

2.1 Subjects

(a) Human Studies. Studies have been made of the effect of P^{32} on hemoglobin and reticulocytes in seven patients with polycythemia rubra vera. In only one case [redacted] studied at the University of Minnesota, was the patient hospitalized; hence, bile pigment excretion studies are limited to this one patient. The other subjects were ambulatory patients studied in the hematology clinic of the University of Chicago. In the present report only those studies that bear directly upon the problem of hemoglobin

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metabolism will be dealt with. Other studies of these patients are described elsewhere in these volumes. (35)

The P^{32} was administered orally in divided doses in all but two instances. In the latter cases it was given intravenously.

(Patients with anemia due to chronic radium poisoning have not been available to us for study. Fecal urobilinogen excretion was found to be normal in one individual with minor bone changes but no anemia. Various radioactivity measurements indicated a total body content of 2.2 micrograms of radium in this patient. It might be pointed out in this regard that patients with so-called aplastic or refractory anemia of unknown etiology, have often been found (10) to have considerable increases in excretion of fecal urobilinogen.)

(b) Animal Studies. The effect on hemoglobin metabolism of both single and multiple doses of total body irradiation was studied in mongrel dogs. The technique of irradiation is described elsewhere (36).

The animals were kept in metabolism cages suitable for the separate collection of urine and feces. The urine was combined in consecutive one to four day periods, the longer periods being used during the chronic stages. The feces were similarly combined in three to six day periods.

The dogs were fed commercial dog biscuits (Friskies), generally supplemented with meat one or two times weekly. Most of the animals were cared for and studied in other

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respects by members of the Biology Section whose reports may be found elsewhere in this volume [37, 38, 39].

2.2 Analytical Procedures.

(a) Routine hematology studies included hemoglobin, red blood cell, hematocrit, and reticulocyte counts. These are reported in more detail elsewhere [37, 38, 39]

(b) Bile pigment excretion studies. These studies, it is generally agreed, yield the most reliable index of the rate of hemoglobin destruction.

(1) Excretion of fecal urobilinogen was determined in both dog and human subjects by using Watson's method of analysis[40].

(2) Bilirubin excretion was quantitated in dog urine employing the method of Malloy and Evelyn as described originally for serum [41]. Readings were taken after 15 minutes and again after the addition of alcohol. Because of the known low renal threshold for bilirubin in dogs [42] a considerable portion of the total bile pigment may be excreted in the urine in these animals.

(c) Red cell protoporphyrin studies, were done because of reports that this substance is significantly altered in hypochromic and infectious anemia [43, 44]. The method of Grinstein and Watson was used [45].

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3. Experimental

3.1 The Effect of P^{32} on Hemoglobin Metabolism in Patients with Polycythemia Rubra Vera.

Case I [redacted] A forty-six year old white female received a total of 19.8 mc of P^{32} from October, 1944 to June, 1945. Ten mc of this amount was administered intravenously on June 9 and 11, 1945. Her hemoglobin on June 12 was 16.2 grams per cent. By July 31 it had fallen to 12.5 with a red count of 5.7 million. Twenty-eight days later it was 8.6 grams, and by October 2 had come down to 6.7 grams with a red count of 2.66 million. Unfortunately no reticulocyte determinations were done until September 27. From this date until October 13 the latter ranged from 3.6 to 8.5 per cent. During this period fecal urobilinogen excretion was two to three times above normal. The bone marrow (biopsy) exhibited a marked normoblastic hyperplasia. Pertinent studies are plotted in Figure 1.

From the above data it is obvious that the patient developed a hemolytic anemia following the final P^{32} administration. This is shown by the elevated fecal urobilinogen excretion as well as by the rapid fall in hemoglobin concentration despite an elevated reticulocyte count. No blood transfusions were given during the period of study.

Cases II to IV. Hemoglobin and reticulocyte studies in three other patients who received oral P^{32} therapy are summarized in figures 2 to 4. It should be noted that there is no indication from the reticulocyte counts of sufficient suppression of erythropoietic activity to account for the

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hemoglobin fall. Actually at least one of these patients (J.B.) had a definitely elevated reticulocyte count during part of the period of hemoglobin fall. Unfortunately, however, the absence of bile pigment excretion studies in these patients makes it impossible to prove with certainty the presence of a hemolytic reaction.

In Figure 5 are plotted the data on an individual (A.O.) whose hemoglobin, following other therapy, had fallen to about 12 grams per cent. Following the administration of 18.6 mc P^{32} over a period of 24 days, his hemoglobin rose steadily to about 17 grams. This study is important because it indicates that normal erythropoiesis may follow the administration of this dose of P^{32}.

In summary it should be pointed out that the per cent fall of hemoglobin values in each of the above patients (except A. O.) averaged about one per cent per day over a period of 14 to 40 days. During the same period, average reticulocyte counts for the individual patients ranged from 0.5 to 1.1 per cent (no reticulocyte studies were done on M.K. during the period of maximum hemoglobin fall).

No consistently significant change was found in the concentration of protoporphyrin in the red blood cells following the administration of P^{32}. The average red blood cell protoporphyrin concentration in these patients was correlated to some degree with the color index; elevated values were found mostly in those patients with a low color index whereas normal values were generally found associated

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with normal color indices. As seen in Figure 6, however, the spread of values at any given color index was very great. It is obvious, too, that other factors must be operative since changes in color index in given patients did not result consistently in simultaneous changes in the protoporphyrin concentration in red blood cells.

3.2 The Effect of Total Body X ray on Hemoglobin Metabolism in Dogs.

(a) Control Dogs. In addition to control studies of treated dogs, fairly prolonged studies of fecal urobilinogen excretion were made in two control dogs. Results in the two instances were essentially similar. The data on one of them are illustrated in Figure 7.

A total of 85 determinations have been done of fecal urobilinogen excretion in 18 control dogs. Most values ranged from 5 to 10 mg per day with extremes of 1 to 25 mg per day.

In working with mongrel dogs, such as were available to us, one must constantly keep in mind the fact that control dogs are not necessarily healthy animals, and that "routine" therapeutic procedures may produce undesirable biochemical effects. This fact was emphasized in the study of one dog whose fecal urobilinogen excretion rose markedly just before beginning the administration of 25 r total body X ray daily. Simultaneously the dog's hemoglobin concentration fell and the reticulocyte count rose sharply. On inquiry

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it was found that seven days, and again three days before x-ray administration began the dog was given 22 cc of Lederle's Canine Antibacterial Serum (Formula #1) in the hope that it would combat an infection and cause the return of an elevated white count to normal. Studies of this animal are presented in Figure 8.

(b) Irradiated Dogs. In most dogs given a single large dose of total body X ray an increased excretion of urobilinogen was found, especially from about the fourth to twelfth day. This is often, though not always, associated with a drop in the reticulocyte count. Data on two such dogs are illustrated in Figures 9 and 10

From the sixth to the tenth day after irradiation, the hemoglobin of dog 43 fell at an average rate of about six per cent per day; that of dog 36 fell at an average rate of about 2 per cent per day from the third to ninth day following irradiation. This compares well with the data on several other dogs whose hemoglobin dropped for several days at a maximum rate of two to five per cent per day [37,38,39].

A third dog (#44) was given 300 r total body X ray. Four determinations during the sixteen day control period averaged 12 mg per day. During the sixteen days after irradiation the average value was 26 mg per day. In the last two day period the concentration rose to 170 mg per cent, but because of marked constipation the per diem value for this period was only 22 mg per day.

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Dog 37 received a single injection of 26.5 mc Sr^{89}. Studies of hemoglobin metabolism in this animal are summarized in Figure 11.

The interpretation of data on excretion of bile pigment is often difficult because many of the dogs become severely constipated. This is illustrated in Figures 12, 13 and 14, in which urobilinogen excretion values are plotted in terms of both milligrams per 100 grams feces and milligrams per day. It will be noted that the former value may rise markedly with little or no significant change in the latter value. All three dogs received 50 r total body X ray daily until death.

Terminal increases in fecal urobilinogen excretion may be associated with internal hemorrhage. This was most strikingly illustrated in dog 26 which died 72 days after daily treatment with 12.5 r total body X ray was started. Subcutaneous hemorrhages were first noted 14 days before death, and considerable internal bleeding was found at the post-mortem examination. During the last 14 days of life the hemoglobin concentration fell precipitously from 11.2 to 4 grams per 100 cc. The fecal urobilinogen excretion during the last eight days averaged 120 mg per day, the highest value yet found in dogs. It is, of course, impossible to say what proportion of this was due to the bleeding as against red cell destruction by the usual mechanisms.

Excretion of urinary bile pigment, likewise, was found

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to be affected by large doses of radiation. E. Painter, of the Biology Section, first called our attention to the excretion of a green urine in a dog to which plutonium had previously been administered. Green urine has been found quite consistently in dogs receiving large doses of radiation. Its appearance has been well correlated with a diminution in food intake. This may be explained by the finding of Kanasaki[46] and others[47,48] that a decrease in liver glycogen results in the excretion of a green biliverdin-containing bile. Beckman spectro-photometric studies of the dog urine by M. Hagedorn indicated that this urine pigment was spectroscopically similar to, if not identical with, biliverdin. Further evidence for abnormal bile pigment excretion in the urine came in the course of studies in excretion of urinary coproporphyrin by these dogs[49]. The ether extract of acidified urine from dogs receiving either external or internal radiation was commonly found to be green. Such a finding is rare in control dogs, but has been observed by S. Schwartz in dogs injected intravenously with hematin or hemoglobin.

Quantitative studies of urinary bilirubin were instituted to measure this effect more accurately. Several hundred determinations on control and irradiated dogs ranged generally from one to four mg per day. Practically all the bilirubin was the prompt-reacting type. Occasional values rose to six mg per day or more. Though values often tended to be higher

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after irradiation, the range was generally similar to that of control dogs. In some dogs, as shown in Figure 15, the increased excretion was fairly pronounced.

These investigations were supplemented by studies of bile pigment excretion in three bile-renal fistula dogs given a single dose of total body X ray. These latter studies were made since the determination of fecal urobilinogen has certain disadvantages, such as the following:

1. The number of analyses is limited because the samples must be combined in periods of several days each.
2. There is a time lag of a few days between the time of blood destruction and the excretion of urobilinogen in feces.
3. The previously noted factor of constipation may make the data difficult to interpret.
4. It is possible that the bilirubin and biliverdin from the bile may not be completely converted to urobilinogen. This possibility was illustrated by the finding of a large amount of green pigment (biliverdin?) in the feces of one animal (Dog #37) given strontium[89].

The bile renal-fistula dogs were prepared for us by Dr. F. G. Allen, according to the method of Kapsinow[(50)]. In all but one animal (Dog #86) the gallbladder was anastomosed to the renal pelvis by means of an incision

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through the kidney cortex. In dog 86 the gallbladder anastomosis was made at the uretero-pelvic junction. It should be pointed out that the quantitative interpretation of the data is limited by the following factors:

1. Complete hematology studies were not possible at this time because of the pressure of other work.
2. All but one of the dogs (SQ-5) showed varying degrees of closure of the fistula opening at autopsy.

All three dogs showed significant increases in bile pigment excretion soon after total body irradiation, indicating the presence of some degree of hemolytic reaction. The data on Dog SQ-5 is illustrated in Fig. 16. The operation performed on this animal was most successful as indicated by post-mortem examination.

Bilirubin excretion by the other two dogs rose to over 50 mg per day in the few days after administration of 250 r total body X ray. These values were at least twice as high as the highest control values in these animals.

4. Discussion

4.1 The In Vitro Study of Radiation - Induced Hemolysis

The *in vitro* hemolytic effect of both alpha rays [51-54] and X rays has been demonstrated repeatedly [55-59]. The red cells thus irradiated were found to be swollen and to show increased permeability, especially to cations [55,56]. In all instances, however, the dose of several thousand r of radiation required to produce this effect was vastly greater

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than any therapeutic dose. Nevertheless, this difference in dose of radiation is not, per se, evidence against the *in vivo* hemolytic action of therapeutic doses of radiation, since these smaller doses may injure the red cells in such a way that they are more easily phagocytized. In this connection it would be of interest to irradiate with X ray portions of whole blood, containing isotopic carbon or nitrogen in the hemoglobin molecule. Its fate, when injected into experimental subjects, might then be compared to the fate of non-irradiated portions of the same blood. We are not familiar with any studies of this type, reported in the literature.

4.2 Other Studies on the Manhattan Project that Point to a Hemolytic Reaction in Radiation Anemia. Lorenz and co-workers[29] at the National Cancer Institute have administered daily small doses of gamma rays to the whole body of guinea pigs, mice, and rabbits, and have studied their effects on the circulating erythrocytes. They believed that the precipitous drop in red cell count that occurred during the last few weeks of life cound not be accounted for by internal bleeding or by the assumption that erythropoiesis was even completely inhibited. Some of the animals in this study were sacrificed at intervals after starting the irradiation treatment. The bone marrow of these animals was reported by Eschenbrenner to be either normal or hyperplastic [60]. Definite hypoplasia, however, was found in many animals in which treatment was continued until they

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died with severe anemia.

Jacobson, et al, have reported the occurrence of severe acute anemia in rabbits given acutely lethal doses of total body x-ray [61]. A hemolytic component was assumed to be an important factor in the rapid development of these anemias. In these animals, as well as in dogs given acutely lethal doses of radiation [62] the bone marrow showed marked destruction of cellular elements and general aplasia.

In animals injected with plutonium, Jacobson reported the finding of hyperplastic marrow associated with chronic low doses, while evidence of hypoplasia was found with larger doses [63].

4.3 Interpretation of Urobilinogen Excretion Data. Certain limitations of urobilinogen excretion studies in the dogs have already been enumerated. These relate to the time lag between blood destruction and urobilinogen excretion, to possible incomplete conversion of bilirubin to urobilinogen, to irregularity in bowel habits, and to inability to identify the nature of the hemolytic process, i.e., whether due to internal bleeding or to more usual mechanisms. Other limitations too, might be considered. Heilmeyer [64] and Watson [25] especially, have described a diminution in urobilinogen excretion in cases of post hemorrhagic anemia. This might be considered either as a pigment sparing action or a "throttling" of blood destruction. Fecal urobilinogen excretion data, too, should be interpreted in terms of the amount of circulating,

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hemoglobin. Therefore, in a dog with severe anemia an apparently normal fecal urobilinogen value may actually represent as increase in the rate of blood destruction, with respect to the total amount of hemoglobin that the dog has.

From the available data it is impossible to determine the relative magnitude of destruction of mature circulating cells as compared to that of immature cells within the bone marrow. In pernicious anemia, for example, it has been suggested that the increased fecal urobilinogen excretion may be due to excessive intramarrow destruction of young red blood cells. It may be, too, that such is the case in patients with anemia due to chronic radium poisoning.

The effect of acute irradiation might be investigated by first giving isotopic nitrogen for a period of several days and then, after two to three months, isotopic carbon. Shortly thereafter either external or internal radiation would be administered. At this time the N/C ratio should be high in the older circulating cells, while the reverse should be the case in the bone marrow cells. By determining this ratio separately in the hemin of circulating cells and bone marrow cells, and again in the excreted bilirubin or urobilin one might ascertain the relative degrees of destruction of young versus old cells.

4.4 Inhibition of Erythropoiesis. In the studies reported here, there is no doubt that erythrocyte regener-

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ation was interfered with. Otherwise, the great regenerative powers of the bone marrow would have been sufficient to maintain more nearly normal hemoglobin and red count levels, even with an increased rate of red cell destruction. It should be pointed out, however, that under some conditions the bone marrow responds even after administration of relatively large doses of radiation. Dog 55 (Figure 7) is illustrative in this regard. Following the development of an acute hemolytic anemia, due possibly to serum administration, the reticulocyte value rose and the anemia improved despite the continued administration of 25 r total body X ray daily. Patients receiving P^{32}, likewise, were able to respond with an elevated reticulocyte count. Such a reticulocyte response, however, was not forthcoming in those dogs which received an acutely lethal dose of radiation. In this regard it might be noted that Jacobson, et al, have shown [65] that rabbits in which erythropoiesis was stimulated by previous production of anemia due to phenylhydrazine or bleeding are often able to recover normally from the anemia despite administration of nearly lethal doses of X ray.

Finally, it should be emphasized again that the present discussion does not aim to minimize the importance of the erythropoietic inhibition caused by irradiation. It is intended, however, to indicate the necessity for considering still other mechanisms to account for both acute

1119539

and chronic radiation anemia.

5. Acknowledgments

We are indebted especially to L. Prosser, E. Painter, P. Swift, R. Zagaria, and R. Ferretti for their assistance in making the animal studies possible. We also wish to thank Miss V. Hawkinson of the Department of Medicine, University of Minnesota, for the analyses on one of the patients [redacted] and Miss L. Warner for preparing the Figures used in the text. The P^{32} used was received from the Clinton Laboratory at Oak Ridge, and further prepared for us by R. Finkle, P. Tomkins, and A. Broido. in the Metallurgical Laboratory at the University of Chicago.

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Figure 1.

The Effect of P^{32} on Hemoglobin Metabolism in a Patient with Polycythemia

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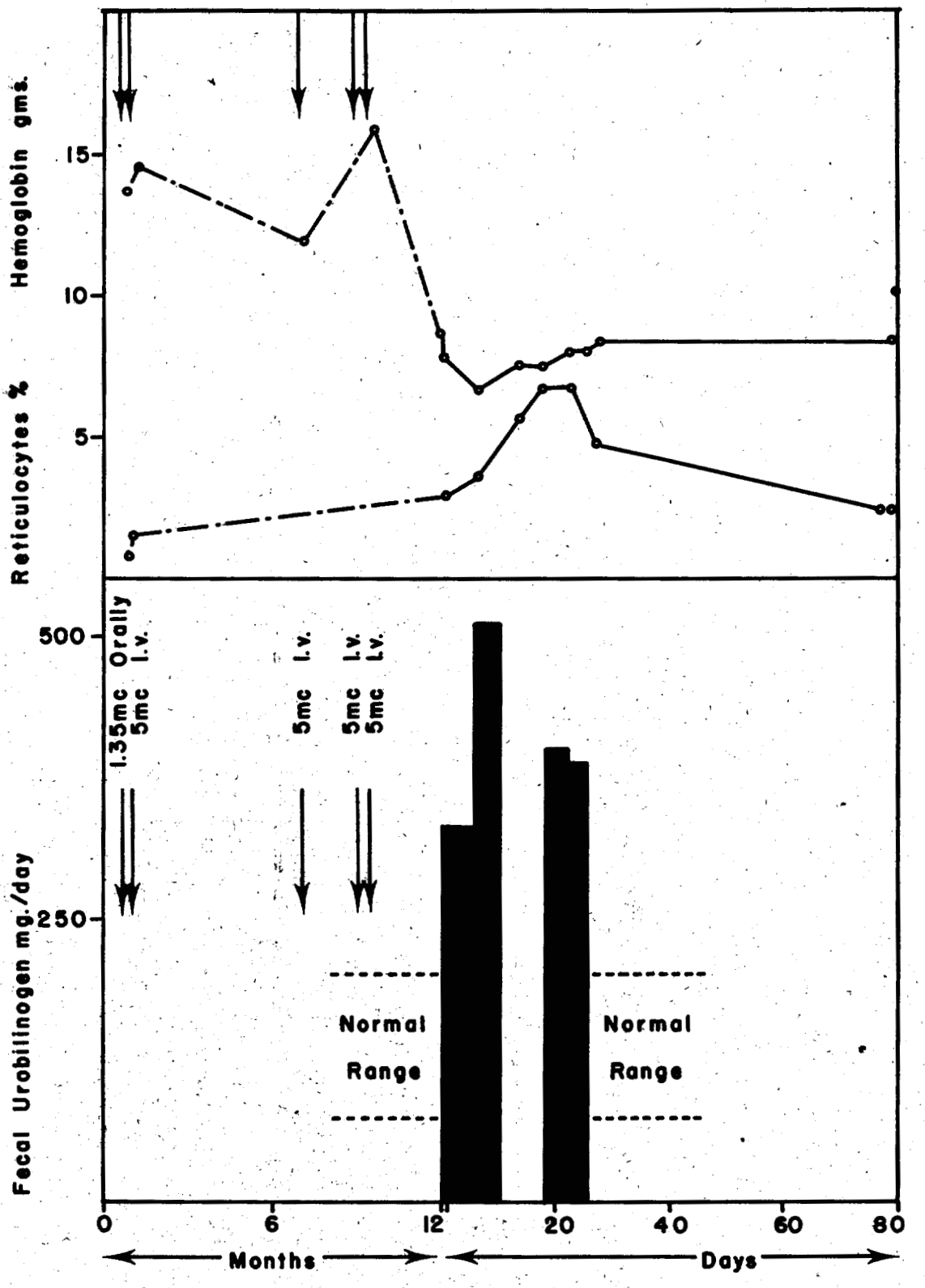

The Effect of P^{32} on Hemoglobin Metabolism in a Patient with Polycythemia

FIG. 1

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Figure 2.

The Effect of P^{32} on Hemoglobin and Reticulocyte Count in a Patient with Polycythemia.

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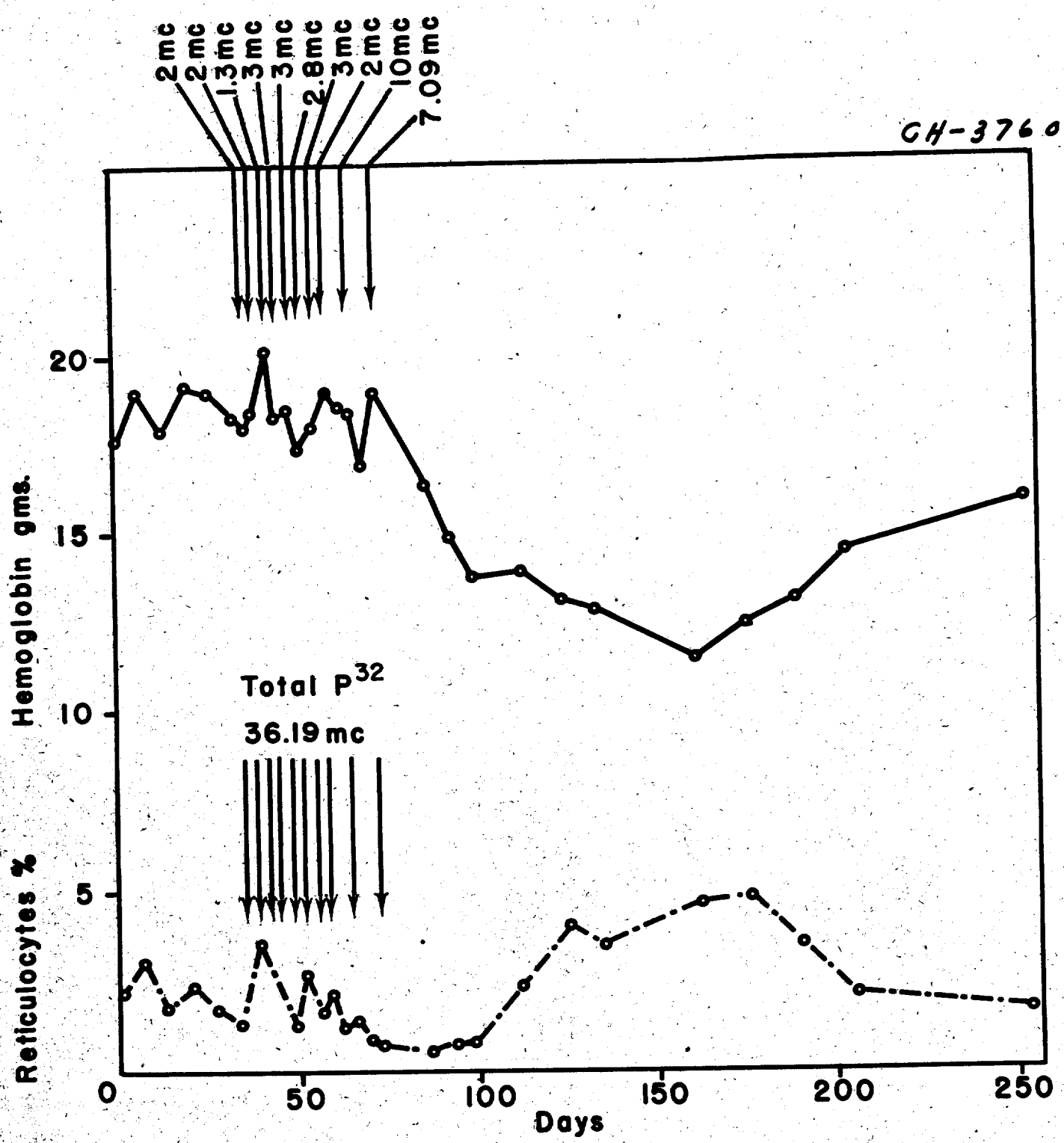

The Effect of P^{32} on Hemoglobin Metabolism in a Patient with Polycythemia

FIG. 2

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Figure 3.

The Effect of P^{32} on Hemoglobin and Reticulocyte Count in a Patient with Polycythemia [redacted]

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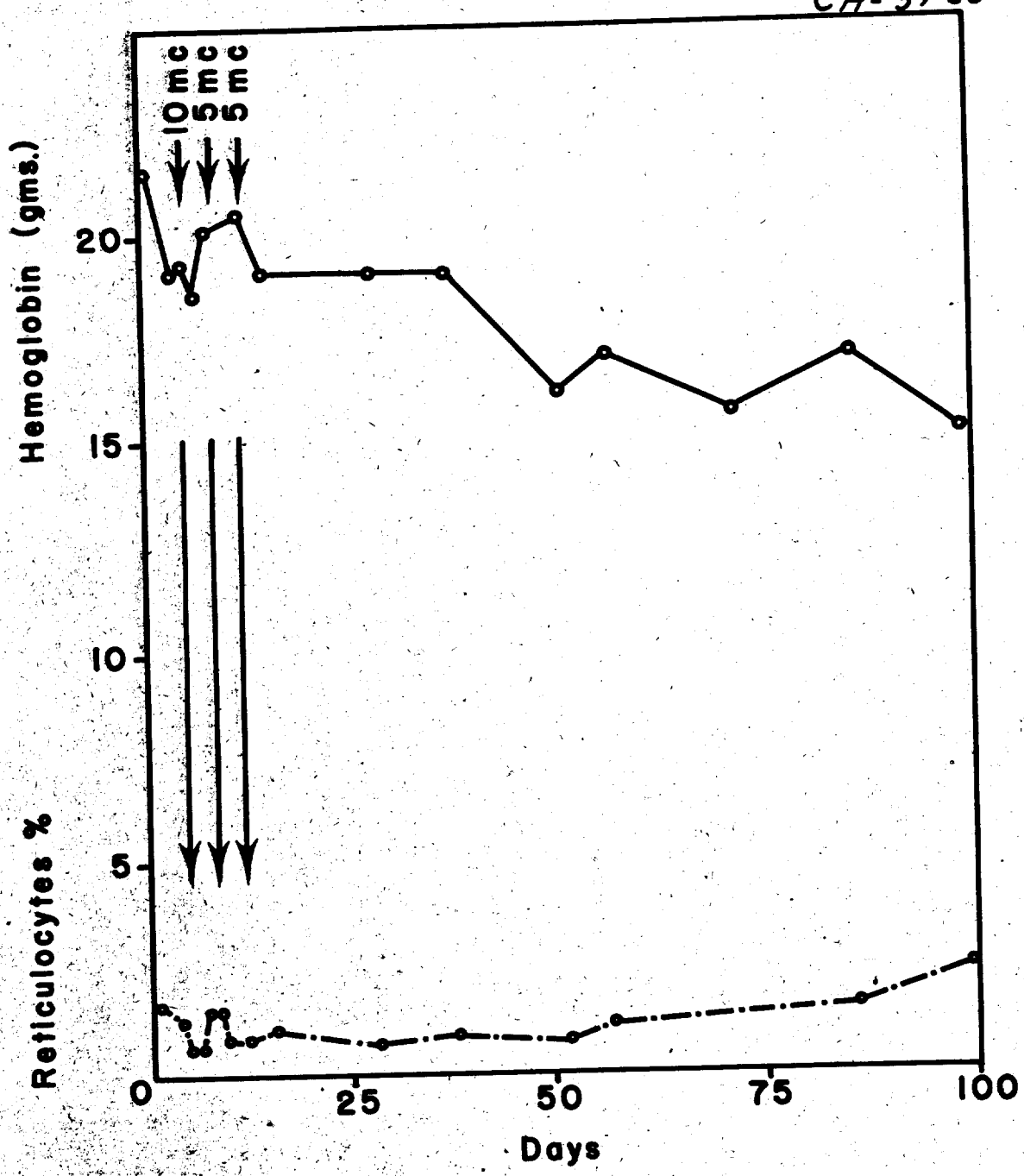

The Effect of P^{32} on Hemoglobin Metabolism in a Patient with Polycythemia

[redacted]

FIG. 3

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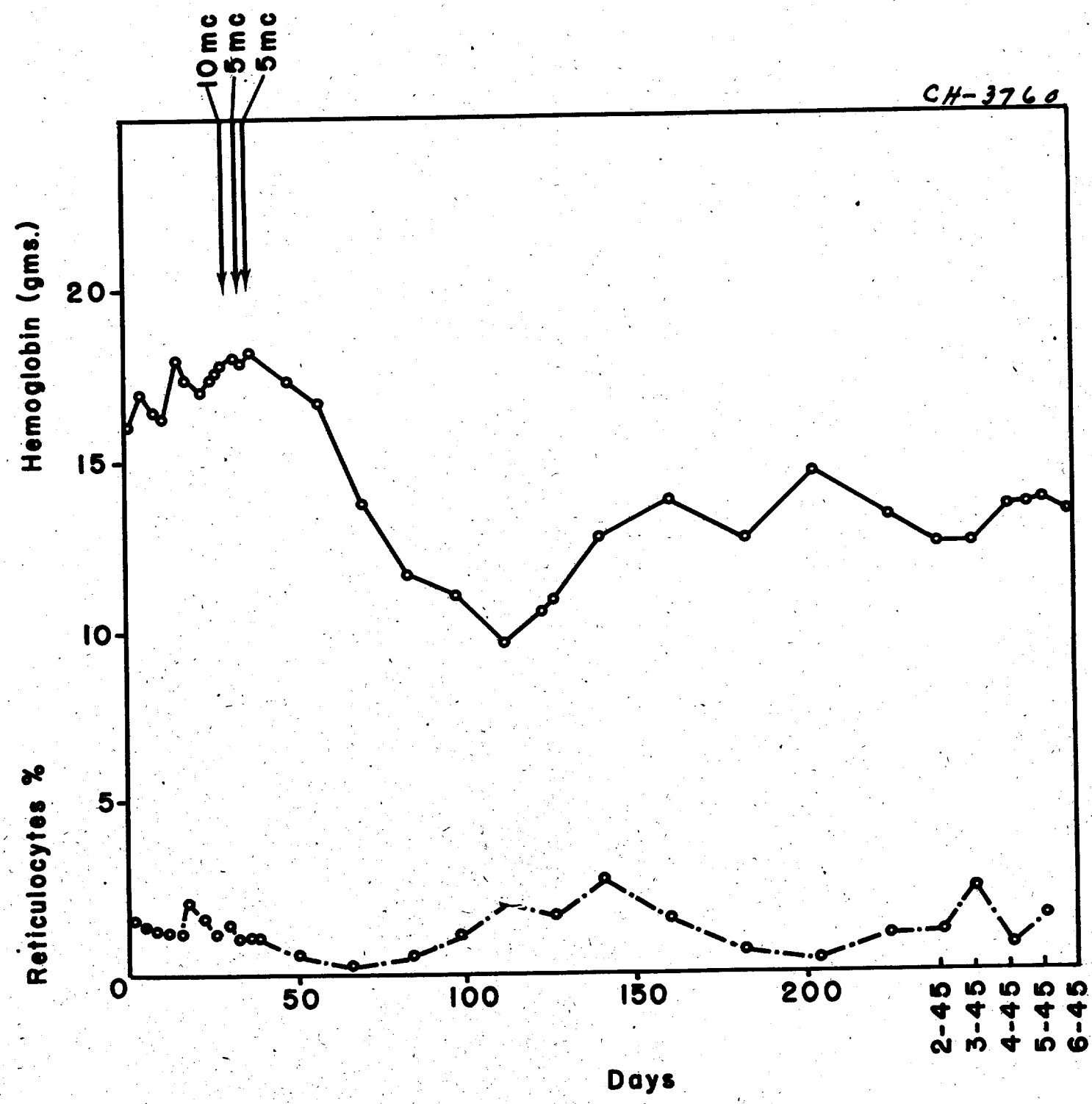
Figure 4.

The Effect of P^{32} on Hemoglobin and Reticulocyte Count in Patient with Polycythemia [redacted]

1119555



The Effect of P^{32} on Hemoglobin Metabolism in a Patient with Polycythemia

FIG. 4

111955b

Figure 5.

Recovery to Normal of Hemoglobin Concentration Following Administration of 18.6 mc P^{32} to a Patient with Polycythemia. [redacted]

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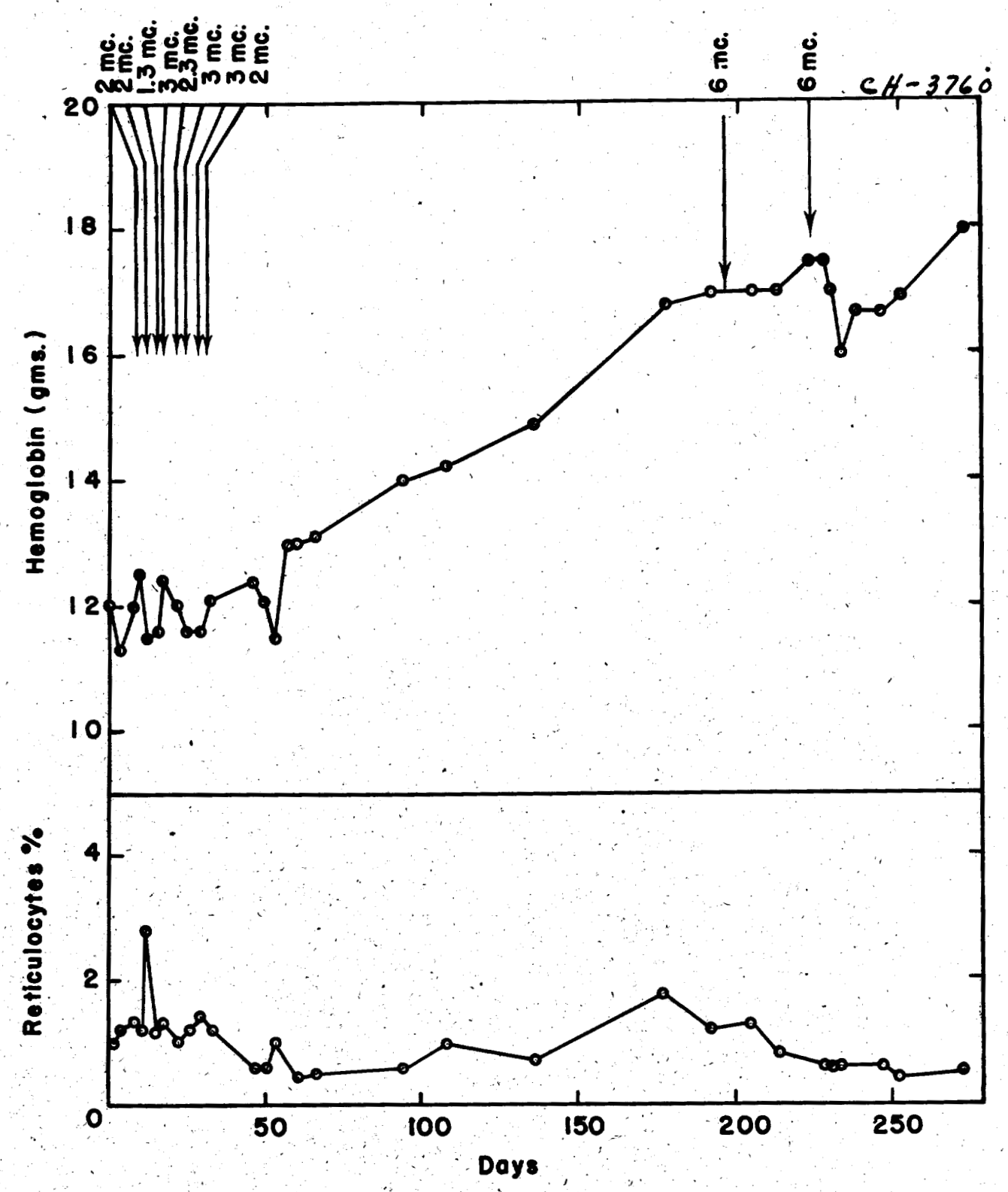

Recovery to Normal of Hemoglobin Concentration Following Administration of 18.6 mc P^{32} to a Patient with Polycythemia.

[redacted]

1119558

FIG. 5

Figure 6.

Correlation of Red Cell Protoporphyrin and Color Index in Patients with Polycythemia Receiving P^{32} Therapy

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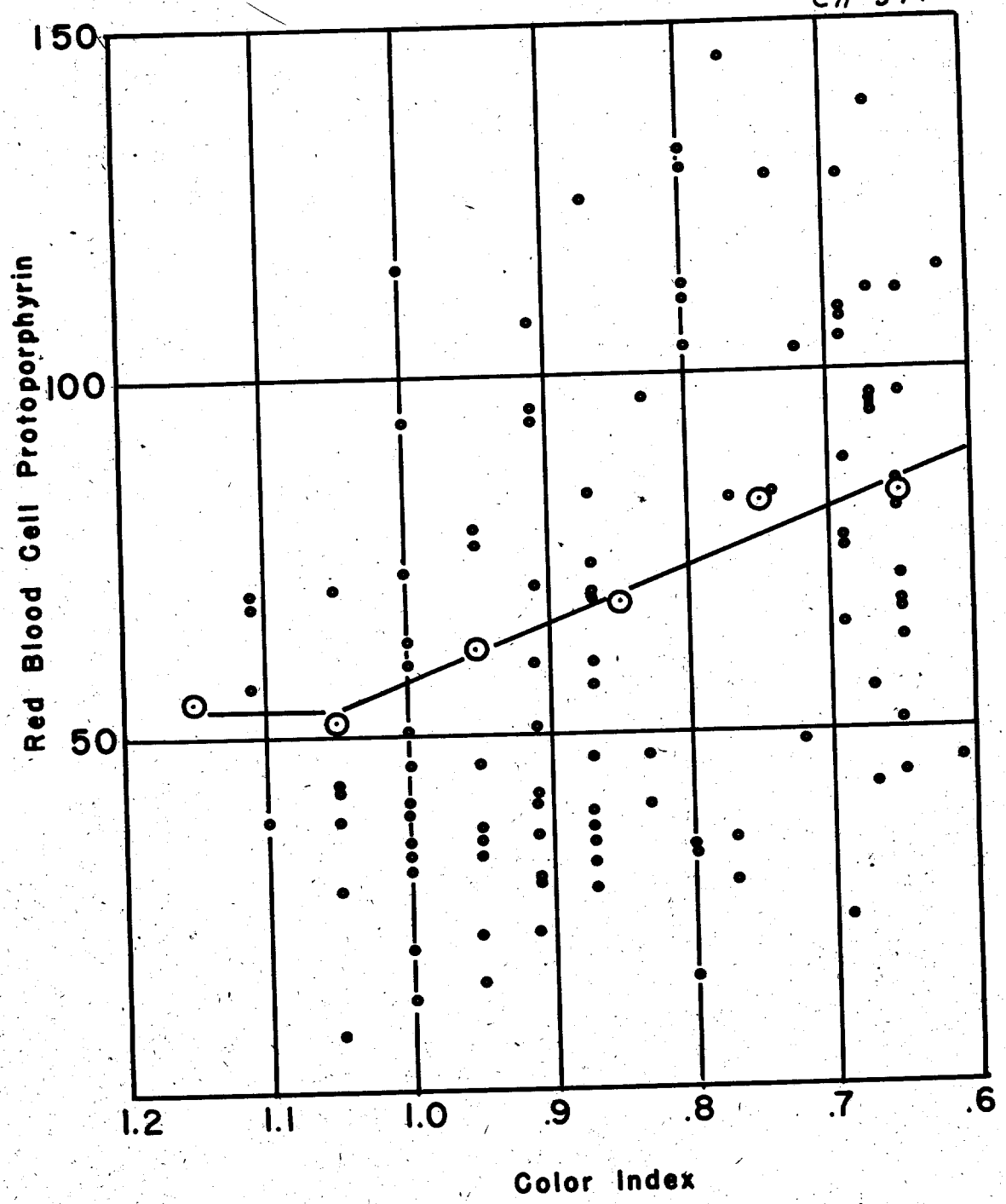

Relation of Red Cell Protoporphyrin and Color Index Values in 5 Patients with Polycythemia Vera

⊙ Average protoporphyrin values for each 0.1 division on abscissa

FIG. 6

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Figure 7.

Hemoglobin Metabolism in a Control Dog (26)

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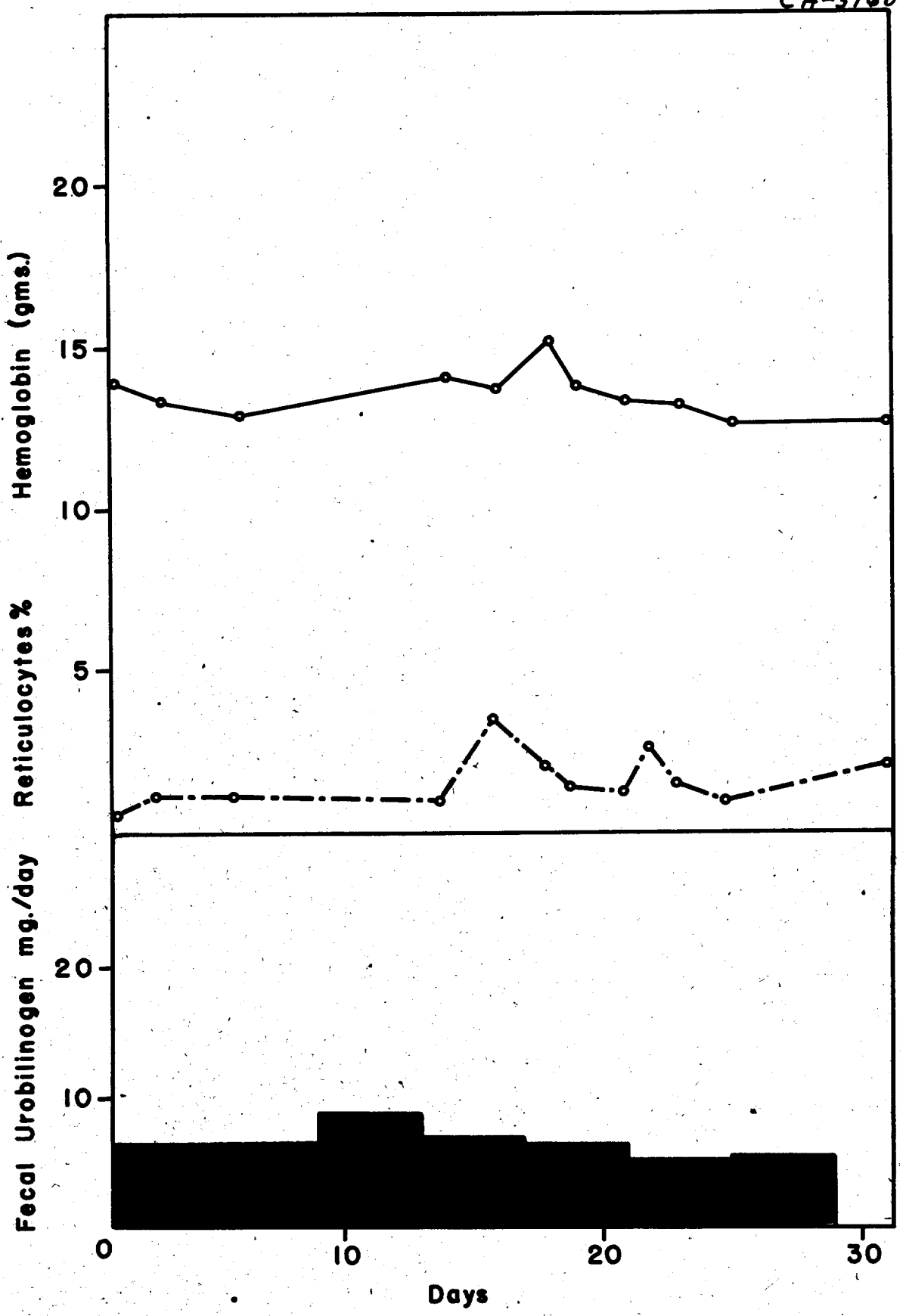

Hemoglobin Metabolism in a Control Dog
(Dog 26)

1119562

Fig. 7

Figure 8.

Homoglobin Metabolism in a Dog with a Hemolytic Reaction Just Before Beginning Treatment with 25 r Daily Total Body X irradiation (55)

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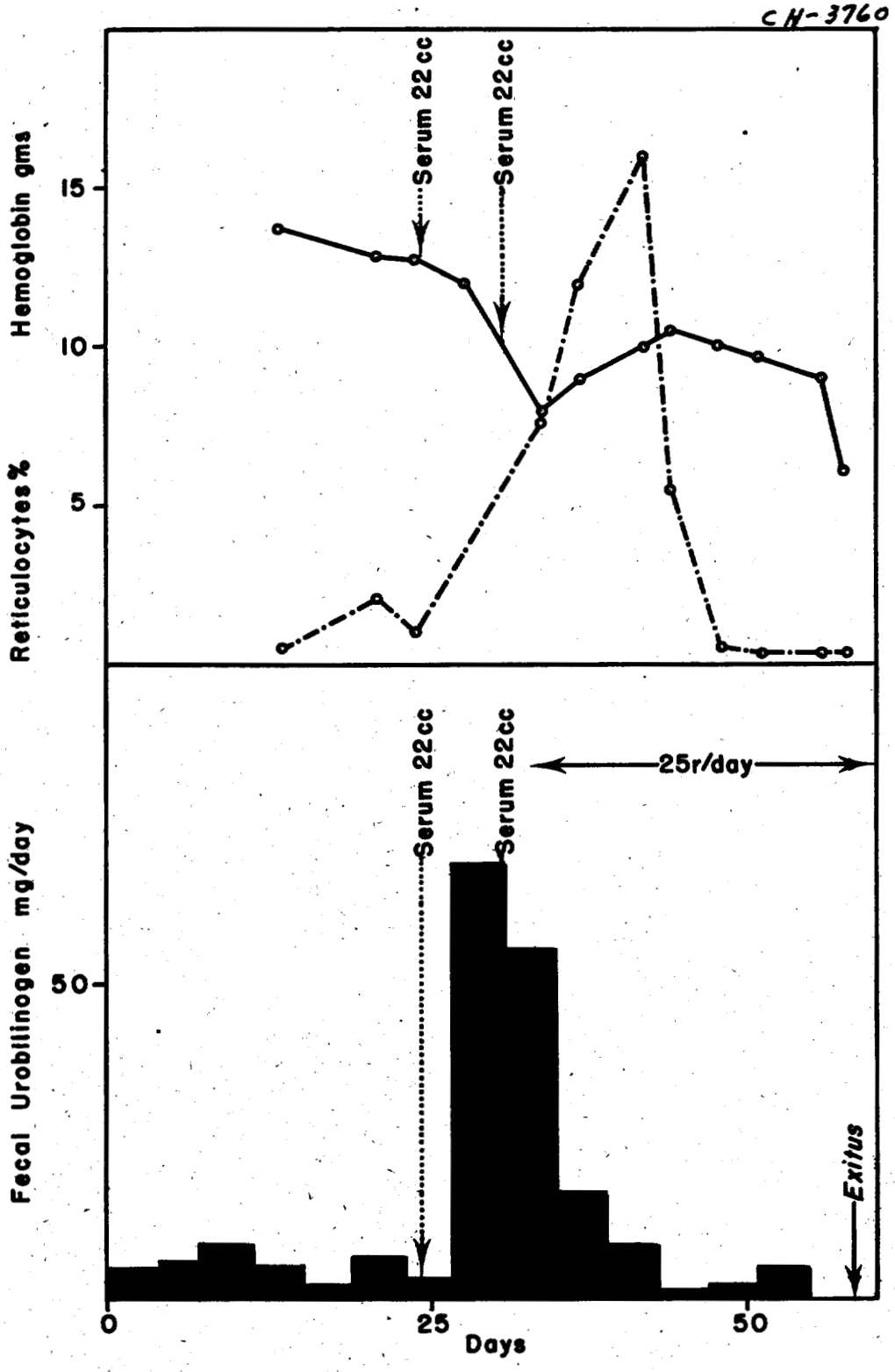

Hemoglobin Metabolism in a Dog with a Hemolytic Reaction just before Institution of 25r Total Body X-ray Daily

FIG. 8

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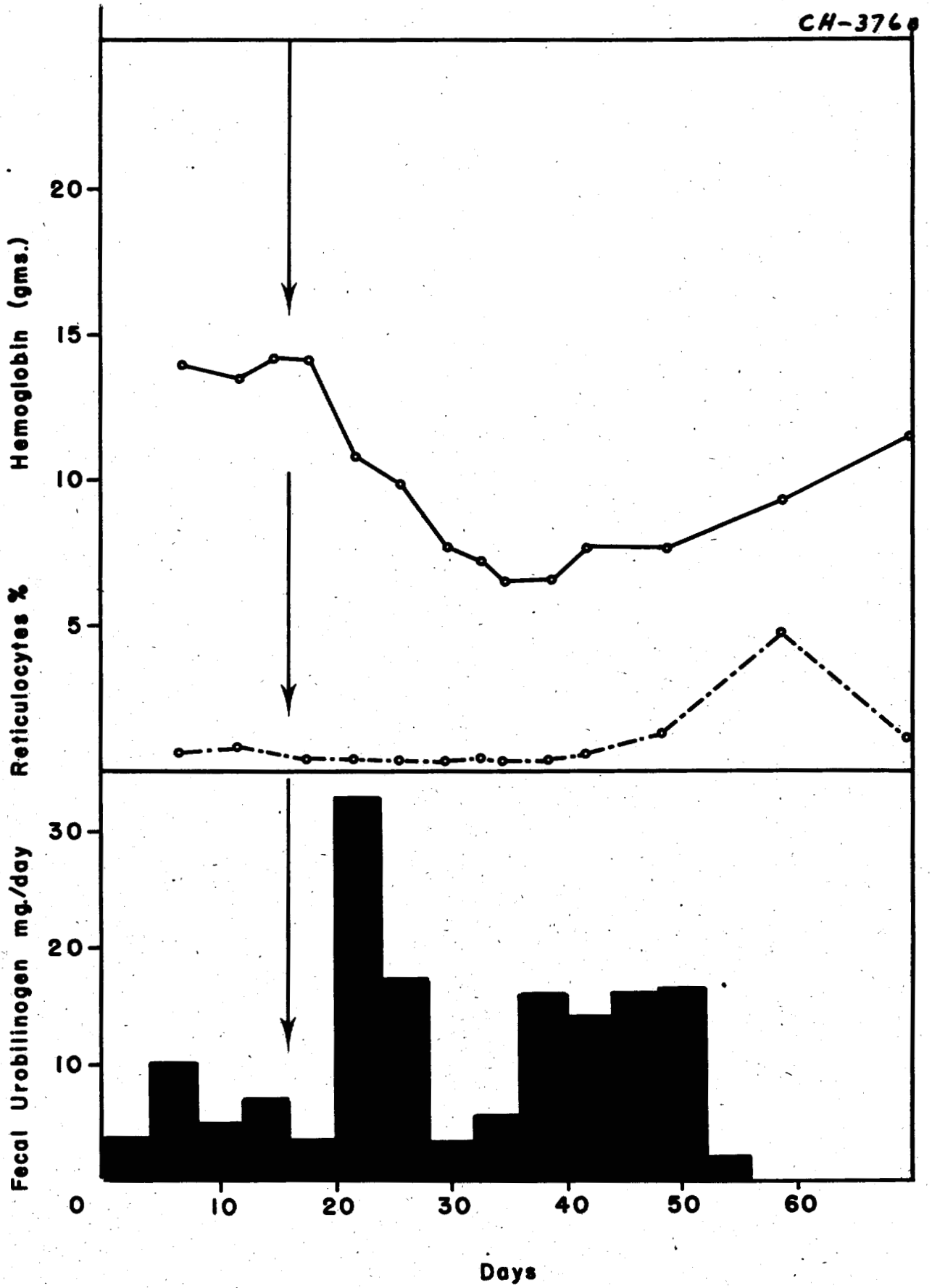
Figure 9.

The Effect on Hemoglobin Metabolism of 300 r Total Body X Irradiation, Single Dose (Dog 43)

1119565



The Effect on Hemoglobin Metabolism of 300r Total Body X-ray, Single Dose (Dog 43)

FIG. 9

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Figure 10.

The Effect on Hemoglobin Metabolism of 209 r Total Body X Irradiation, Single Dose (Dog 36)

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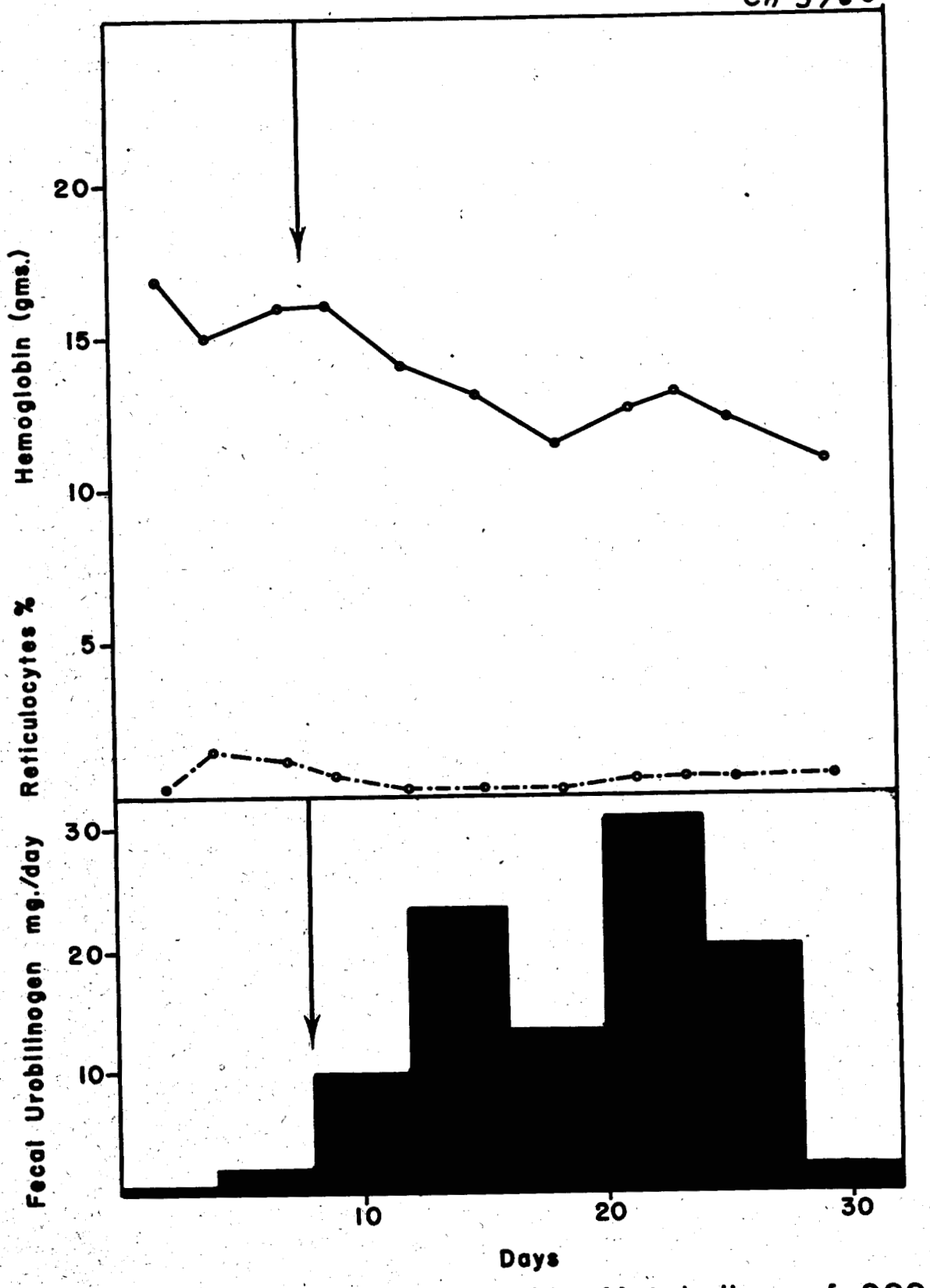

The Effect on Hemoglobin Metabolism of 209r Total Body X-ray, Single Dose (Dog 36)

FIG. 10

1119568

Figure 11:

The Effect on Hemoglobin Metabolism of 26.5 mc Sr^{89},

Single Dose (Dog 37)

1119569

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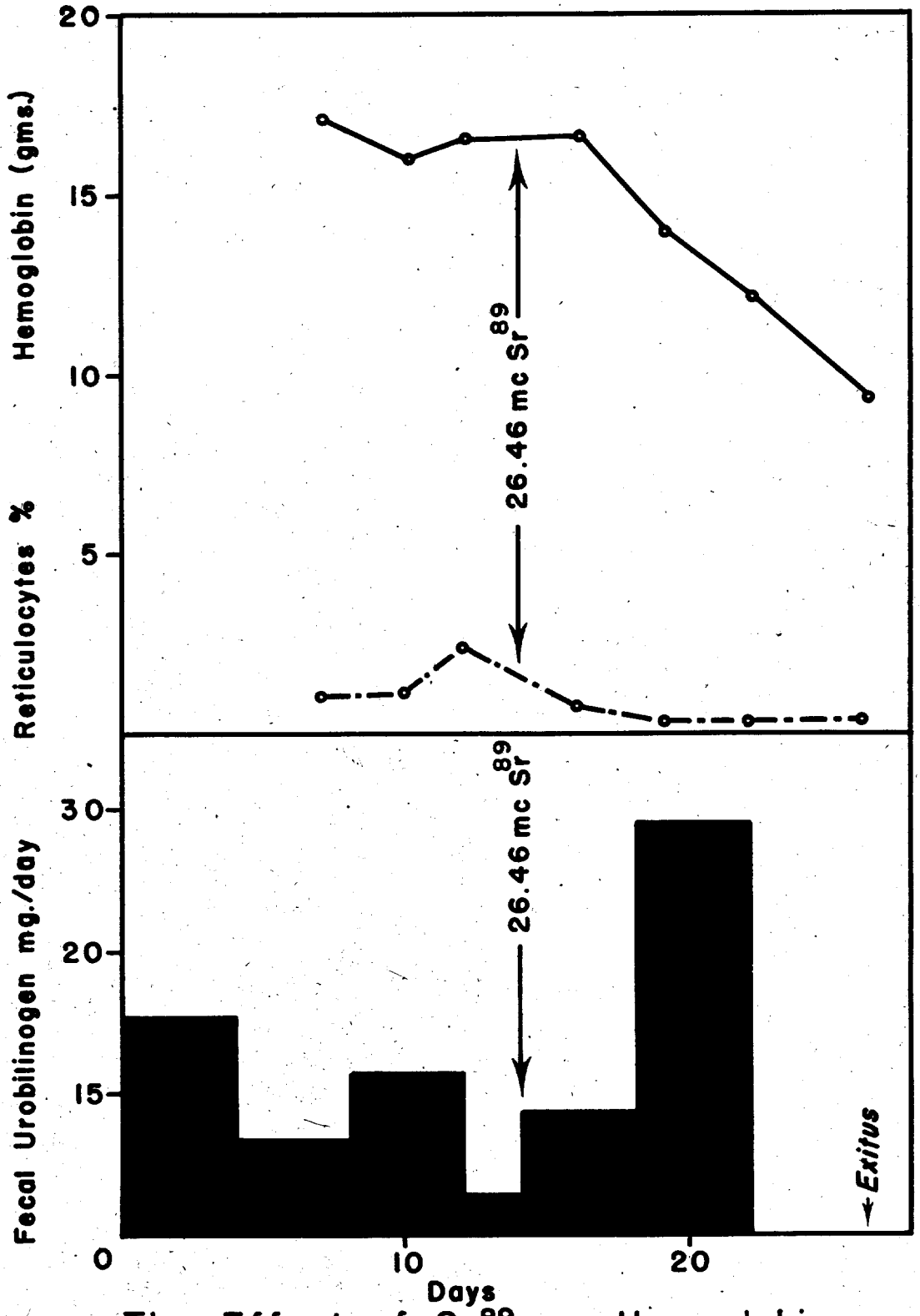

The Effect of Sr^{89} on Hemoglobin Metabolism in a Dog

(Dog 37)

FIG. 11

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Figure 12.

The Effect on Hemoglobin Metabolism of 50 r Total Body X Irradiation Daily (Dog 29)

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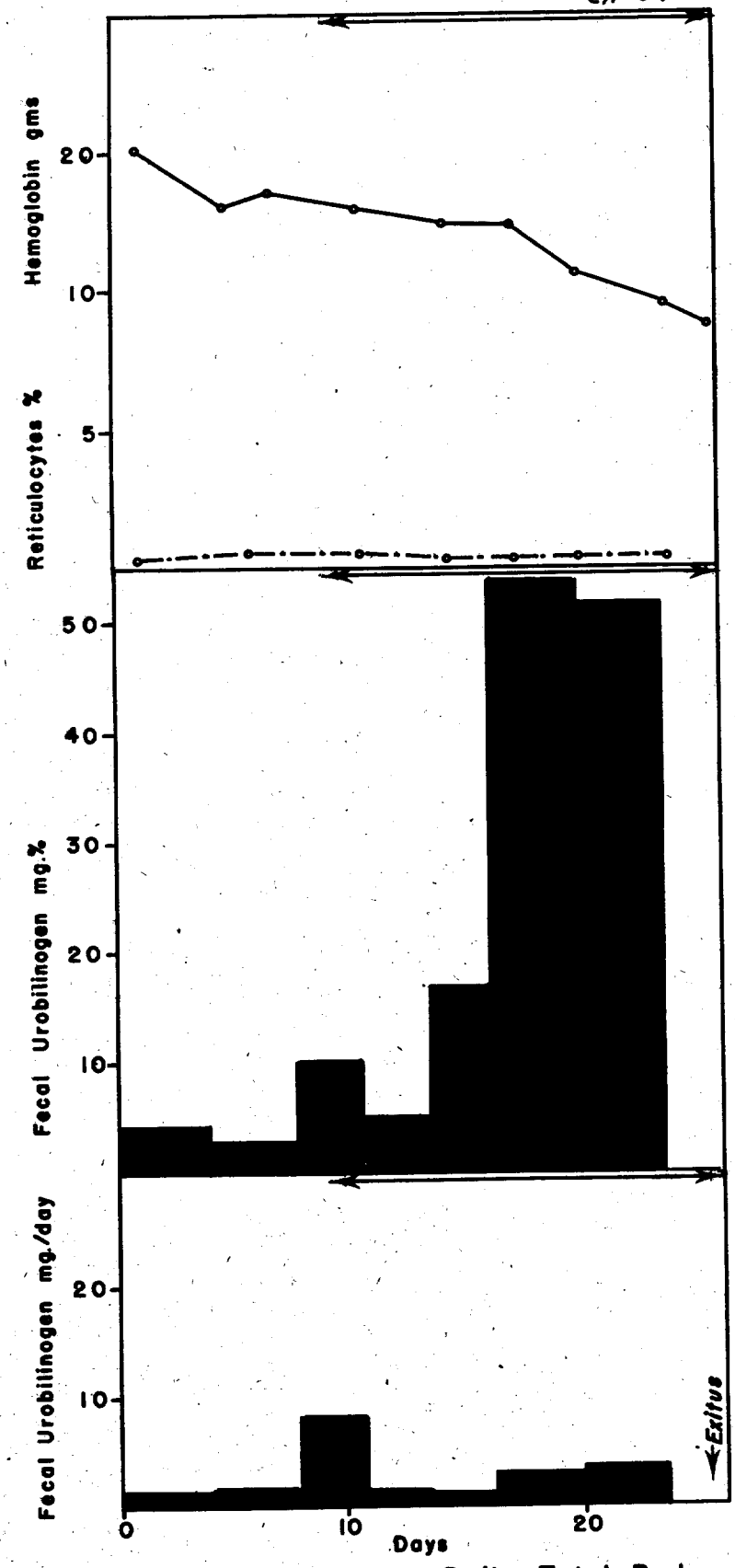

The Effect of 50r Daily Total Body X-ray on Hemoglobin Metabolism in a Dog

(Dog 29)

FIG. 12

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Figure 15

The Effect on Hemoglobin Metabolism of 50 r Total Body

[illegible]

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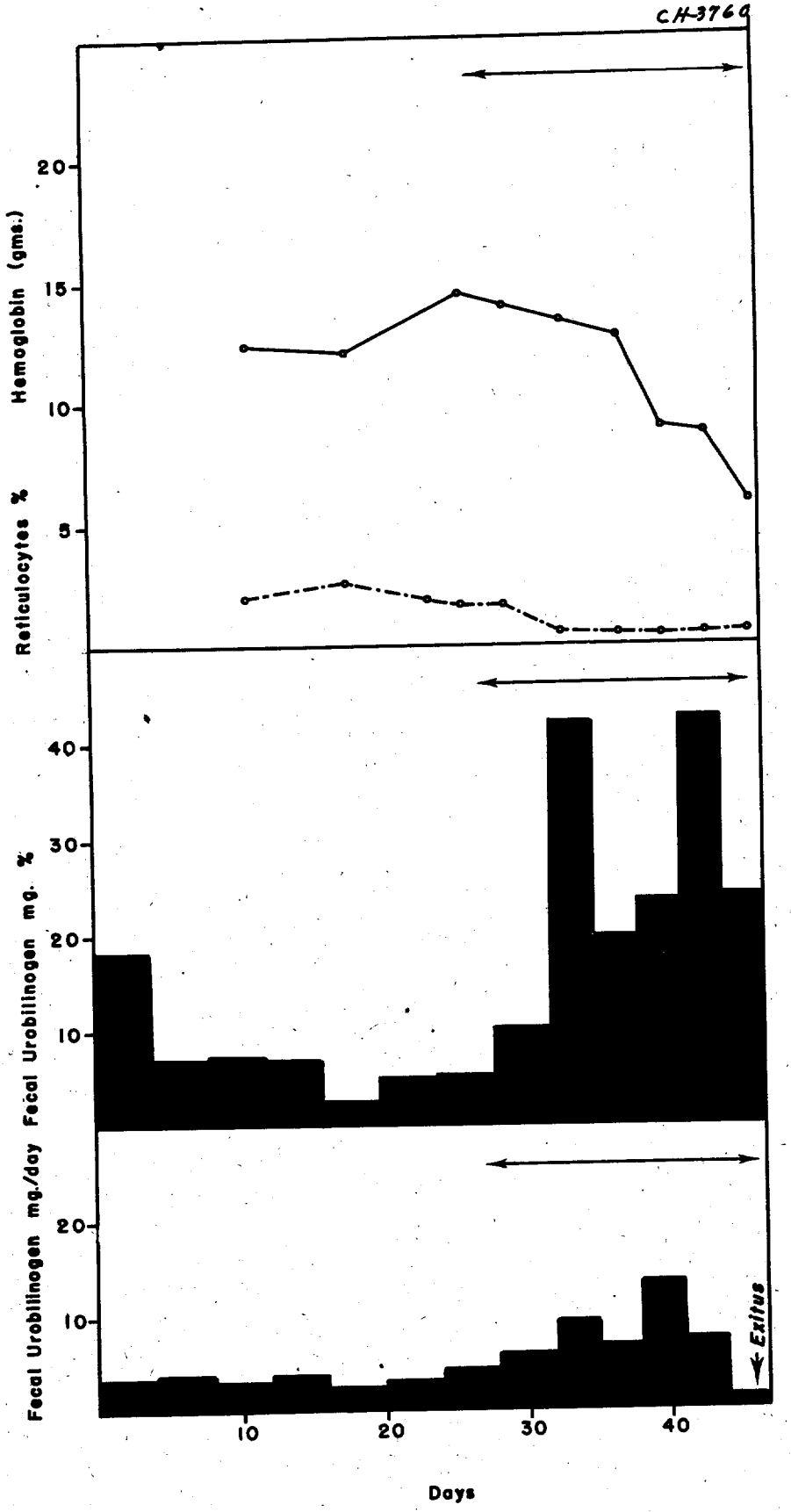

The Effect on Hemoglobin Metabolism of 50r Daily Total Body Radiation
(Dog 47)

FIG. 13

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Figure 14

The Effect on Hemoglobin Metabolism of 50 r Total Body X ray Daily (Dog 30)

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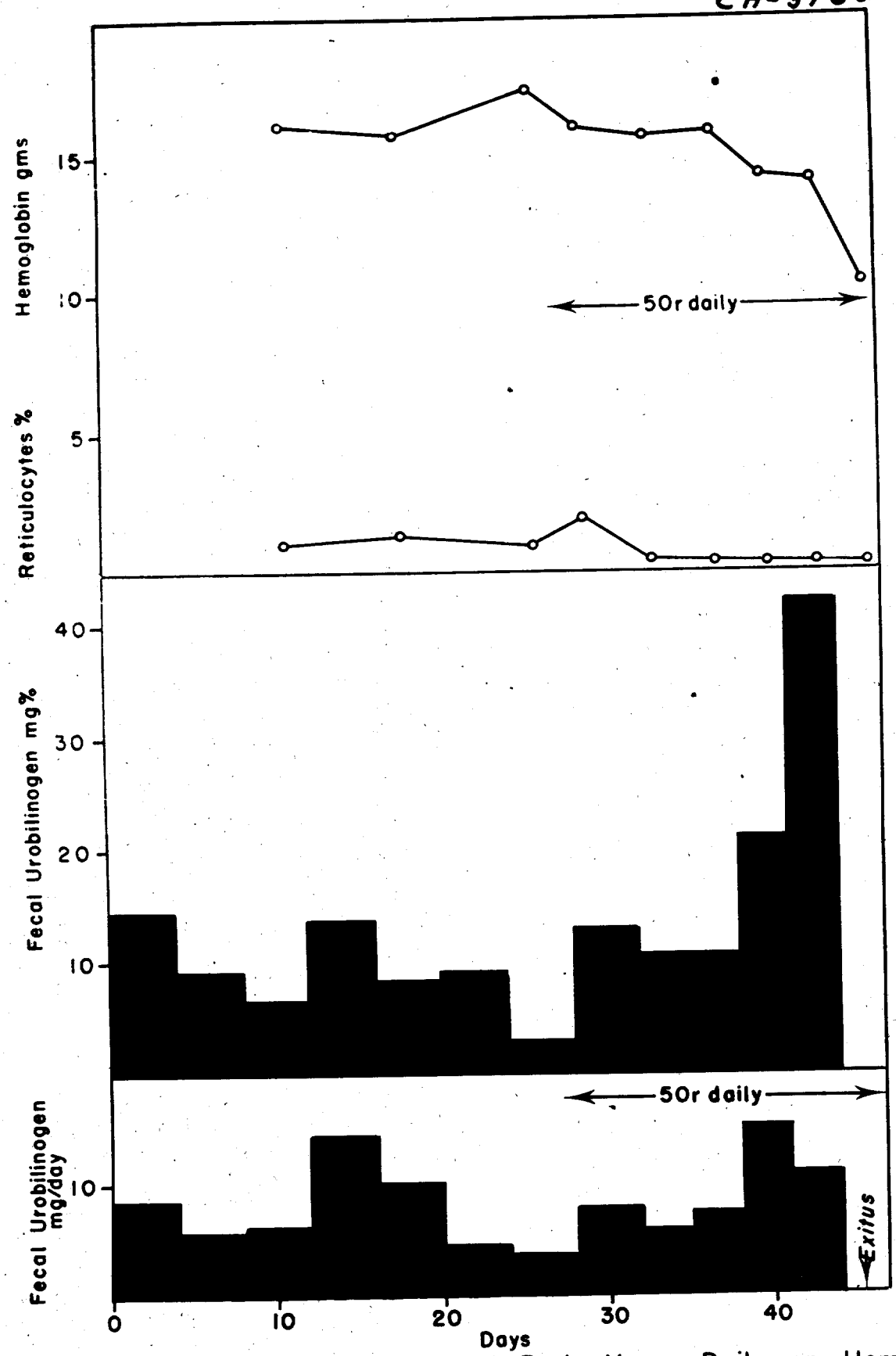

The Effect of 50r Total Body X-ray Daily on Hemoglobin Metabolism in a Dog

(30)

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FIG. 14

Fig. 15 Urinary Excretion of Bilirubin in 2 Dogs Injected with Plutonium.

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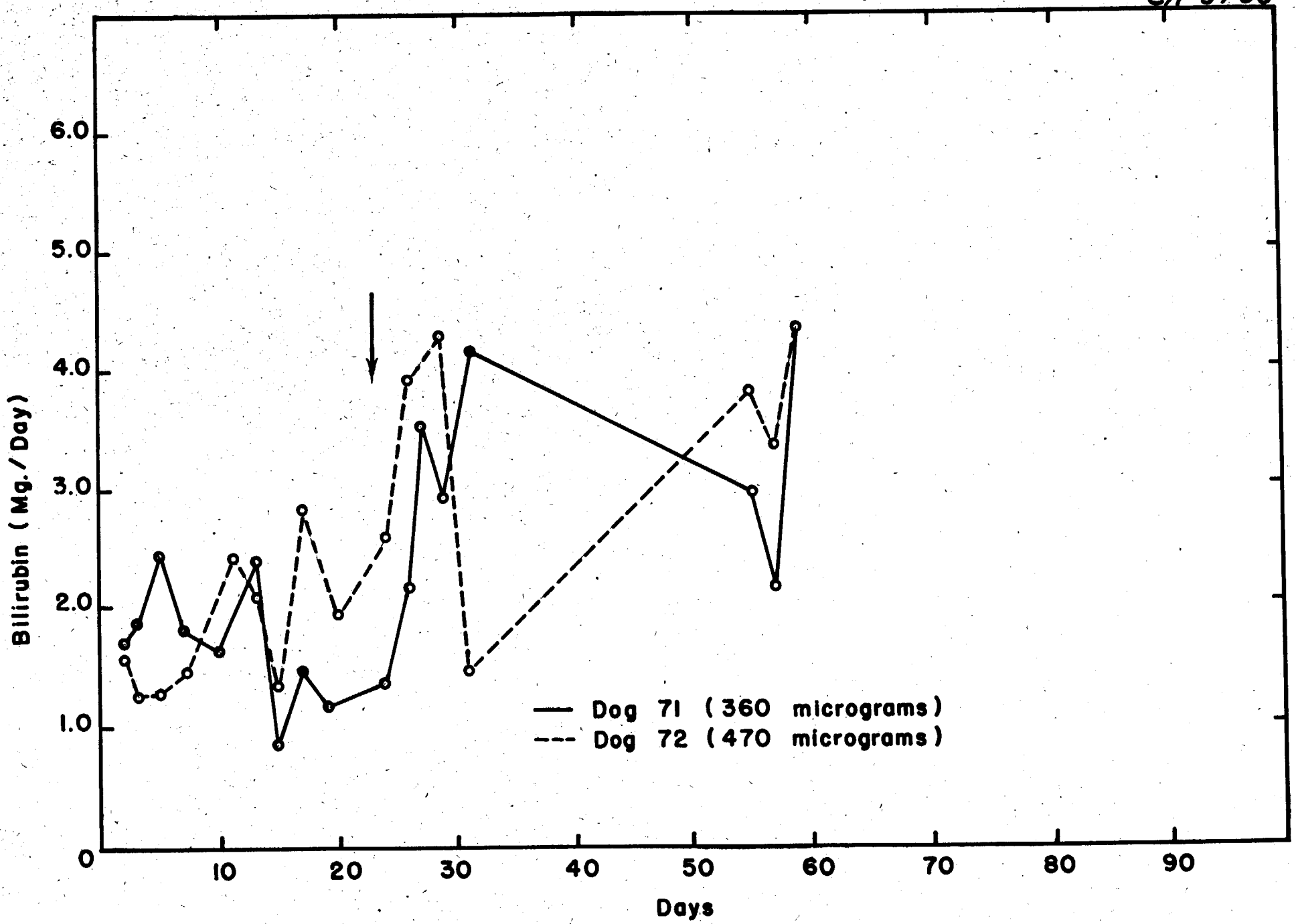

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FIG. 15

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Figure 16.

The Effect of 450 r Total Body X ray, Single Dose,

on Hemoglobin Metabolism in a Bile-Renal Fistula Dog (SQ5)

1119579