

EVALUATION OF YOUNG CALF AS AN ASSAY TOOL

FOR BONE MARROW POISONS: QUALITATIVE AND QUANTITATIVE

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by

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INTRODUCTION

Cancer is a neoplastic disease condition in which the ultimate goal of patient and physician alike is a cure by chemotherapeutic agents, surgery, ionizing radiation, or other means. Most of the cancer chemotherapeutic agents in use today which have a degree of efficacy in controlling neoplastic conditions also cause a marked depression of other active proliferating tissues of the patient, the bone marrow, lymphoid tissue and intestinal mucosa. The use of these toxic chemotherapeutic agents causes a decrease in the body's defense mechanisms by their action upon the bone marrow and other hematopoietic tissues. They may also cause significant alterations in the body fluid and electrolyte balance by action upon the intestinal mucosa. Both of these conditions, if allowed to progress, can lead to death of the patient. The basic problem in the use of the current cancer chemotherapeutic agents is to achieve with a given drug adequate anti-tumor activity with the minimum of toxic side effects.

The depression of the bone marrow by these cancer chemotherapeutic agents causes a condition called "aplastic anemia". Although "aplastic anemia" is a term that presently enjoys common usage, the condition produced is actually an atrophy of the bone marrow. Aplasia indicates that the cells in the marrow are primordial in nature and small, but atrophy denotes that the cellular constituents have decreased in size and/or number after achieving the normal morphology.

Agents that cause an atrophic bone marrow in animals and man have been called "bone marrow poisons". The fact that most useful cancer

chemotherapeutic agents have marrow poisoning properties has required extensive test or assay systems to evaluate the marrow poisoning potentials in addition to the anti-tumor potentials of the various compounds.

For obvious reasons, humans have not been used as the initial screening system for the anti-tumor potentials and toxic potentials of various experimental compounds. Laboratory animals have been used in rather elaborate screening systems and assay techniques developed for the detection of potential anti-cancer agents. The mouse has been an ideal animal for this evaluation because neoplastic conditions can be induced in them and the response to the potential anti-cancer compounds can be evaluated. Concurrently this same assay system is used for the detection of the gross toxicities of the compounds.

Currently much attention is being directed to relating the anti-tumor activities of compounds to details of their molecular structure, to facilitate the development of more efficient anti-tumor agents. In contrast little emphasis is being given to relating the marrow poisoning activities of these same agents to details of their molecular structure. Conceivably, exploitation of this latter approach could facilitate the detection and subsequent elimination of the undesirable toxic structural groups associated with marrow poisoning properties of the compounds with retention of their anti-tumor capabilities. Utilization of this approach will require the development of an assay system designed specifically to quantitate the bone marrow depression induced by chemical compounds.

Little work has been done in the common laboratory animals to establish quantitatively the effects upon the bone marrow of the various structural moieties of anti-cancer agents. In contrast, the calf has been used extensively to quantitate the effects of structural analogs of several marrow poisons upon the bone marrow.

The calf has been shown to be very sensitive and quantitative in its response to the bone marrow poisons, trichloroethylene-extracted soybean oil meal (TCESOM) and S-(dichlorovinyl)-L-cysteine (DCVC). The calf is also sensitive to ionizing radiation and its response pattern indicates that the calf is on the order of twice as sensitive to radiation as other species tested. The calf's quantitative response to several bone marrow poisons and its marked sensitivity to the effects of ionizing radiation indicate that the calf might be a very sensitive system for the quantitative assay of the marrow poisoning potential of anti-cancer compounds.

It is proposed in this investigation to evaluate the ability of the calf to sense and respond quantitatively to the bone marrow poisoning properties of several cancer chemotherapeutic agents in use today.

LITERATURE REVIEW

Assay of Bone Marrow Poisons

Bone marrow toxicity assays are initiated by administering the potential bone marrow poison to an animal by any of a variety of routes, i.e., intravenous, intraperitoneal, intramuscular, or oral (51). The assays are completed by evaluating the effects of the compound for its marrow poisoning properties either directly by examining the bone marrow for changes in the normal morphological picture, or indirectly by examining the changes in the cellular constituents of the peripheral blood which reflect the morphological changes in the bone marrow (41, 42).

Administration of compounds

Potential bone marrow poisons have been administered to animals by all logical routes (7). The route of administration with the slowest absorption, i.e., the oral route, in most cases, has caused an increased toxic effect because of the larger dosages which are required to reach the desired drug level in the fluids bathing the tissues. The prolonged duration of blood levels of the compound due to the decreased rate of absorption also leads to an increased toxic effect of the compound (24, 16).

The intravenous route of administration allows a more rapid attainment of desired drug levels in the fluids bathing the tissues with less compound per unit of body weight. The lesser amount of compound injected plus the speed at which compounds can be eliminated from the body after getting into the blood can aid in decreasing the toxicity of the administered compound (15, 16).

The intraperitoneal route has absorption and elimination rates comparable to the intravenous route and can be used unless the compound is an irritant and is capable of producing an aseptic peritonitis (16).

Theoretically, all routes can be used on any animal, but generally the choice of convenient routes is dependent upon the experimental animal employed. The oral route can be used in all animals if the compound can be placed in capsules or in the food (51). The intraperitoneal route, on the other hand, is the method of choice for injection into the small rodents because of the inaccessibility of blood vessels for intravenous injection. The monkey and dog lend themselves readily to intravenous injections (51).

Single dose or multiple dose regimens of administration of marrow poisons can both lead to the production of toxic conditions as indicated by the bone marrow response (14, 21, 22). Ferguson, et al., (14) have shown that the sequence of occurring events in both types of dosage programs, the timing of the appearance of the various responses of the marrow due to the effects of the bone marrow poison, may be different.

Experimental animals

The common laboratory animals; rats, mice, dogs and monkeys have been used as assay animals for bone marrow poisons (8, 14, 31, 38, 41, 42). The choice of the various animals has been shown to depend upon several criteria (51).

The first criterium is sensitivity of the animals to the compound. Although all of the common laboratory animals appear to be sensitive to

most marrow poisons, the dog has been found to be the most sensitive (14, 38, 41, 42, 50).

The mouse has been used extensively for the simultaneous assay of bone marrow toxicities and anti-tumor potentials of experimental compounds (51). Neoplastic conditions can be induced in mice and the response of the neoplasm to the test compounds can be determined (21, 22, 24). The hematopoietic tissues of the mouse are also quite sensitive to marrow poisons (51). This sensitivity coupled with the size, availability and genetic uniformity of the laboratory mouse has resulted in its wide acceptance as an assay system for marrow poison evaluations.

The size of the animal is an important criterium which must be taken into consideration. The allowable frequency of sampling of blood, bone marrow or tissues is dependent upon the size of the animal. Large animals can provide samples at frequent intervals and enable the investigator to determine the progressive response of the animal(s) to the effects of the administered compound (38, 41, 42). The smaller animals, on the other hand, are suitable if sampling is done only at necropsy (8).

Evaluation of toxicity

The evaluation of the morphological changes of the bone marrow in response to marrow poisons is accomplished by bone marrow aspirations either in the live animal or at necropsy (49). Marrow smears are prepared and studied to determine if a change has occurred in the normal

ratio between the myeloid cells and the erythroid cells at 2:1 to 4:1. The change in the number of megakaryocytes present can also be noted at the same time.

Deviations from the normal morphological characteristics detected in the bone marrow in response to marrow poisons are an acellularity due to a decrease in the myeloid series and megakaryocytes. This may be followed by a decrease in the erythroid series depending upon the length of the assay period and the toxicity of the compound (21, 48, 50).

The peripheral blood commonly reflects the atrophy of the bone marrow. This reflection occurs because the cells in the marrow have been shown to be precursors of the cells in the peripheral blood (49). The thrombocyte decrease is the first response of poisoned marrow detectable in the peripheral blood (28, 41, 42), and reflects a megakaryocyte decrease. A leukopenia due to a decrease in the myeloid series and an impairment of lymphoid tissue, and an anemia corresponding to the erythroid series decrease are also detectable.

Bone marrow poisons also affect other tissues characterized by rapid proliferation causing a decrease in growth and function. These tissues are characterized by the stratified squamous epithelium of the skin and the gastrointestinal mucosa (20, 28, 41, 42).

Death and the time of death have been used as qualitative and quantitative criteria in marrow toxicity assays in addition to the detectable marrow responses (14, 41, 42).

The atrophy of the bone marrow and other hematopoietic tissues due to the marrow poisons can lead to a decrease in the activity of the

animal's defense mechanisms. The effects of the marrow poisons upon the intestinal mucosa can alter the body fluid and electrolyte balance. Both of these conditions if not corrected can lead to the death of the animal. Death as a result of these secondary sequela due to the effects of bone marrow poisons has given the most qualitative and quantitative responses (14, 41, 42).

Early death has been shown to occur if very large amounts of bone marrow poisons have been given, but the mechanism(s) of action in this early death has not been established (7). Calabresi and Welch (7) have postulated that the bone marrow poisons in some manner affect and alter metabolic activities concerned with the homeostatic process. They further state that the alterations in the homeostatic processes, can lead to death.

Calf Assay Systems

Young calves have been utilized as experimental animals in a wide variety of assay techniques. They have been used as the assay animals in nutrition experiments (3, 12, 19, 35) and in toxicological experiments with insecticides (29).

The young calf was found to be the ideal animal for the detection of toxic trichloroethylene-extracted soybean oil meal (TCESOM), the toxic principle of which turned out to be a potent bone marrow poison (33, 34, 36). Assay techniques using the calf were developed to determine the amount of toxic principle in various preparations of the toxic meal (36), and also to determine the amount of toxic principle

found in various fractions of the toxic meals (34, 46). These findings led to its being used as an assay system for other bone marrow poisons; S-(dichlorovinyl)-L-cysteine (DCVC) (25, 26, 27, 43) and bracken fern, Pteridium aquilinum (13).

TCESOM and DCVC assays

The hematopoietic system of the young calf has been shown to be very sensitive to the effects of the "aplastic anemia" producing substance(s) in toxic TCESOM (30, 33, 34, 36) and to DCVC (25, 26, 27, 43). Laboratory animals, mice, rats, guinea pigs, rabbits and dogs in contrast did not show the typical response of an aplastic anemia or a blood dyscrasia with TCESOM (37). Likewise, the rat failed to show the typical aplastic anemia response with DCVC (11).

The calf responds to the bone marrow poisons TCESOM and DCVC, like common laboratory animals respond to other bone marrow poisons with an immediate thrombocytopenia and a leukopenia with a relative lymphocytosis (26, 27, 43). The thrombocytopenia seen in calves in response to DCVC and TCESOM is much more severe than the thrombocytopenic response of laboratory animals due to other bone marrow poisons. Prominent hemorrhagic signs were observed consistently along with the thrombocytopenia in calves (30, 46). Following the thrombocytopenia and leukopenia with a relative lymphocytosis, a severe leukopenia and an anemia ensued if the assay period was prolonged.

Investigations have also shown that the occurrence of death and the time of death of the calf in response to the administration of TCESOM

and DCVC were also excellent criteria for quantitative assays of discrete dosage levels of both substances (25, 26, 27, 42).

The young calf has been found to respond quantitatively to large single dose or smaller daily dose regimens of TCESOM (25, 26) and DCVC (27, 42). The response of the calf has been shown to be very predictable for both programs.

Schultze, et al., (43) have conducted thorough comparative evaluations in calves of the changes observed in the cellular constituents of the peripheral blood and the changes observed in smears from bone marrow aspirations in response to bone marrow poisons, TCESOM and DCVC. They found that the changes in the peripheral blood reflected accurately the morphological changes observed in the bone marrow smears.

Gamma radiation response

The young calf has been shown to be sensitive to gamma radiation (39). The sequence of events observed in the peripheral blood in response to the gamma radiation was a very severe leukopenia within two days with a thrombocytopenia occurring later. This response in the calf was similar to that observed in other species (9, 23).

The reported sensitivity of the calf to gamma radiation and the observed radiomimetic response of the calf to TCESOM and DCVC led Schultze and co-workers (44) to determine in detail the effects of gamma radiation on the young calf. They were able to ascertain that not only was the calf sensitive to the effects of gamma radiation quantitatively, within prescribed limits, but also that the young calf was on the order of a factor of two times more sensitive than other mammalian species.

Laboratory investigations have demonstrated that the calf was extremely sensitive to two bone marrow poisons, and that the calf appeared to be appreciably more sensitive than other animals to the effects of gamma radiation. The reported increased sensitivity of the calf suggests that this animal might be the animal of choice for a very sensitive assay system for bone marrow poisons.

Cancer Chemotherapeutic Agents

Modes of action

Certain effective cancer chemotherapeutic agents which are also bone marrow poisons have been shown to act in metabolic pathways involving nucleic acids (5, 20). Investigators have demonstrated that these compounds block nucleic acid synthesis, interfere with the transcription of the genetic information from desoxyribonucleic acids (DNA) to ribonucleic acids (RNA) and cause an abnormal translation of the message into peptide chains and proteins. These interferences with nucleic acid metabolism have been shown to block normal mitotic activity (20). While the mechanisms of action of most anti-neoplastic agents are those mentioned above, new chemotherapeutic agents have been employed whose mechanisms of action have not been established or are only partially known. These new anti-cancer compounds have also been shown to be bone marrow poisons (5, 7, 20).

Neoplasms or tumors have been demonstrated as conditions in which the active proliferation of the affected cells is increased or at least altered in some manner (2). Abraham (1) has postulated that

chemotherapeutic agents which appear to interfere with these active proliferating tumor cells might adversely affect tissues in the body which normally show active proliferation. Brockman (5) has cited several instances in which it has been established that these compounds affect not only the neoplasms but also the bone marrow, the intestinal mucosa and the epithelium of the skin.

Classification

Many different types of compounds have been used as anti-cancer agents (7). With few exceptions, these agents can be placed into two main groups: alkylating agents and antimetabolite compounds.

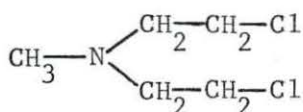
Alkylating agents which have been in common use as chemotherapeutic agents in the treatment of cancer for many years have mechanisms of action which have not been clearly elucidated (28). Karnofsky and Clarkson (20) have cited several suggested mechanisms of action for alkylating agents. They indicated that the major site of action is probably the alkylation of the 7-nitrogen of guanine. It was suggested that alkylation at this point may cause an easier scission of the sugar bond possibly leading to a break in the DNA chain.

The antimetabolites on the other hand are placed in two categories; purine and pyrimidine base analogs and dietary essential analogs. The purine and pyrimidine analogs, which may be typified by 6-mercaptopurine and 5-fluoro uracil (5-FU), have been shown to act as inhibitors of the corresponding purine or pyrimidine bases in nucleic acid metabolism. This inhibition can block DNA or RNA synthesis and interfere with protein synthesis (5, 8). The dietary essential analogs,

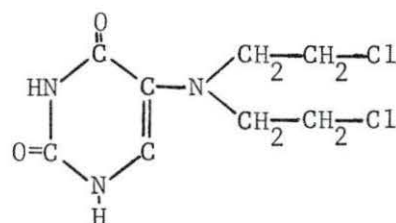
of which the anti-folic acid compounds are typical, have been shown to interfere with those reactions in which the nutrient is required as a co-factor (5, 18).

Mechlorethamine·HCl and uracil mustard

Mechlorethamine·HCl (methyl-bis(β-chloroethyl)amine) and uracil mustard (5-bis(2-chloroethyl)-amino uracil) are alkylating agents which have been used as cancer chemotherapeutic agents (28). The structures of these two compounds are as follows:



Mechlorethamine·HCl



Uracil mustard

These two compounds are polyfunctional alkylating agents, i.e., they have more than one active site. Both of these agents have been shown to cause cross-linking between two different guanines on the same DNA chain or on two separate DNA chains (20). Shanbrom, *et al.*, (47) indicate that uracil mustard because of its carrier structure, uracil, may also interfere with tumor metabolism by acting as a pyrimidine analog.

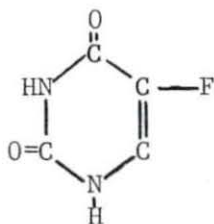
Subacute and chronic toxicity studies with mechlorethamine·HCl and uracil mustard have indicated that both cause similar qualitative responses in assay animals (24, 41, 42). The two compounds cause an initial hematopoietic tissue depression followed by gastro-intestinal upsets and death. The first sign of the hematopoietic tissue depression

was a thrombocytopenia. This was followed by a granulocytic and lymphocytic leukopenia with an ensuing depression of the erythrocyte counts and hemoglobin value.

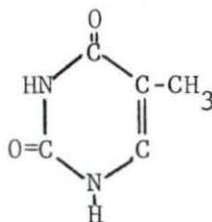
Rats injected intraperitoneally with uracil mustard at dosage levels of 1 mg./kg./day developed toxicity symptoms (24). Quantitative and qualitative studies in monkeys and dogs treated with mechlorethamine·HCl at six dosage levels between 0.021 and 0.672 mg./kg. daily have indicated that both the monkey and the dog respond in a similar qualitative manner at all of the dosage levels used. The dog as indicated by the response of the thrombocytes and the gastro-intestinal mucosa appeared to be more sensitive to the effects of this nitrogen mustard than the monkey. Death occurred earlier in monkeys even though they did not show the early signs of sensitivity exhibited by the dog to this compound (41, 42).

5-Fluoro uracil (5-FU)

5-Fluoro uracil (5-FU) is a pyrimidine analog, very similar in structure to thymine. The structures of these two compounds are shown below:



5-Fluoro uracil



Thymine

Most of the evidence has indicated that the mechanism of action of 5-FU is to inhibit the enzymes involved in nucleoside and nucleotide synthesis, specifically those of pyrimidine nucleosides and nucleotides (10). The most profound inhibitory effect of 5-FU has appeared to be in the synthesis of DNA thymidine. The 5' deoxyriboside of 5-FU has been shown to cause an irreversible inhibition of the enzyme thymidylate synthetase (8). Several other effects of 5-FU have been demonstrated due to its incorporation into ribonucleotides and polyribonucleotides, among which have been demonstrated are abnormalities in protein synthesis (5).

Subacute and chronic toxicity studies with 5-FU in mice, rats, dogs, and monkeys (49) and in humans under clinical study (17, 48) have indicated that the primary sign is a hematopoietic tissue depression. This is followed by intestinal mucosa damage and death.

Mice treated with 5-FU at a dosage level of 40 mg./kg./day for 6 days have shown severe depressions of thrombocytes, neutrophils, lymphocytes and erythrocytes (22). With dosages of 25 mg./kg./day, for 14 days only mild changes occurred in the peripheral blood (22).

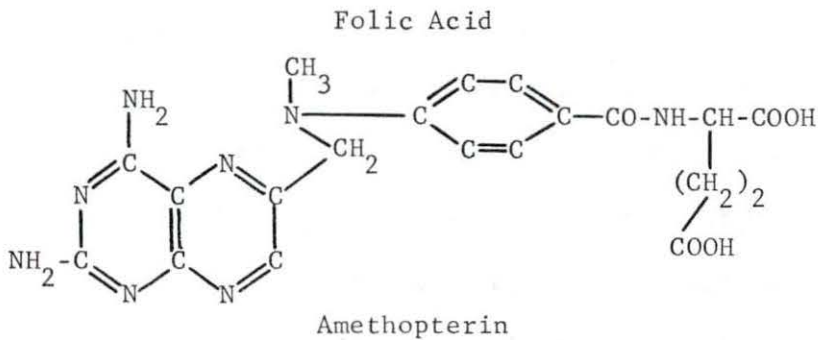
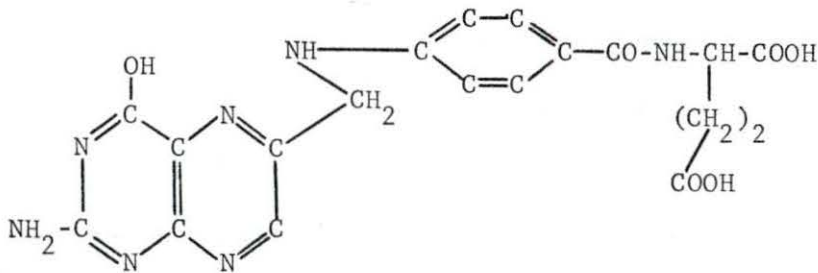
Dogs injected intramuscularly with 5-FU at levels of 2.5 mg./kg./day for 30 days showed toxicity signs, but monkeys required 10 times that amount to produce toxicity signs (50).

Humans with cancer have been given 5-FU at dosage levels of 4, 6 or 8 mg./kg./day for periods of 14 to 42 days (17, 48). Mild toxicity signs of slight hematopoietic tissue depression were observed in patients treated at the lower dosage schedules. More severe signs of increased

hematopoietic tissue depression and gastro-intestinal upsets were observed with the progressively higher dosage levels.

Amethopterin

Amethopterin is an analog of folic acid which has a molecular structure similar to that of folic acid (pteroylglutamic acid) as shown below:



The structural differences between folic acid and amethopterin are an amino group attached to position 4 and a methyl group attached to position 10. Amethopterin prevents the reduction of folic acid by inhibiting dihydro-folic acid reductase (18). Jukes (18) has presented evidence to indicate that this enzyme has an affinity for amethopterin about 100,000 times greater than folic acid. This reduction step has

been shown to be a necessary reaction in the methylation of deoxyuridine monophosphate (dUMP) to form thymidine monophosphate (TMP).

Comparative studies with amethopterin have been conducted with mice, rats and dogs (14, 31, 38). Additional information has been derived from clinical studies on humans (28). These studies indicated that the initial signs of a toxicity in response to amethopterin are those of hematopoietic tissue depression. These initial signs are followed by digestive upsets and death.

Evidence has been presented that the dog is more sensitive to amethopterin than are rodents (38). Intra-muscular injections of amethopterin of 1.0, 0.2 and 0.05 mg./kg./day elicited varying toxicity responses in the dog. The onset of the toxicity signs was shown to be dependent upon the amount of compound given. Animals receiving the greatest amount of compound per unit body weight showed the earliest signs. The time of death of these animals appeared to reflect the dosage levels quantitatively (14).

MATERIALS AND METHODS

Experimental Animals

Male Holstein calves weighing 32 to 45 kg. were purchased from a single local source. Upon receipt, the calves were given a preventative 2 ml. injection of an antibiotic.¹ A second 2 ml. injection of the antibiotic was given at a later time if necessary.

The calves were housed, two per stall, in clean, well ventilated concrete stalls which were bedded with straw. These stalls were 8 feet by 8 feet with corresponding outside stalls of the same type for access in good weather.

The rations consisted of a commercial powdered milk preparation² with grain, alfalfa, a mineralized salt block and fresh water available at all times.

After receipt, the calves were conditioned for a period of one to two weeks. This conditioning period was essential to make sure the calves were healthy. This period was also used to check the peripheral blood picture several times to determine if it fell within the recognized normal limits described by Schalm (40).

During the experimental procedures, the rectal temperatures and the weights were taken weekly. Clinical examinations were made daily on

¹Penstrep, Merck; Procaine penicillin G, 200,000 units and the sulfate salt of dihydrostreptomycin, 0.25 gm. per ml. of solution.

²Dari Dri; Milk Specialties, Inc., Dundee, Illinois.

each calf to detect signs which might have been due to the effects of the test compounds.

The hair on the necks of the calves was kept closely clipped over the jugular vein to allow adequate disinfection of the area. Blood samples were collected from the jugular vein with sterile, siliconized¹ needles and syringes. A drop of blood was placed immediately on a paraffin block for thrombocyte count dilutions. Five milliliters of blood were then placed in a rubber stoppered glass container which was siliconized and contained 5 mg. of the dipotassium salt of ethylenediamine-tetraacetic acid as the anticoagulant. Blood samples were obtained at least twice a week from the test animals and more often as changes occurred in the cellular constituents of the peripheral blood. The control calf was bled once a week.

Test compounds were administered by injection into the jugular vein. Daily doses were given on the basis of a prescribed amount of compound per unit of body weight. The amount of compound injected daily was adjusted each week following the weighing to allow for the change in weight.

Test Compounds

The anti-cancer compounds used in this investigation were obtained from a local retail source.

¹Siliclad; Clay-Adams, Inc., New York.

Mechlorethamine·HCl (Mustargen-Merck)

The mechlorethamine·HCl was furnished in sterile 10 mg. vials. This compound was reconstituted daily with 20 ml. of sterile water to make a final concentration of 500/ μ g. per ml. The excess solution was discarded.

Uracil mustard (Upjohn)

The uracil mustard was supplied in one mg. capsules. The contents of the capsules were emptied into sterile volumetric flasks of a sufficient size. One ml. of dimethylacetamide was added for each mg. of compound as suggested by Schumacher and O'Connell (45) to increase the solubility of the uracil mustard. Sterile water was added to adjust the final concentration to one mg. per 5 ml. An additional solution consisting of one ml. of dimethylacetamide in 5 ml. of sterile water was prepared. Both solutions were made fresh daily and the excesses discarded.

5-Fluoro uracil (Fluorouracil-Roche)

5-Fluoro uracil was furnished in 10 ml. vials in an injectable form. The concentration was 50 mg. per ml. The solutions after opening were stored at room temperature until used, or until a white precipitate formed in the bottom of the vial.

Amethopterin (Methotrexate sodium-Lederle)

The amethopterin was supplied in 50 mg. sterile vials. The compound was reconstituted with 20 ml. of sterile water to make a final concentration of 2.5 mg. per ml. The solutions were stored at room temperatures until used.

Peripheral Blood Evaluations

The parameters measured in the peripheral blood were total thrombocyte counts, total erythrocyte counts, total leukocyte counts, differential leukocyte counts, hematocrit and hemoglobin determinations. These techniques have been described by Wintrobe (49) or Schalm (40).

Reagents

1. Rees-Ecker dilution fluid (49)

Sodium citrate	3.8 Gms.
Brilliant cresyl blue	0.05 mg.
Formaldehyde, 40%, neutral	0.2 ml.

A sufficient quantity of water to make a total volume of 100 ml.

2. 5% Acetic Acid

Glacial Acetic Acid	5.0 ml.
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A sufficient quantity of water to make a total volume of 100 ml.

3. Drabkin's Solution for hemoglobin determination (48)

Sodium bicarbonate C.P.	1.0 Gm.
Potassium cyanide C.P.	52 mg.
Potassium ferricyanide C.P.	198 mg.

A sufficient quantity of water to make a total volume of 1000 ml.

This solution was stored in a brown bottle.

4. Wright's Stain¹5. Phosphate buffer solution¹

Giordano pH 6.4

¹Prepared by Hartman-Leddon Company, Philadelphia, Pa. and obtained from A. H. Thomas Co., Philadelphia, Pa.

Thrombocyte counts

The Rees-Ecker method of thrombocyte enumeration described by Wintrobe (49) was the procedure followed. The dilutions were made directly at the time of obtaining the blood sample. Five groups of 16 squares in the hemocytometer were counted and the total number of thrombocytes was multiplied by 5,000 to calculate the number of thrombocytes per cu. mm.

Erythrocyte counts

The erythrocytes were enumerated using the method described by Schalm (40). The Rees-Ecker diluting fluid was used instead of the described diluting fluid.

Total leukocyte counts

The leukocytes were counted by the method described by Schalm (40). Five percent acetic acid was used as the diluting fluid and hemolyzing agent.

Differential leukocyte counts

Blood smears were made as described by Schalm (40). The Wright's staining method was used. The stain was applied for a one-minute period. A similar quantity of the buffer solution was mixed with the stain and allowed to stand on the slide for a five-minute period. The slide was washed with water, air dried and examined by oil immersion techniques.

Hematocrit

The hematocrit or packed cell volume (PCV) was determined by the

micro-hematocrit method (40). A Clay-Adams¹ microhematocrit centrifuge and micro-hematocrit reader were used in this procedure.

Hemoglobin determinations

The cyan-methemoglobin (49) method for hemoglobin determinations was used in this procedure. The B & L Spectronic 20² was used to measure the percent transmission.

Necropsy Techniques

Necropsies were performed on all experimental animals. All observed gross lesions were noted and recorded for subsequent evaluations.

¹Clay-Adams, Inc.

²Bausch and Lomb, Inc., Rochester, N.Y.

EXPERIMENTAL

The experimental plan developed for this investigation was designed to supply for each of the cancer chemotherapeutic compounds tested answers to the following questions:

1. Can the calf sense the marrow poisoning activity of the compound?
2. Can one or more of the responses observed in the calf to the compound be quantitated?
3. Are the responses to the compound observed in the calf comparable to those observed in other species of laboratory animals for the same compound?
4. Are the responses to the compound observed in the calf comparable to those observed in calves poisoned with TCESOM and DCVC?

The initial goal for each compound tested was the determination of a level of daily compound administration that would allow the depression of the marrow and hematopoietic tissues to occur at a rate that would result in the death of the calf between 30 and 60 days. At these rates of marrow and hematopoietic tissue depression in the calf, the signs and symptoms characterizing this depression have been shown to appear in an orderly and progressive manner. Hematologic signs in the peripheral blood, a progressive thrombocytopenia, leukopenia and relative lymphocytosis, are followed by clinical signs of a decrease in the rate of gain, general loss of condition, hemorrhage, temperature spike, diarrhea, and death. At the necropsy examination, the classical signs associated with a marrow depression are commonly observed. Petechial and ecchymotic

hemorrhages observed throughout the body cavities and tissues and a blood tinged fluid in the body cavities are common findings. That the calf is capable of manifesting this orderly qualitative response to marrow poisons has been thoroughly substantiated by extensive studies with TCESOM (33, 36), DCVC (44) and ionizing radiation (39, 45).

The second of the goals for each of the compounds was to attempt to obtain an indication as to whether any of the responses observed could be quantitated with dosage levels. Evaluation of the ability of the calf to respond in a quantitative manner to graded dosages of a given compound involves the selection of several dosage levels that will reflect with one or more of the various signs associated with marrow depression, a dose-degree of response-time relationship that is quantitative in nature. Extensive studies with TCESOM and DCVC have established that the calf is capable of such a quantitative response to these poisons (33, 43).

The selection of compounds for evaluation from the array of cancer chemotherapeutic compounds in use today was guided by the following criteria:

1. Compounds selected should have demonstrated a degree of success in human therapy.
2. Compounds selected have been evaluated for marrow toxicity in other experimental animals.
3. Compounds selected should include both the alkylating and anti-metabolite types.
4. Compounds selected should be suitable for intravenous injection.

Mechlorethamine·HCl and uracil mustard were selected as being representative of the alkylating type compounds. Uracil mustard is a structural analog of mechlorethamine·HCl and might have a different degree of activity due to the substitution of the uracil moiety for the methyl group. It is also possible that the substituted uracil might act as an antimetabolite in pyrimidine metabolism.

The compounds, 5-fluoro uracil and amethopterin, are representatives of the antimetabolite class of cancer chemotherapeutic agents. 5-Fluoro uracil is also a structural analog of uracil mustard since in both cases, the uracil moiety is substituted at the 5' position. Amethopterin is an antimetabolite of folic acid.

The dosage regimen selected was that employed by McKinney, et al., (25) with DCVC, daily doses of test compound administered by the intravenous route. The selection of the initial daily dosage levels for administration of the compounds to calves was based on approximations derived from levels employed in human chemotherapy (7). Subsequent dosage levels were increased or decreased by factors of 2 until the desired rates of depression of the marrow were attained.

All of the experimental data, exclusive of necropsy observations, have been compiled in graphic form for presentation. These data, grouped according to the compound tested, appear in the appendices. Summary tabulations extrapolated from these raw data appear as tables in the text.

Control Calf

The calf 947 was maintained throughout the investigation as a control for the purpose of establishing the normal variations that might

appear in the hematologic and clinical parameters being measured due to the ages of the calves, experimental manipulations and environmental factors. The parameters measured in this calf were total thrombocyte counts, total erythrocyte counts, total leukocyte counts, differential leukocyte counts and hematocrit and hemoglobin determinations. The differential leukocyte counts were converted to absolute values for graphic presentation. None of the erythrocyte data obtained for the control and experimental calves has been presented in graphic form in the appendices because no significant variations were observed.

The one to two week conditioning period allowed each animal to serve as its own experimental control. This conditioning period was used to establish the normal, pre-test parameters for each calf.

The control calf was also observed daily along with the experimental calves for the appearance of the signs and responses that have been reported as being observed in laboratory animals poisoned with the specific marrow poisons under investigation, as well as the signs and responses observed in the calf due to the effects of administration of DCVC.

Mechlorethamine·HCl

The data presented in Appendix II and summarized in Table I give the biological responses of the young calves given graded daily doses of mechlorethamine·HCl.

The mechlorethamine·HCl administered to calf 844 (14.85 $\mu\text{g./kg./day}$) and calf 942 (30.8 $\mu\text{g./kg./day}$) appeared to produce a slight hematopoietic

Table I. The biological responses of young calves to mechlorethamine·HCl

Calf No.	844	942	977	953	958
Dosage data					
Amount/kg./day ($\mu\text{g.}$)	14.85	30.8	59.4	118.8	118.8
(μ moles)	0.0772	0.1599	0.3884	0.6178	0.6178
Total Cpd. given (mg.)	146.88	126.014	162.786	81.594	59.832
Weight of calf-initial	128	101	115	115	90
max.	289	157	130	115	90
Final	289	157	130	115	90
DAYS TO DEVELOP					
Hematologic signs					
Thrombocytopenia					
Initial decline mm^3	33	11	23	never	never
Below 200,000/ mm^3	60	54	34	never	never
150,000/ mm^3	never	never	34	never	never
100,000/ mm^3	never	never	37	never	never
50,000/ mm^3	never	never	47	never	never
Leukopenia					
Below 5,000/ mm^3	never	22	4	5	1
3,000/ mm^3	never	never	20	9	5
2,000/ mm^3	never	never	20	never	5
1,000/ mm^3	never	never	43	never	never
Lymphocytosis					
Above 80%	52	22	20	8	2
85%	never	22	20	never	never
90%	never	never	41	never	never
95%	never	never	41	never	never
Clinical signs					
Temperature spike	never	never	39	11	7
Diarrhea	never	never	37	8	6
Death	108*	73*	47	13	12
Necropsy					
Lesions observed	no	no	yes	yes	yes
Typical lesions of a marrow hypoplasia	no	no	no	no	no

*This animal was killed, and had a normal appearance.

depression. The dosage levels were apparently too low to elicit the desired hematologic responses and the times of death were far in excess of the desired 30 to 60 days. The increased dosage did appear to materially affect the growth rate of calf 942, which can be observed by comparing Figures 7 and 10 of Appendix II.

The compound administered at the next higher level, calf 977 (59 $\mu\text{g.}/\text{kg.}/\text{day}$), produced an orderly and progressive hematopoietic tissue depression, diarrhea, temperature spike and death. The time of detection of a significant leukocyte depression was sooner than the occurrence of a significant thrombocytopenia. All of the responses occurred within the 30 to 60 day period indicating that this dosage level is within the assay range.

The dosage level of 118.8 $\mu\text{g.}/\text{kg.}/\text{day}$ administered to calves 953 and 958 appears to be too high to elicit the desired hematologic effects in the calves. A very rapid onset of a leukopenia was observed in both calves, but the thrombocytopenia and relative lymphocytosis apparently did not have sufficient time to develop prior to death. The animals were markedly affected by this level of the compound as evidenced by the lack of growth and the early appearance of the temperature spike and the severe diarrhea.

The gross lesions observed at necropsy in calves 977, 953 and 958 consisted of moderate numbers of petechial hemorrhages on the serosal surfaces and in the heart. Severe pneumonia-like lesions were observed in the lungs of calf 977, and moderate lung lesions were observed in calf 953. There was an apparent splenic hypoplasia observed in calf 977

which was indicative of a hematopoietic tissue depression. While petechial hemorrhages of the serosal surfaces are typical findings in calves poisoned with TCESOM and DCVC, these lesions appear only after extended periods of a severe thrombocytopenia. Of these calves, only calf 977 exhibited a thrombocytopenia of a degree suggesting any association of the hemorrhagic appearance with the administered compound.

The pneumonia-like lesions were thought to be due to an acute septicemia caused by an overwhelming bacterial infection, possibly due to a decrease in the animals' defense mechanisms resulting from the activity of the compound on the hematopoietic tissues.

The 59.4 $\mu\text{g.}/\text{kg.}/\text{day}$ level of the mechlorethamine $\cdot\text{HCl}$ produced an ideal response in the peripheral blood indicating an orderly depression of the hematopoietic tissues leading to death of the animal. The responses observed in the calf at this level are comparable to those reported in monkeys and dogs (41, 42). These responses did not appear to be as severe as those observed in calves due to DCVC.

A quantitative assay range for mechlorethamine $\cdot\text{HCl}$ was not established but it appears as if the 59.4 $\mu\text{g.}$ level could be approximately in the middle of the desired range. These data indicate that levels could be surveyed at higher and lower levels than the 59.4 $\mu\text{g.}$ level to determine the desired range. The time of death is suggestive of a quantitative response to this compound but insufficient numbers of calves were observed to allow a definite conclusion to be made.

The calf appears to be more sensitive to mechlorethamine $\cdot\text{HCl}$ by a factor of at least 2 than the monkey and dog. The assay range for the

monkey and dog is 21 to 672 $\mu\text{g.}/\text{kg.}/\text{day}$ (41, 42), but a range of 15 to 119 $\mu\text{g.}/\text{kg.}/\text{day}$ produced similar responses in calves.

The response of the calf to mechlorethamine·HCl in comparison to the responses of the calf to DCVC are somewhat different. Hematopoietic tissue depression and death are the common responses, but the visible hemorrhages, fecal blood and typical lesions of an aplastic anemia described by McKinney, et al., (25) were not observed in these investigations.

Uracil Mustard

The data presented in Appendix IV and summarized in Table II give the biological responses of young calves given graded daily doses of uracil mustard.

Uracil mustard is but sparingly soluble in water. Dimethylacetamide has been used to increase the solubility for purposes of intravenous injections in human therapy and experimental work (45). Since the effect of dimethylacetamide on the hematopoietic tissues of the calf was unknown, dimethylacetamide in sterile water was injected intravenously to calf 945 at levels corresponding to amounts recommended for its use as a solublizing aid for uracil mustard intravenous administration (45). The amount of dimethylacetamide administered to calf 945 was 0.022 ml./kg./day. Comparison of the data in Appendix II for calf 945 with the data presented in Appendix I for the control calf indicates that dimethylacetamide did not elicit a hematologic response in calf 945. To complete the evaluation of the possible effects of the use of dimethylacetamide as a solublizing aid for uracil mustard administration,

Table II. The biological responses of young calves to uracil mustard

Calf No.	946	979+	952	956	955
Dosage data					
Amount/kg./day ($\mu\text{g.}$)	22	22	44	88	88
(μ moles)	0.0833	0.0833	0.1663	0.3326	0.332
Total Cpd. given (mg.)	153.95	131.900	151.06	69.8	48.0
Weight of calf-initial	100	95	99	72	72
max.	229	202	150	90	72
Final	229	202	148	90	68
DAYS TO DEVELOP					
Hematologic signs					
Thrombocytopenia					
Initial decline ³	27	16	27	15	never
Below 200,000/mm ₃	31	23	29	18	never
150,000/mm ₃	never	61	41	22	never
100,000/mm ₃	never	61	41	22	never
50,000/mm ₃	never	never	never	never	never
Leukopenia					
Below 5,000/mm ₃	11	16	6	2	1
3,000/mm ₃	45	26	27	2	4
2,000/mm ₃	62	65	34	5	10
1,000/mm ₃	62	never	63	17	never
Lymphocytosis					
Above 80%	41	30	13	22	3
85%	52	65	34	never	3
90%	90	65	34	never	never
95%	94	79	57	never	never
Clinical signs					
Temperature spike	never	never	53	12	12
Diarrhea	never	never	61	9	12
Death	104*	101*	66	23	17
Necropsy					
Lesions observed	no	no	yes	yes	yes
Typical lesions of a marrow hypoplasia	no	no	no	no	no

+ Dimethylacetamide was not used as a solvent.

* Calf was killed. The animal had a normal appearance.

uracil mustard was administered with and without added dimethylacetamide to two calves, calf 946 (uracil mustard at 22 $\mu\text{g./kg./day}$ with dimethylacetamide) and calf 979 (uracil mustard at 22 $\mu\text{g./kg./day}$ without dimethylacetamide). The data in Table II show that both calves exhibited comparable progressive and orderly depressions of the cellular constituents of their peripheral blood. There did not appear to be any significant differences that could be ascribed to the addition of dimethylacetamide or to any increase in solubility of the uracil mustard. While the level of uracil mustard (22 $\mu\text{g./kg./day}$) was high enough to elicit a definite hematopoietic tissue response, it was too low to produce death between 30 and 60 days.

It was decided to use the dimethylacetamide for the higher dosage levels of uracil mustard to facilitate dispersion of the compound for intravenous injection.

The uracil mustard administered at the next higher level to calf 952 (44 $\mu\text{g./kg./day}$) elicited a very marked depression of the hematopoietic tissues as evidenced in the peripheral blood up until the time of death. The occurrence of a temperature spike and a diarrhea prior to death was also evidence that this compound was directly affecting the animal. This dosage level appears to be on the low side of the assay range as determined by the time of death.

The next higher level of administration of uracil mustard, 88 $\mu\text{g./kg./day}$, to calves 956 and 955, produced early death in these animals. In spite of the early deaths, there were indications of rapid hematopoietic tissue depression, especially in calf 956 which exhibited an orderly and

progressive decrease in the cellular constituents of the peripheral blood. Both calves exhibited the clinical signs of diarrhea and temperature spike prior to death.

The necropsy findings observed in calves 952, 956 and 955 were very similar in that there were many petechial hemorrhages on the serosal surfaces, lesions indicative of a severe pneumonia and splenic hypoplasia in all calves. Calf 952 was the only calf exhibiting an extended period of thrombocytopenia which would be expected to produce the extensive petechiation. The splenic hypoplasias observed are indicative of hematopoietic tissue depression. The appearance of the pneumonia-like lesions in the lungs was attributed to a secondary bacterial infection occurring due to a decreased ability of the defense mechanisms to function.

All of the levels of uracil mustard administered produced a marked depression of the hematopoietic tissues. The 44 $\mu\text{g.}/\text{kg.}/\text{day}$ level produced desirable responses of hematopoietic tissue depression followed by death within the desirable period of time. The experimental findings suggest that the calf does respond to this compound in a manner comparable to other laboratory animals.

A comparison of equal molar levels of mechlorethamine·HCl and uracil mustard injected into the calf indicates that uracil mustard appears to be more toxic to calves by a factor of about 2. It is postulated that the substitution of the uracil for the methyl group could produce more efficient alkylation or that the uracil moiety may also act as an antimetabolite affecting pyrimidine synthesis, thereby supplementing the alkylating properties of the compound.

It appears that the assay range for uracil mustard in the calf should fall between the 44 $\mu\text{g.}$ and the 88 $\mu\text{g./kg./day}$ dosage levels. The times at which death occurred in response to the 44 $\mu\text{g.}$ and 88 $\mu\text{g.}$ levels are very suggestive of quantitative responses.

The calf does appear to be more sensitive to the effects of uracil mustard than the mouse or rat. Reports of other laboratory animals tested with uracil mustard do not appear in the literature. Investigations have shown that the mouse responds quantitatively at levels of 1 mg./kg./day (24). It has been shown that rats could tolerate dosage levels of 0.25 to 0.30 mg./kg./day for long periods of time with only moderate toxic manifestations (24).

The response of the calf to uracil mustard does differ with the response to DCVC (44) and TCESOM (36) in that the very severe hemorrhagic lesions were not observed in these calves due to the uracil mustard.

5-Fluoro Uracil

The data presented in Appendix V and summarized in Table III give the biological responses of young calves given graded doses of 5-fluoro uracil (5-FU).

The initial dosage selected of 5-FU administered to calf 750 (3.96 mg./kg./day) produced an almost simultaneous depression of the cellular constituents of the peripheral blood. The decreased rate of gain, temperature spike and diarrhea were evidences that this level of 5-FU was severely affecting the calf. The time of death indicated that this level of the compound was too high to elicit the orderly depression desired.

Table III. The biological responses of young calves to 5-fluoro uracil

Calf No.	750	950	948	951	788	980
Dosage data						
Amount/kg./day (μ g.)	3.96	1.98	1.98	1.49	1.49	1.49
(μ moles)	27.9	13.95	13.95	10.46	10.46	10.46
Total Cpd. given (mg.)	2.764	2.5812	3.0951	2.263	1.2543	0.9679
Weight of calf-initial	83	83	102	91	101	92
max.	87	94	112	106	101	92
Final	85	88	112	97	90	84
DAYS TO DEVELOP						
Hematologic signs						
Thrombocytopenia						
Initial decline	5	4	5	30	8	6
Below 200,000/mm ³	10	4	5	33	10	12
150,000/mm ³	16	25	24	never	10	never
100,000/mm ³	16	25	24	never	13	never
50,000/mm ³	never	never	never	never	never	never
Leukopenia						
Below 5,000/mm ³	12	30	20	22	6	never
3,000/mm ³	15	31	25	33	13	never
2,000/mm ³	never	31	never	35	never	never
1,000/mm ³	never	never	never	never	never	never
Lymphocytosis						
Above 80%	12	29	27	35	never	never
85%	12	30	30	never	never	never
90%	13	30	30	never	never	never
95%	14	31	30	never	never	never
Clinical signs						
Temperature spike	15	23	22	never	15	never
Diarrhea	16	18	23	23	15	10
Death	18	32	31	35	18	16
Necropsy						
Lesions observed	no	yes	yes	no	yes	yes
Typical lesions of a marrow hypoplasia	no	no	no	no	no	no

The lower dosage of 5-FU administered to calves 948 and 950 (1.98 mg./kg./day) produced an orderly and progressive hematopoietic tissue depression with marked thrombocytopenia occurring prior to the leukopenia. The clinical signs observed in these two calves indicated that this compound was directly affecting the animals. The times of death of these two animals were within the desired period of time and were also indicative of a quantitative response to the dosage level.

The lowest dosage level of 5-FU (1.49 mg./kg./day) was administered to calves 951, 788 and 980. Although there were hematological findings which suggested a response to the compound, there were no consistent signs observed except the decreased growth rate.

The times of occurrence of death in all animals injected with 5-FU were very inconsistent. The inconsistent hematological and clinical signs exhibited in all calves were suggestive of a lack of quantitative response in calves to this compound.

The necropsy findings observed in the calves treated with 5-FU were very inconsistent. The most consistent finding was that of dehydrated and emaciated cadavers. Calf 948 exhibited the most extensive lesions which consisted of petechial and ecchymotic hemorrhages of the serosal surfaces, white spotted kidneys, swollen liver, pneumonia and splenic hypoplasia. The significance of the wide array of lesions was not understood, but was attributed to a chronic condition. Calves 950, 788 and 980 had patches of pneumonia-like lesions of the lungs and calf 980 also had white spotted kidneys.

The appearances of the emaciated and dehydrated cadavers of all of the 5-FU treated calves were highly indicative of a direct effect of 5-FU upon the calves. These findings are suggestive of a very marked interference with the homeostatic mechanisms.

The responses of the calf to 5-FU of an emaciation, diarrhea, retarded growth rate and hematopoietic tissue depression are very similar to the responses reported for mice, dogs and monkeys (51). The hematologic responses produced in the calf due to 5-FU are not as orderly and progressive as have been shown in the calf in response to TCESOM (36) and DCVC (44).

The responses of the calf to the graded levels of 5-FU administered indicate that this animal might not be capable of quantitating toxic levels of this compound. The calf does appear to be somewhat more sensitive to the effects of 5-FU than the dog and much more sensitive than other animals. Slight toxicity responses have been elicited in the dog in 30 days at levels of 2.5 mg./kg./day (51), but in the calf, dosage levels from 1.49 mg. to 3.96 mg./kg./day caused death within 35 days. The comparison of dosage levels on a mole basis indicated that the 5-FU is not as toxic to the calf as uracil mustard and mechlorethamine·HCl.

Amethopterin

The data presented in Appendix VI and summarized in Table IV give the biological responses of calves to graded daily doses of amethopterin.

Amethopterin at a dosage level of 1.109 mg./kg./day (calves 784 and 792) was the highest level administered and the only dosage level that

Table IV. The biological responses of young calves to amethopterin

Calf No.	842	943	978	784	792
Dosage data					
Amount/kg./day (μ g.)	0.0693	0.143	0.277	1.109	1.109
(μ moles)	0.1528	0.3153	0.6114	2.4453	2.4453
Total Cpd. given (mg.)	874.9	572.62	1,6668	366.88	335.98
Weight of calf-initial	110	91	97	91	94
max.	286	172	225	91	94
Final	286	172	225	85	85
DAYS TO DEVELOP					
Hematologic signs					
Thrombocytopenia					
Initial decline	5	never	never	never	never
Below 200,000/mm ³	18	never	never	never	never
150,000/mm ³	never	never	never	never	never
100,000/mm ³	never	never	never	never	never
50,000/mm ³	never	never	never	never	never
Leukopenia					
Below 5,000/mm ³	never	never	never	3	never
3,000/mm ³	never	never	never	6	never
2,000/mm ³	never	never	never	never	never
1,000/mm ³	never	never	never	never	never
Lymphocytosis					
Above 80%	never	never	72	never	3
85%	never	never	never	never	3
90%	never	never	never	never	3
95%	never	never	never	never	7
Clinical signs					
Temperature spike	never	never	never	never	6
Diarrhea	never	never	never	2	2
Death	106*	78*	87*	7	8
Necropsy					
Lesions observed	no	no	no	no	no
Typical lesions of a marrow hypoplasia	no	no	no	no	no

*Calf was killed. The animal had a normal appearance.

indicated some hematopoietic tissue depression. There were clear clinical indications of an affect due to this level of compound consisting of severe weight losses, very early diarrheas and the rapid occurrences of death. The necropsy findings of these two calves were normal.

The lower levels of amethopterin administered to calves 842, 943 and 978, did not produce any significant hematologic or clinical responses.

Typical lesions of marrow hypoplasias have been produced in monkeys, rats and dogs (14). These lesions consisted of hematopoietic depression and hemorrhagic syndromes. None of these lesions were observed in calves in response to amethopterin.

High levels of amethopterin, 1.109 mg./kg./day, do cause death in the calf in a very short period of time, but none of the other responses observed in laboratory animals were observed in the experimental calves.

No quantitative responses due to the effects of amethopterin at the levels administered were observed in the calves. It is postulated that a bacterial synthesis of folic acid in the developing rumen was the probable cause of a lack of response of the calf to amethopterin.

DISCUSSION

The classical response, almost textbook in nature, of the hematopoietic system of the young calf to TCESOM, DCVC, bracken fern and ionizing radiation has repeatedly suggested itself to those familiar with the calf's capabilities in this respect, that the calf might be the animal of choice for the qualitative and quantitative assay of bone marrow poisons. The need for a better assay system is readily apparent when considerations are made to the omnipresence of marrow poisoning properties of many of the cancer chemotherapeutic agents in use today.

Experimental findings did show that the cancer chemotherapeutic agents, mechlorethamine·HCl, uracil mustard and 5-fluoro uracil, depressed hematopoiesis in the calf. It was of significance to note that the depression of hemopoiesis resulted in the evolution of a pattern of signs paralleling those reported for the mouse, rat, dog and monkey in response to these same compounds, rather than the pattern of signs characteristically produced in the calf due to the administration of TCESOM, DCVC, and bracken fern or exposure to ionizing radiation. The essential difference in the two patterns of signs is the occurrence of an early and profound thrombocytopenia ultimately resulting in the severe hemorrhagic syndrome characteristic of calves poisoned with DCVC. The fact that the calf responds to ionizing radiation in a manner similar to other mammalian species and that laboratory animals respond in a different manner than the calf to DCVC suggests that the DCVC response picture in the calf is atypical.

In contrast to the other three compounds tested in the calf, the antimetabolite, amethopterin, did not elicit any of the typical signs of depression of hematopoiesis evidenced in laboratory animals even though levels were established that indicated the compound was profoundly toxic to the calf.

Assay ranges for the compounds tested were not established. The experimental results did suggest levels that might be suitable for the assay of the two alkylating agents, mechlorethamine·HCl and uracil mustard. With these two compounds, dose-degree of response-time relationships were suggested for the signs, thrombocytopenia, leukopenia and death. The results obtained for 5-FU gave no indications of degrees or orders of responses suitable for quantitation. The manifestations of the hemorrhagic signs associated with an early and profound thrombocytopenia appear not to be available as additional indicators of effect versus dosage for these cancer chemotherapeutic compounds.

The fact that the data obtained indicated that the calf was more sensitive than other laboratory animals to three of the four compounds evaluated, by factors of at least 2 to 5, suggests that it may be a very sensitive species for evaluation of certain types of marrow poisons. The ability of even the unrefined calf assay to differentiate the marrow poisoning toxicities of the structural analogs, uracil mustard and mechlorethamine·HCl, establishes a difference unreported in the literature. An increased ability to evaluate differences in marrow poisoning activities of cancer chemotherapeutic agents as related to their structures would assist in the attainment of the goal of cancer chemotherapy.

Utilization of this approach could lead to the elimination or reduction of toxic effects of anti-cancer agents if these toxic activities are independent of the anti-tumor activities.

If the calf could be established as even a better system for assaying the marrow poisoning capabilities, especially potential cancer chemotherapeutic agents, its disadvantages of size and management problems would be of little consequence.

SUMMARY

1. The experimental findings indicate that the young calf is very sensitive to the hematopoietic tissue depressing properties of the cancer chemotherapeutic compounds, mechlorethamine·HCl, uracil mustard and 5-fluoro uracil.
2. The experimental results show that the calf responds to the effects of these three cancer chemotherapeutic agents in a manner similar to mice, rats, monkeys and dogs.
3. The marked hemorrhagic lesions which are typical of the response of the calf to the marrow poisons, TCESOM and DCVC, are not exhibited in response to the anti-cancer compounds.
4. The hematopoietic tissues of the young calf are not sensitive to amethopterin at the levels tested.
5. Assay ranges for mechlorethamine·HCl and uracil mustard in calves were not established although assay ranges were suggested by one or more of the exhibited responses of leukopenia, thrombocytopenia or death.
6. The inconsistent responses observed with 5-fluoro uracil, gave no indication of the existence of a quantitative assay range in the calf for this compound.
7. The only quantitative criterium, evidenced in response to the effects of the administration of amethopterin to calves, was death, observed only at the highest dosage levels.
8. The observations that the calf is capable of responding in a dose-degree of response-time relationship to the alkylating agents, uracil

mustard and mechlorethamine·HCl, are highly suggestive of the calf being a more sensitive assay animal than the dog and monkey. These responses indicate that the calf is more sensitive than these two species by a factor of 2 to 5.

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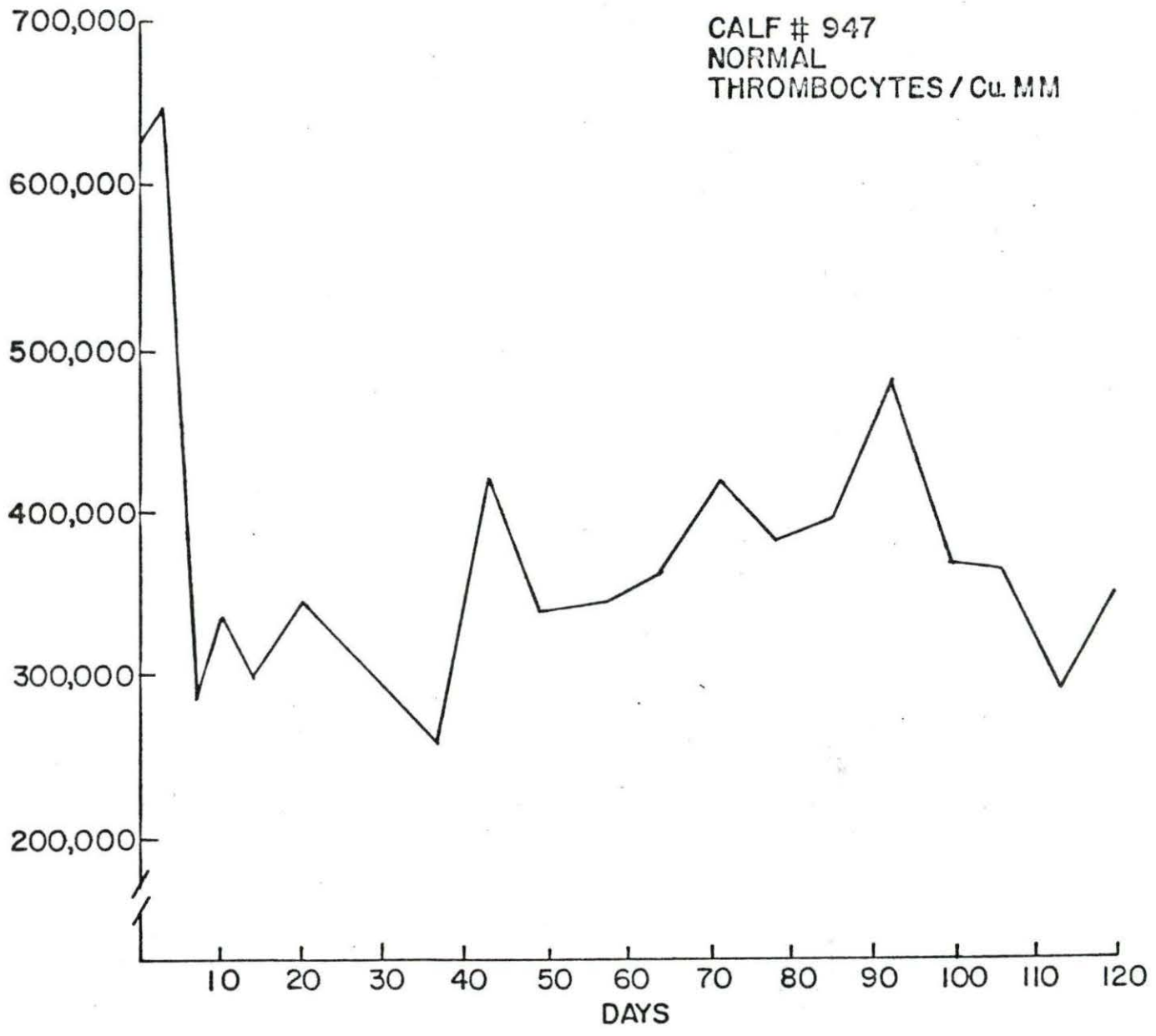
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ACKNOWLEDGMENTS

The author is grateful to Dr. Joseph C. Picken, Jr. for his guidance and encouragement given during this investigation.

APPENDIX I. CONTROL CALF

Figure 1. Calf 947. Thrombocyte counts



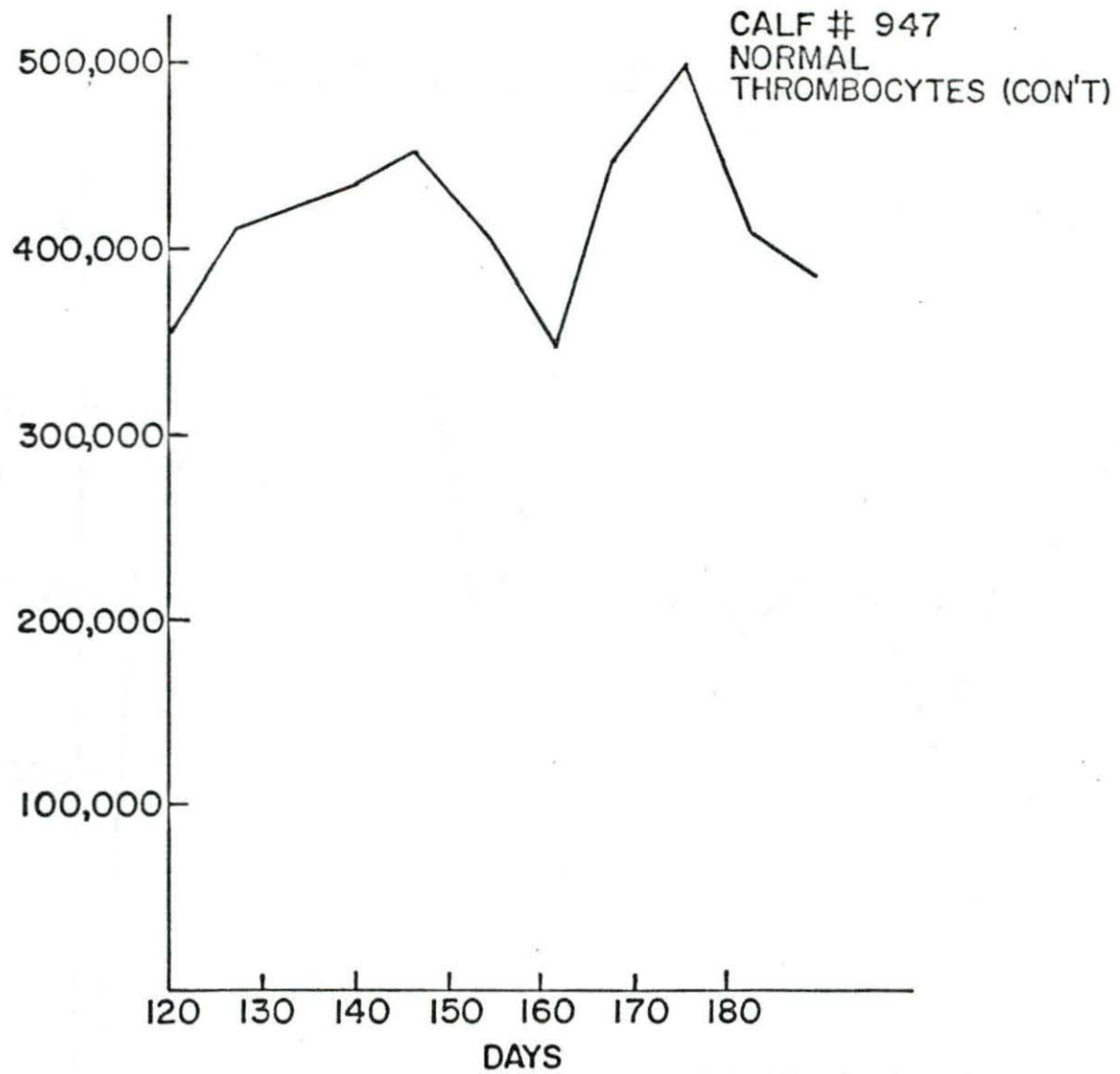


Figure 1. (Continued)

Figure 2. Calf 947. Leukocyte counts

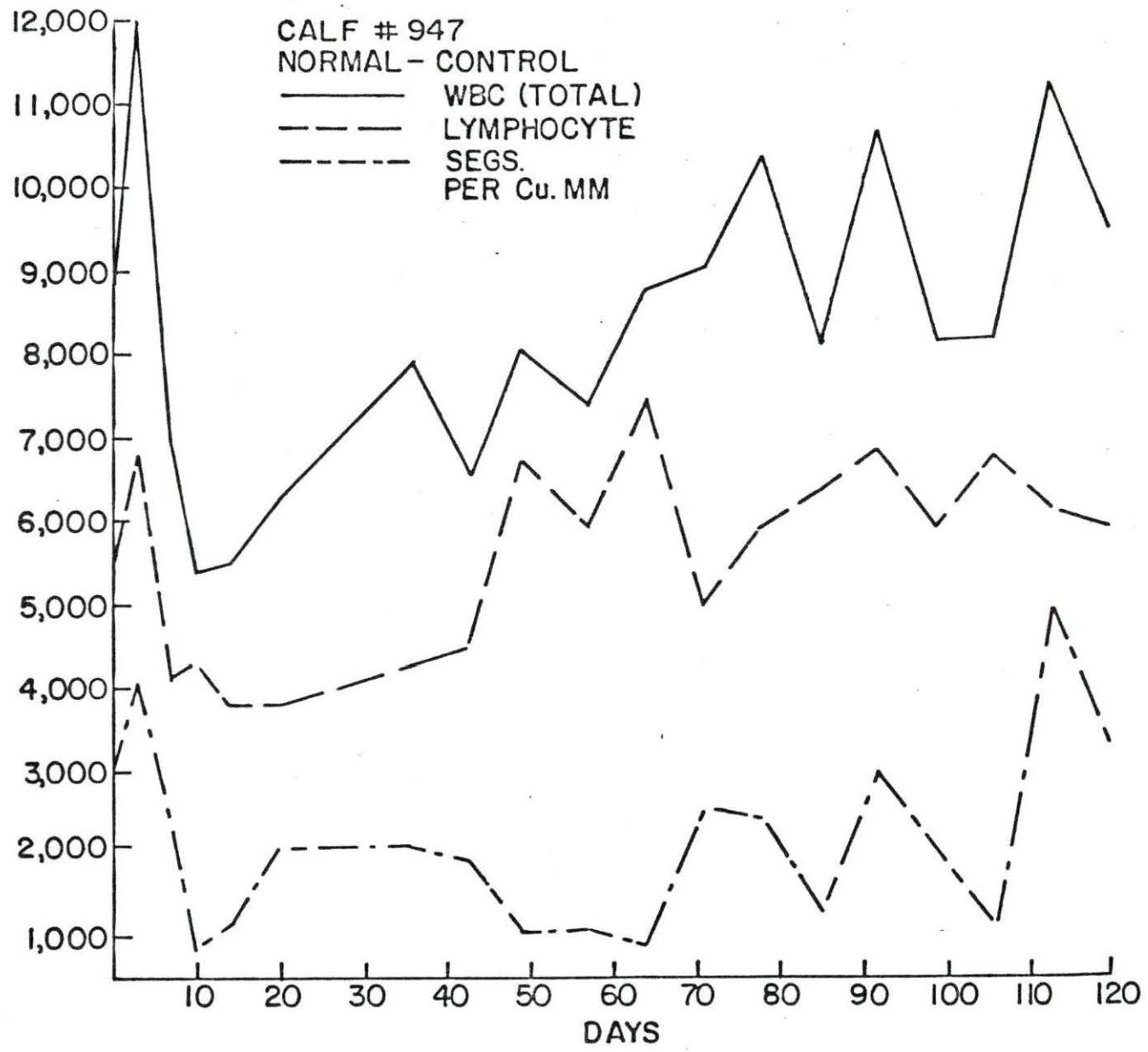
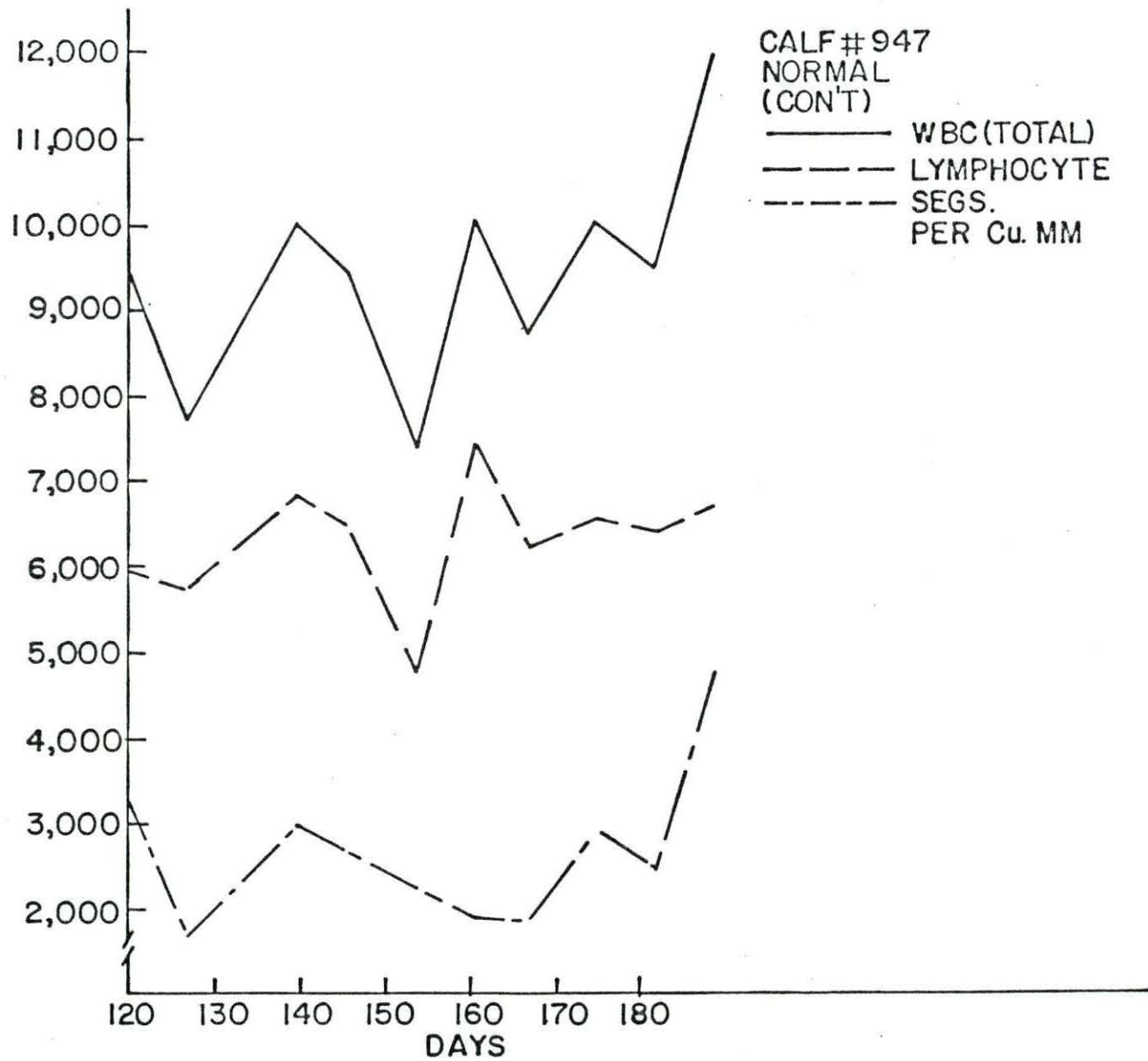


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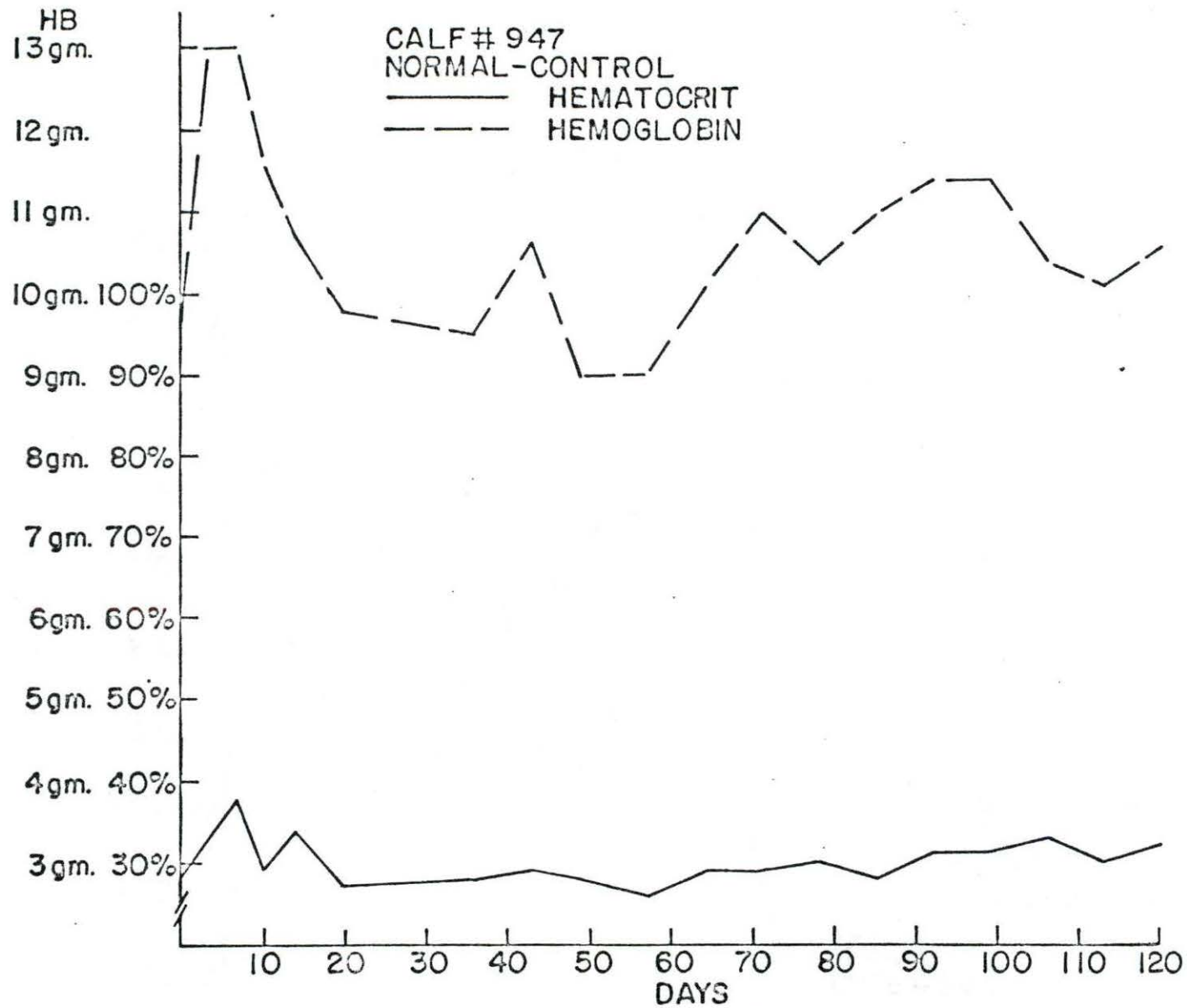
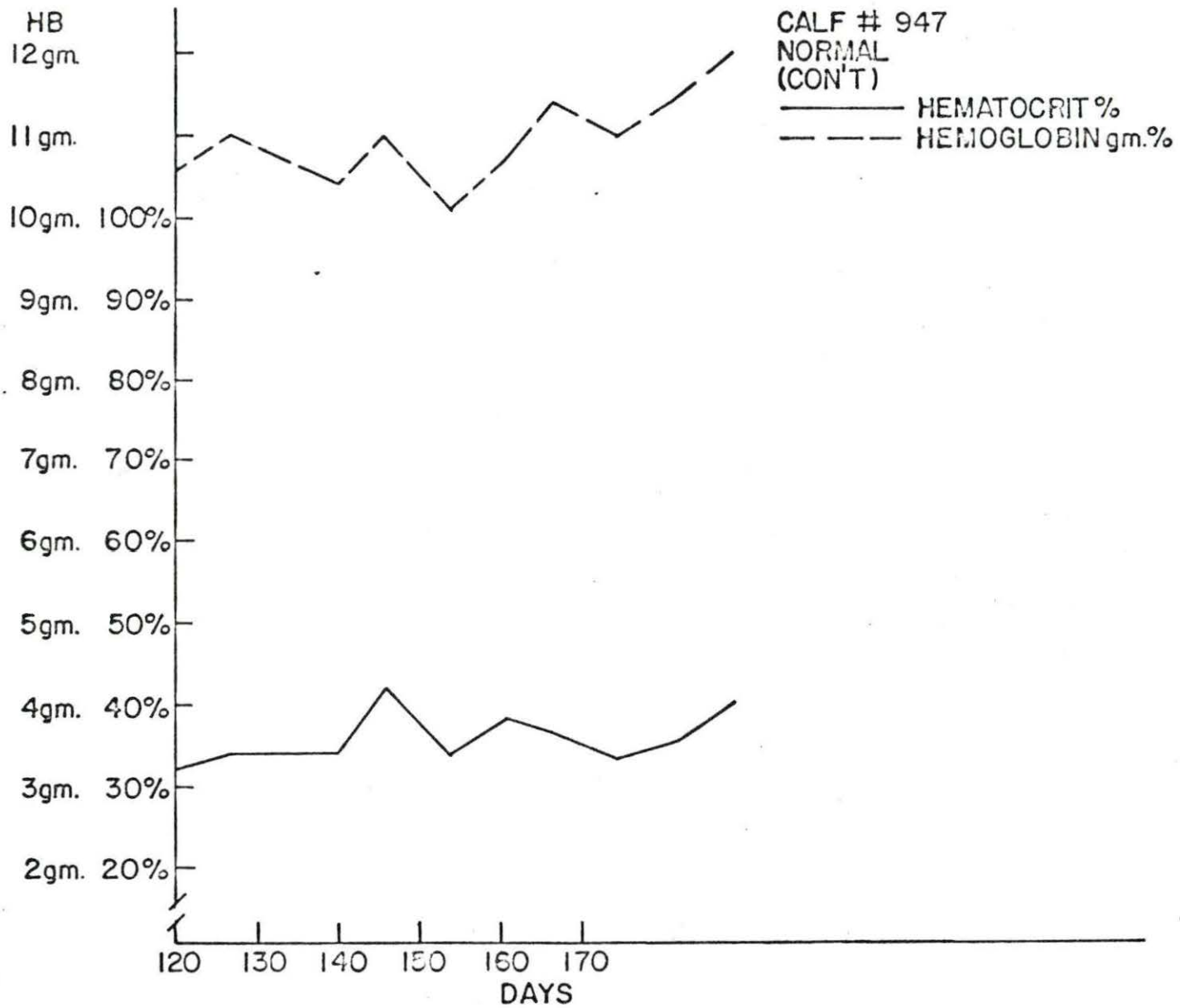


Figure 3. Calf 947. Hematocrit and hemoglobin

Figure 3. (Continued)



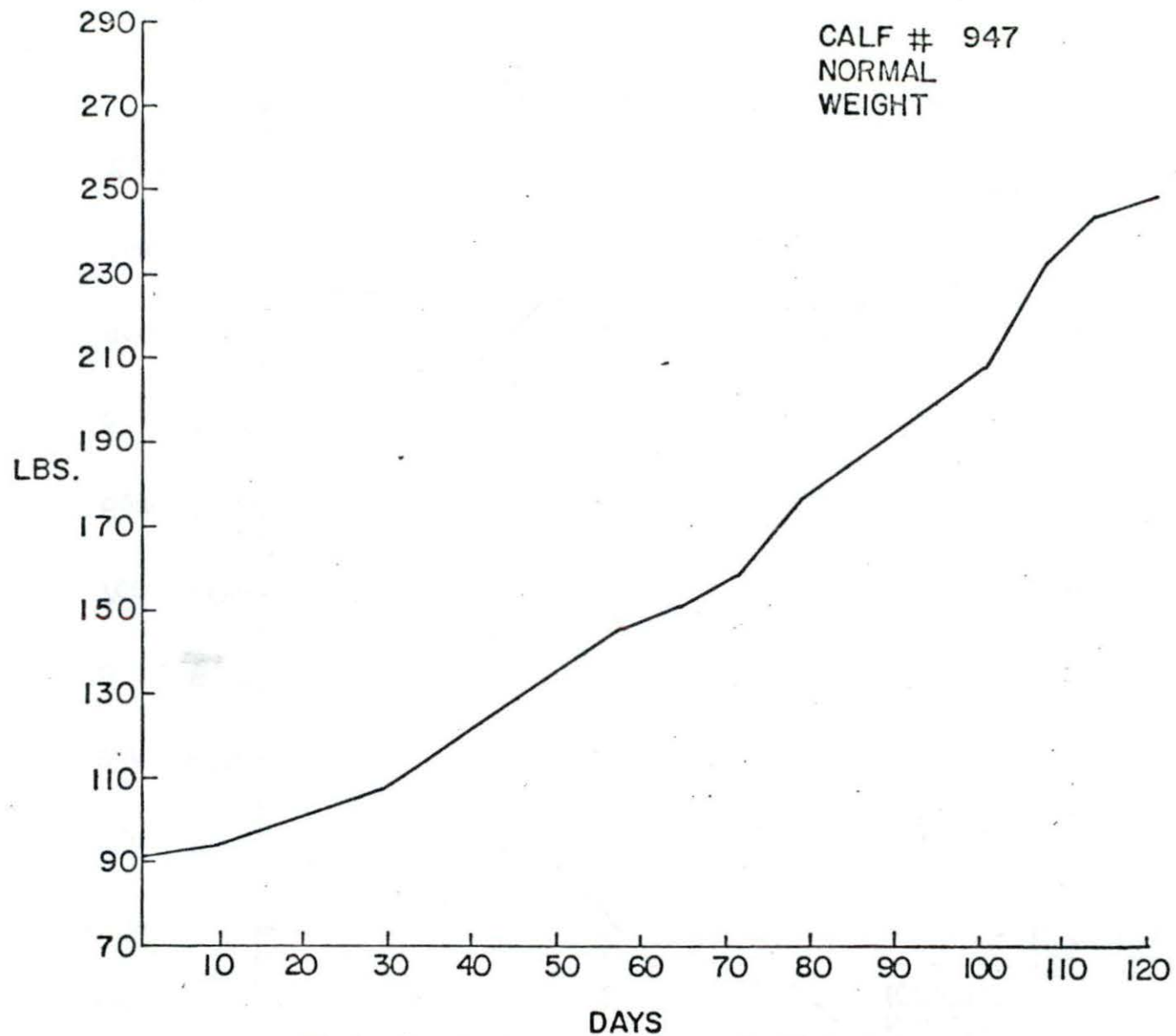


Figure 4. Calf 947. Weight and clinical data

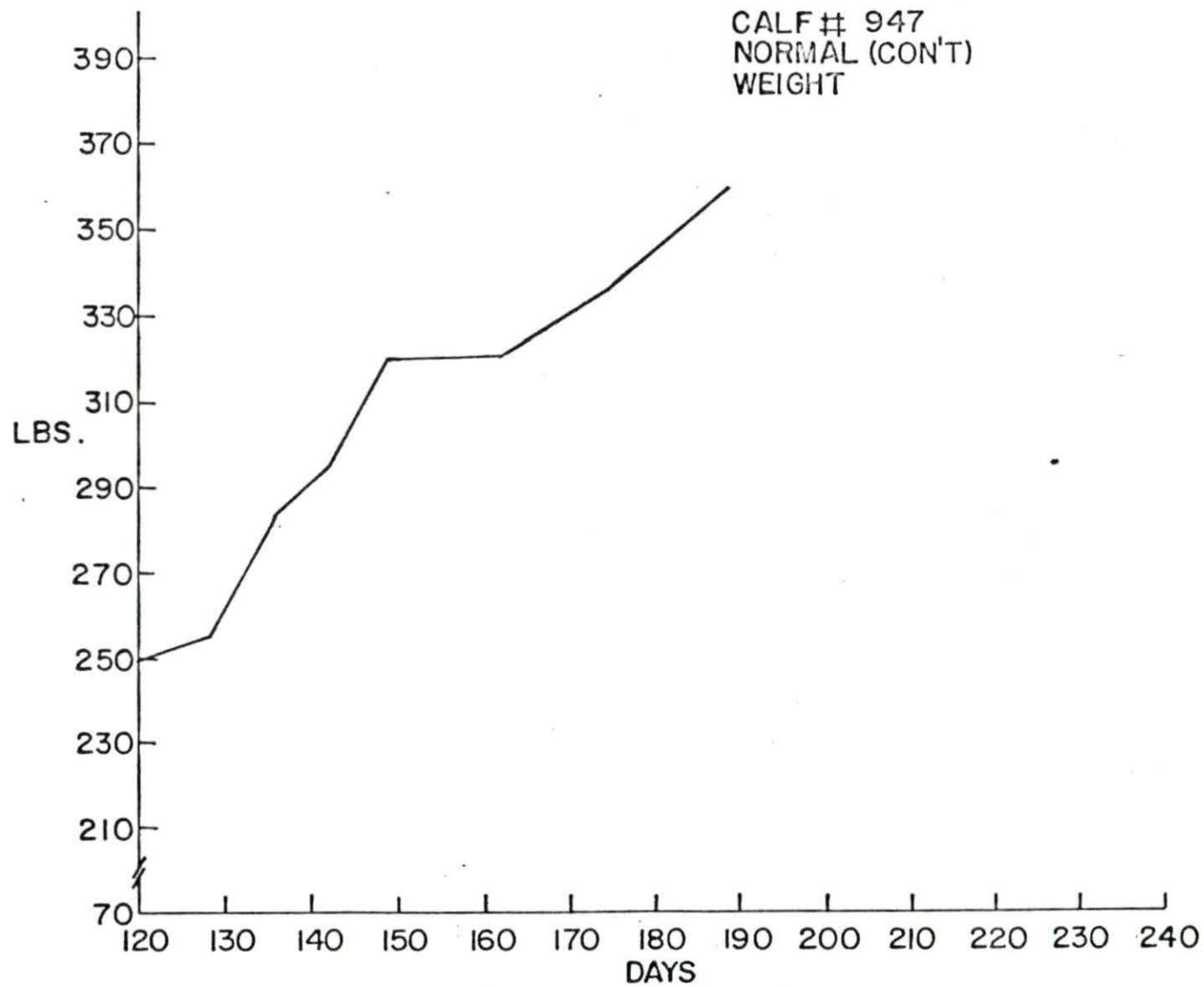


Figure 4. (Continued)

APPENDIX II. CALVES TREATED WITH MECHLORETHAMINE · HCl

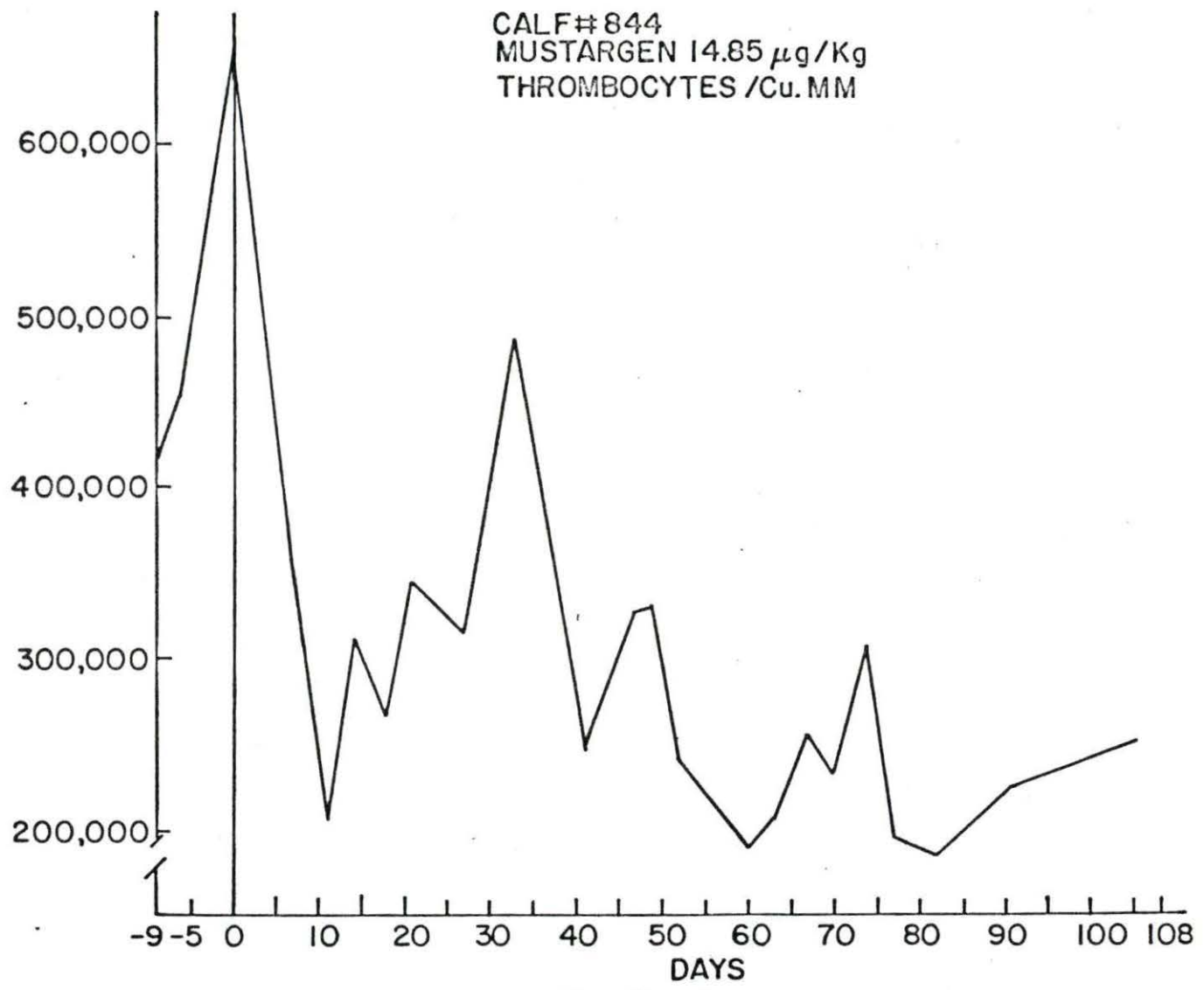


Figure 5. Calf 844. Thrombocyte counts

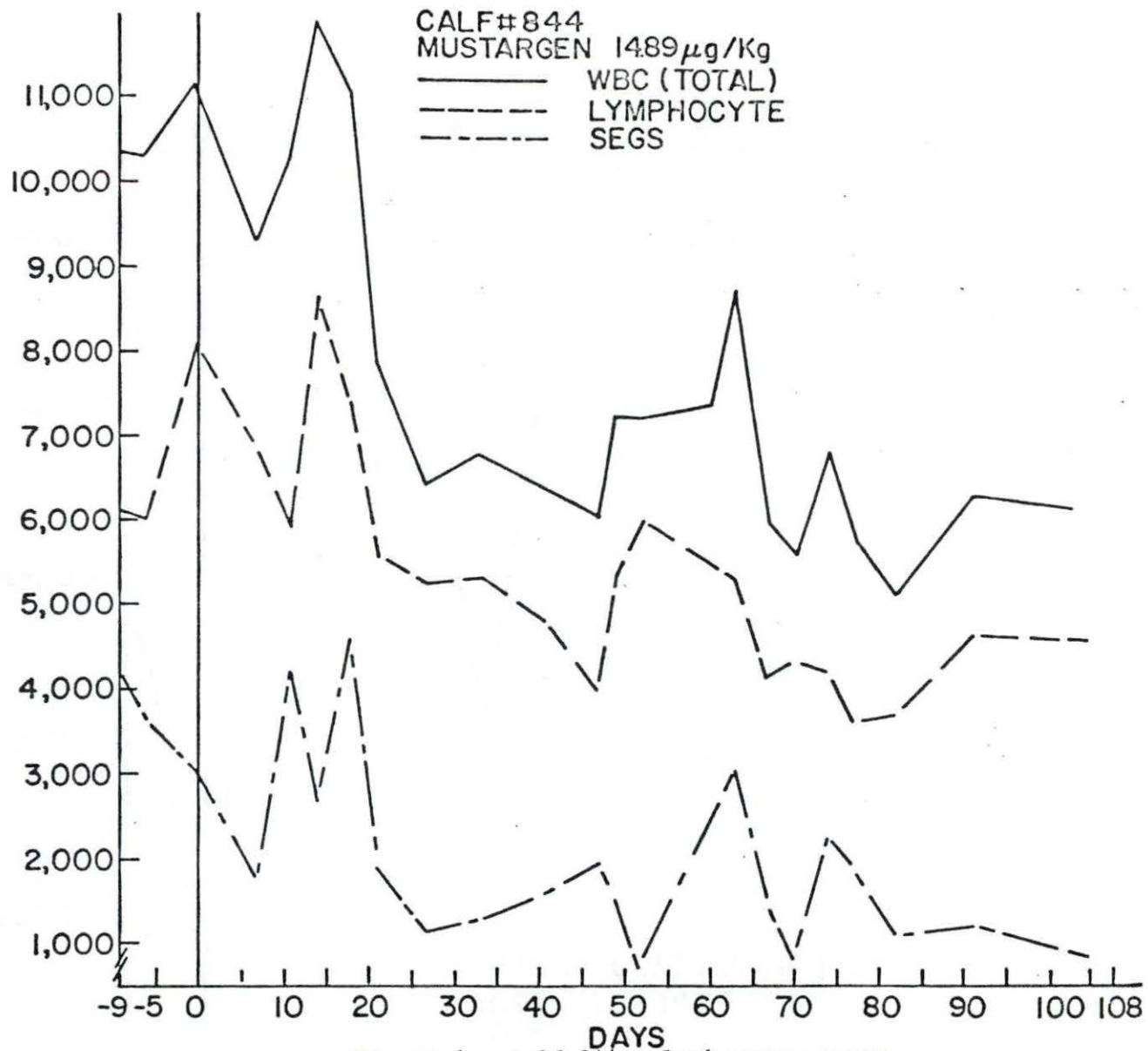


Figure 6. Calf 844. Leukocyte counts

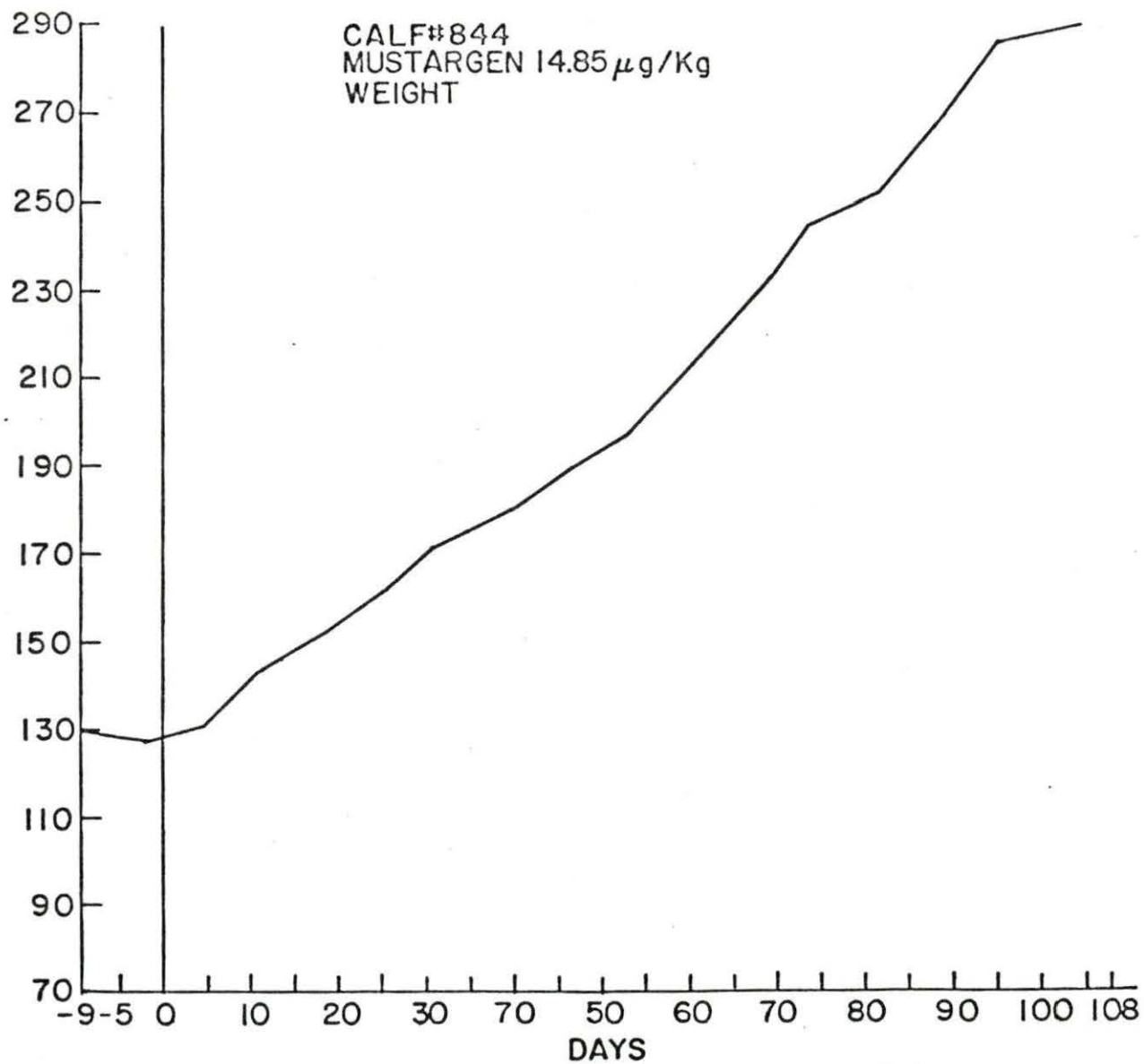


Figure 7. Calf 844. Weight and clinical data

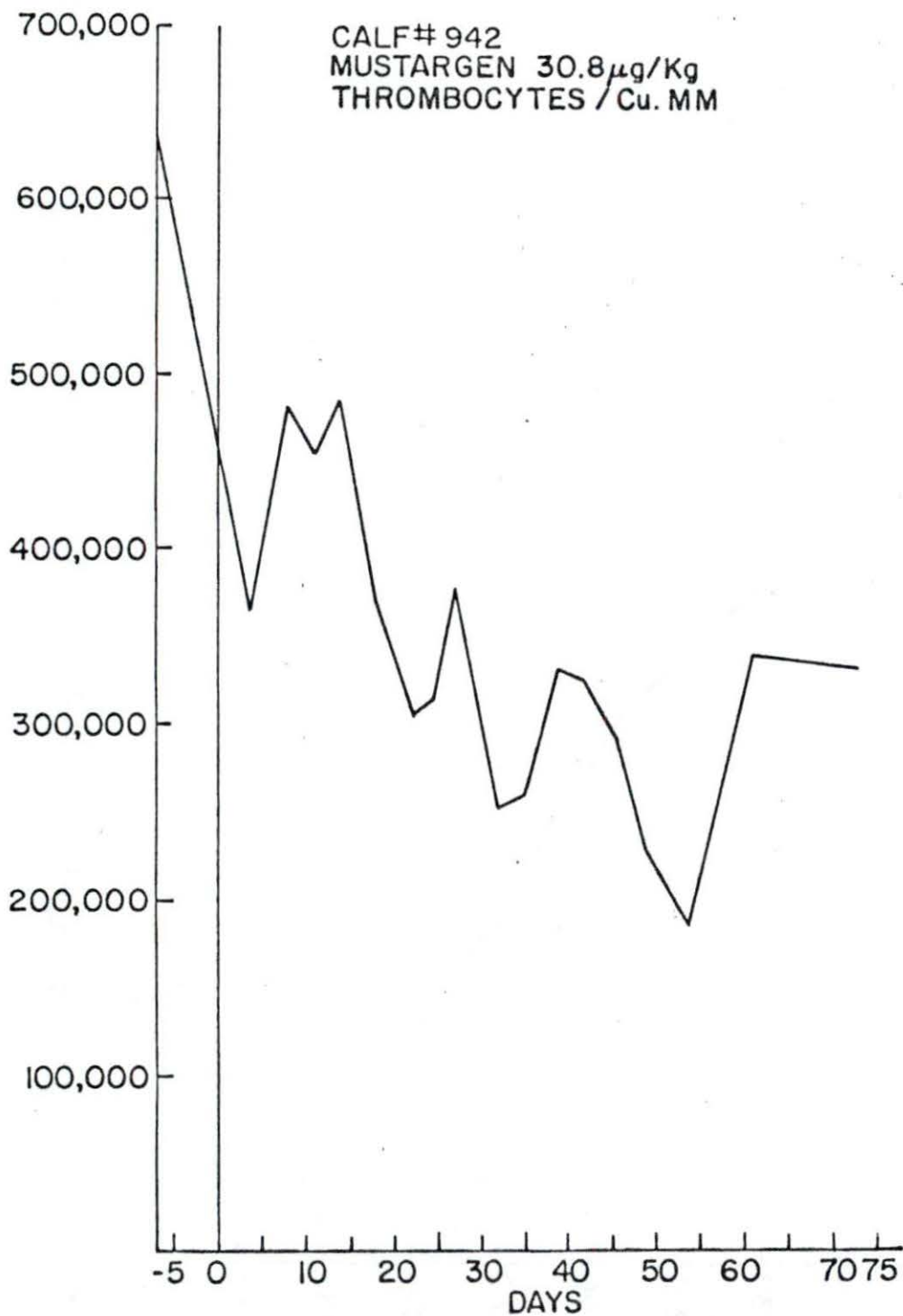


Figure 8. Calf 942. Thrombocyte counts

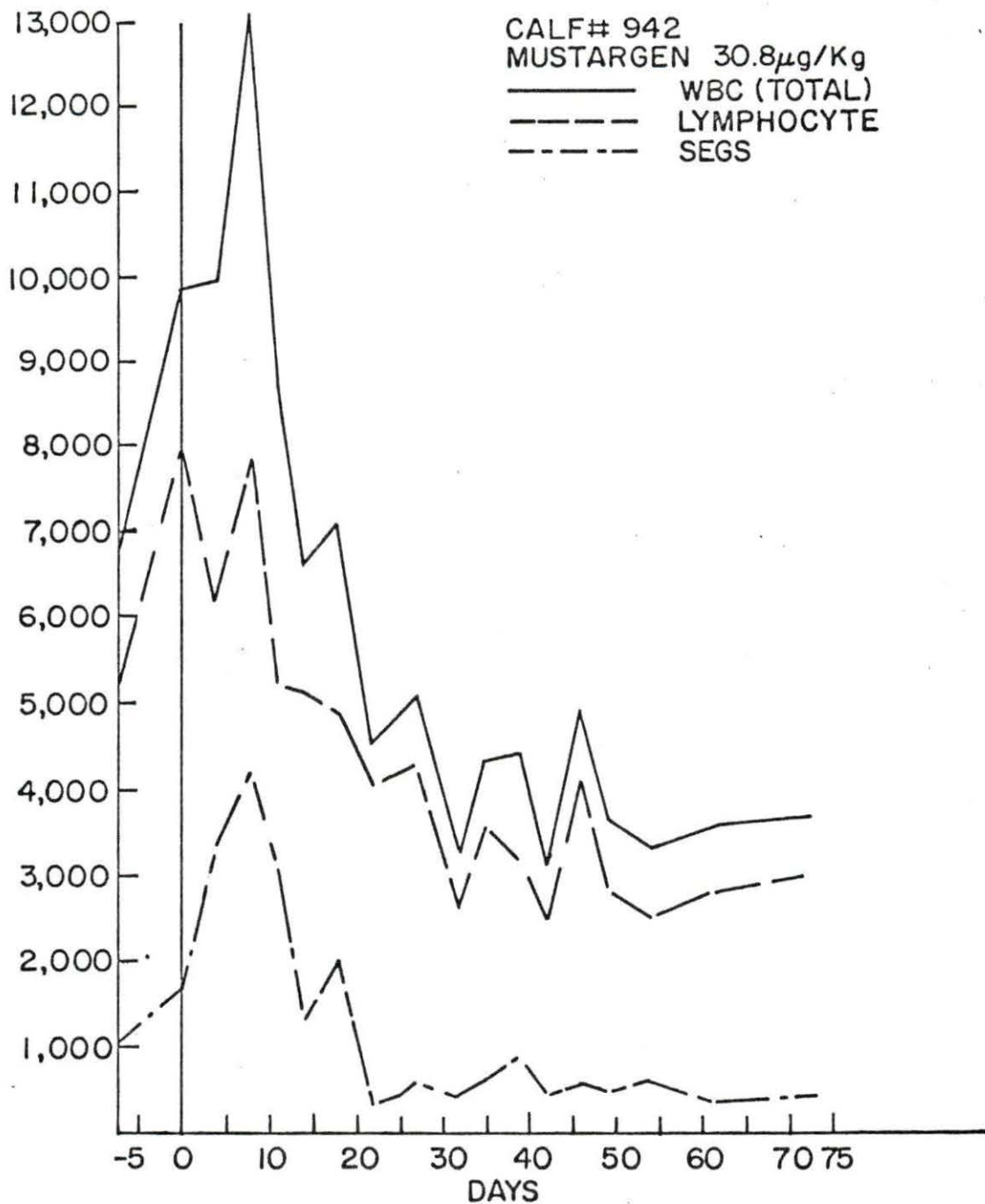


Figure 9. Calf 942. Leukocyte counts

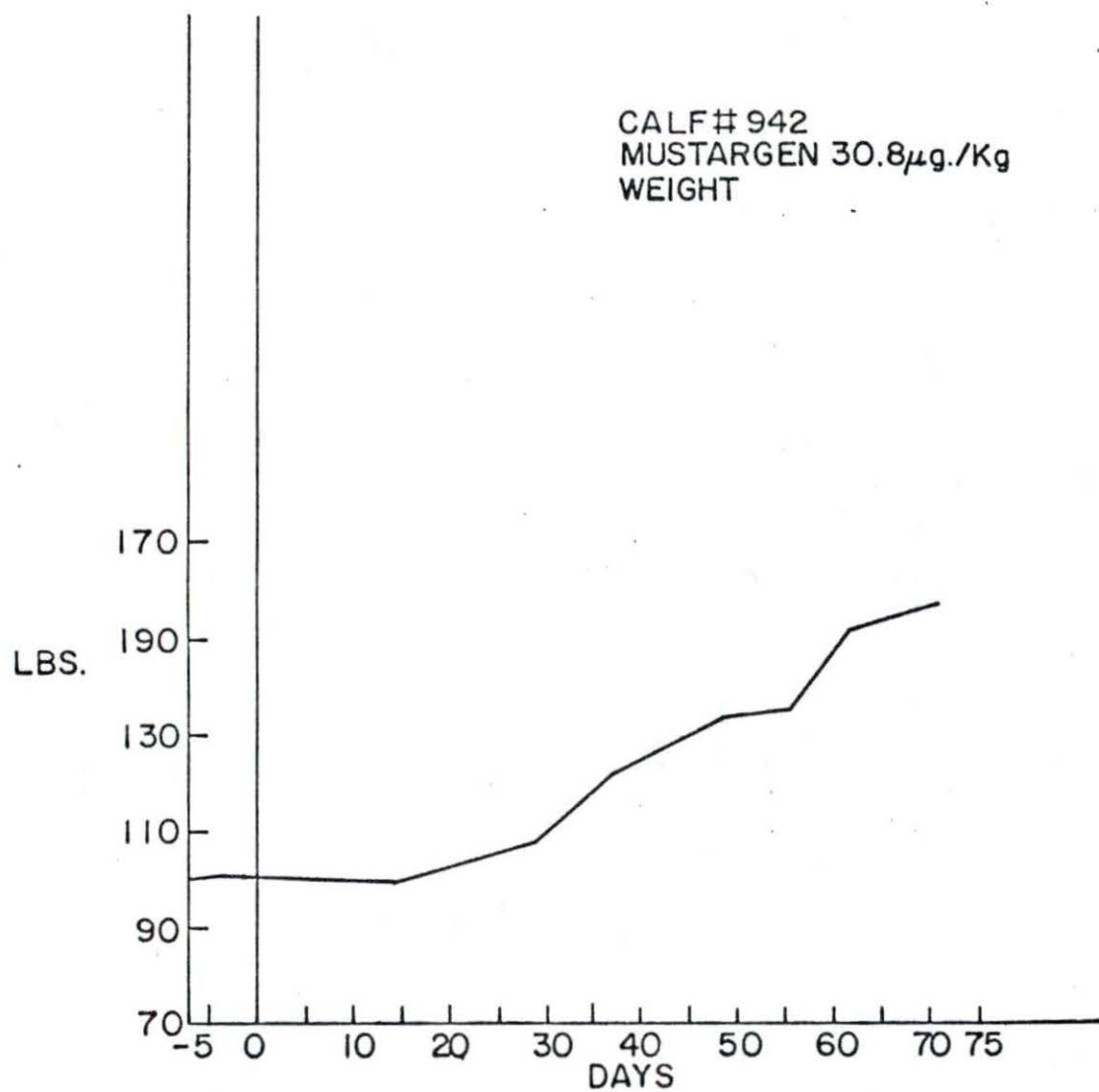


Figure 10. Calf 942. Weight and clinical data

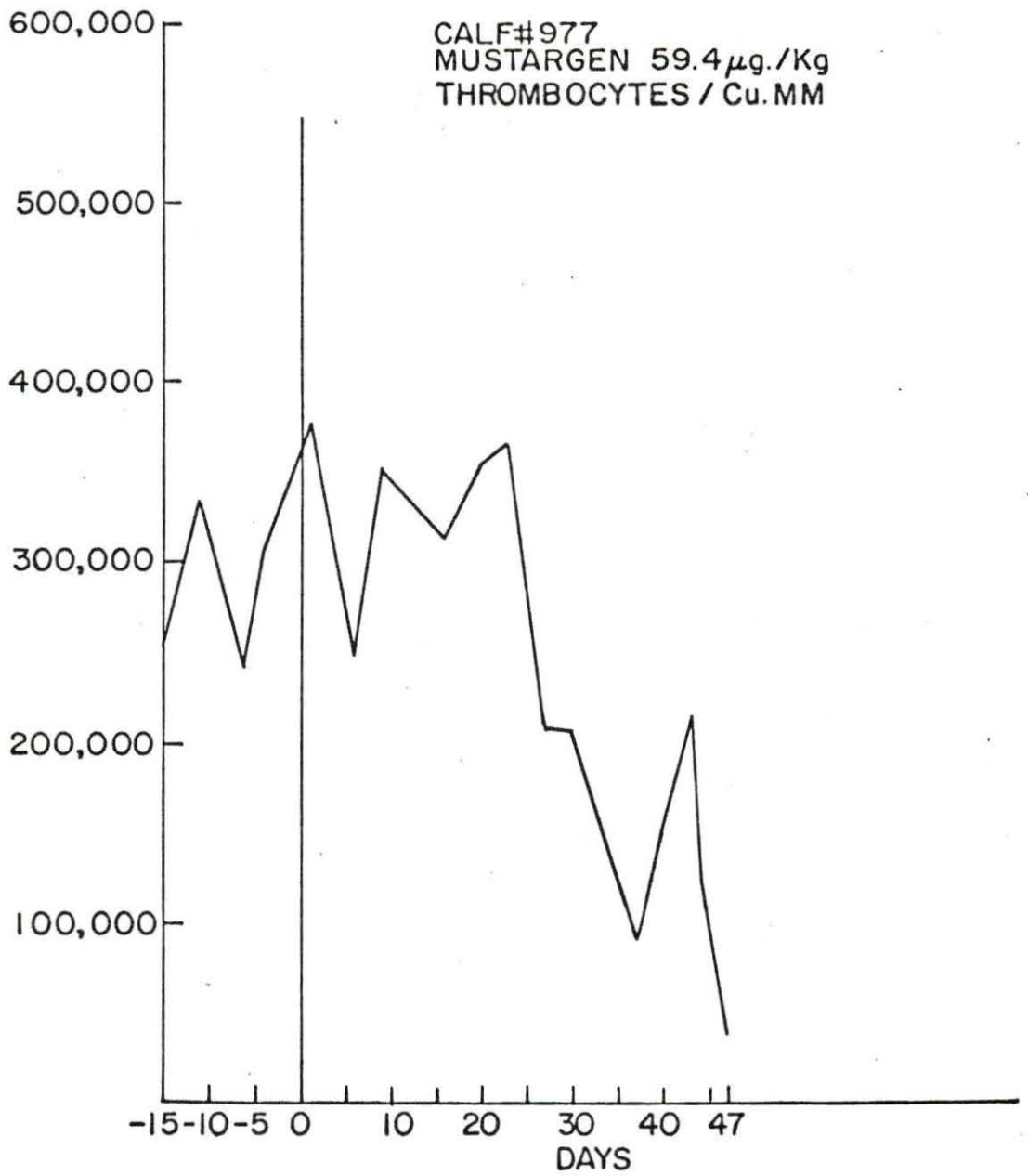


Figure 11. Calf 977. Thrombocyte counts

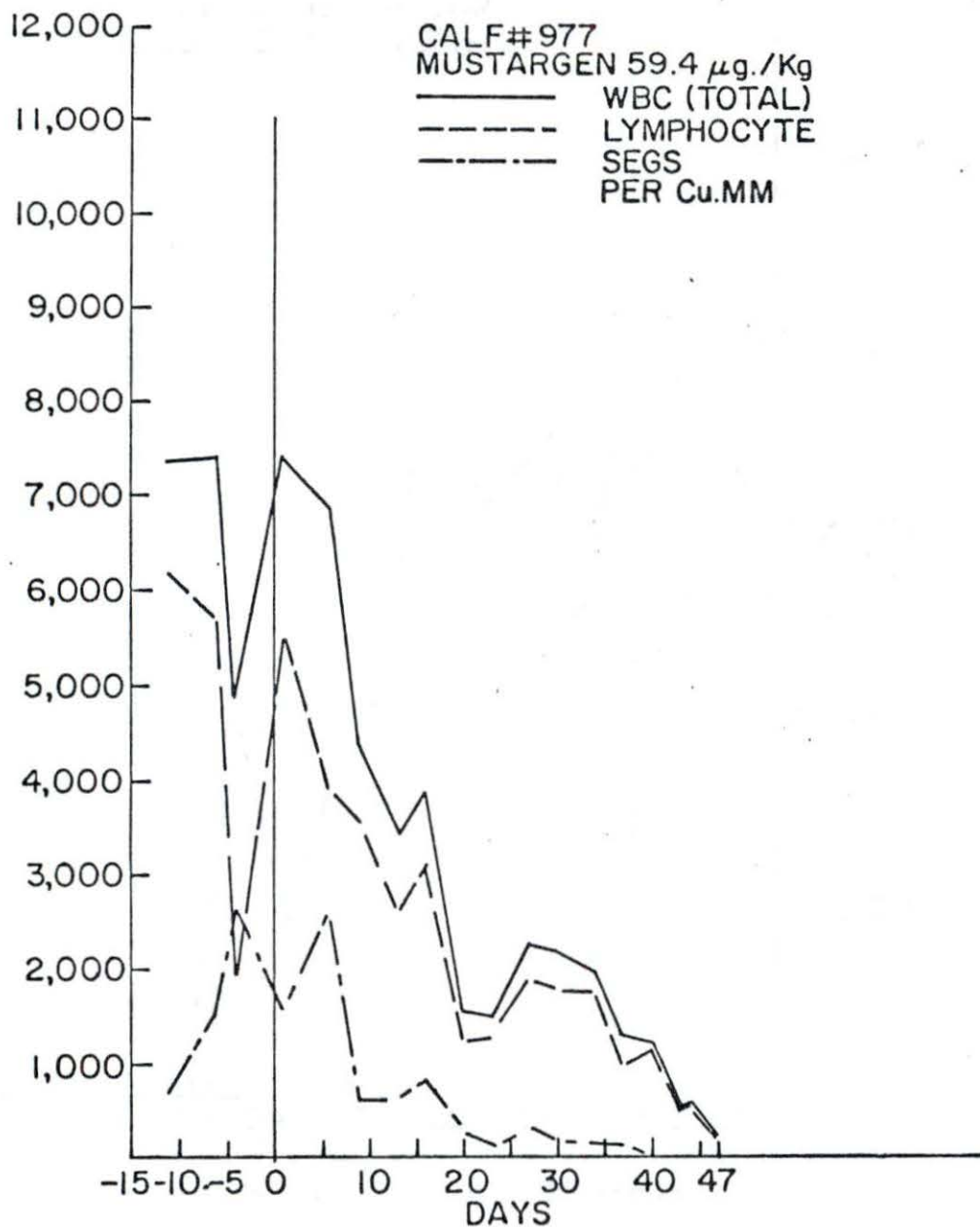


Figure 12. Calf 977. Leukocyte counts

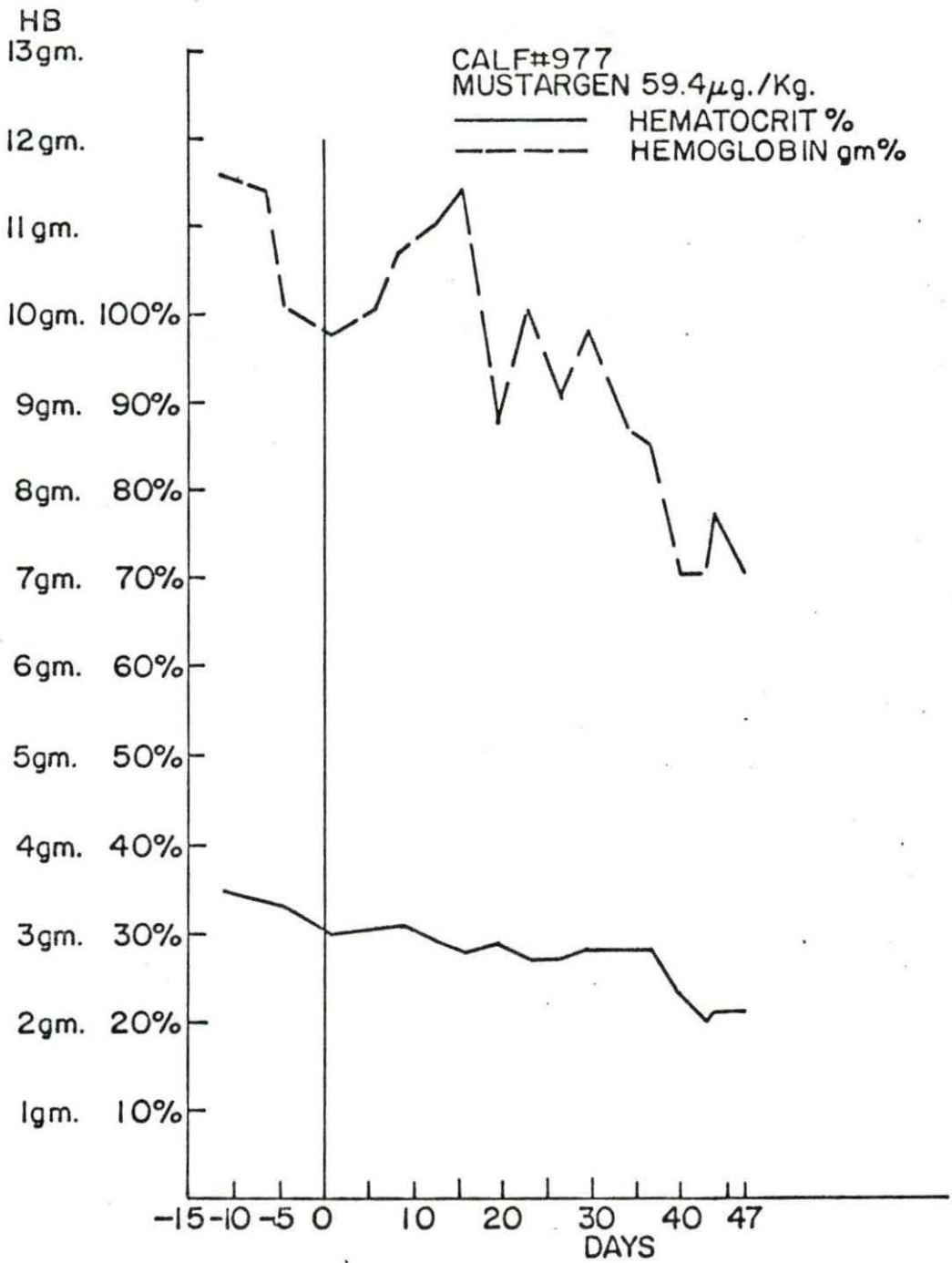


Figure 13. Calf 977. Hematocrit and hemoglobin

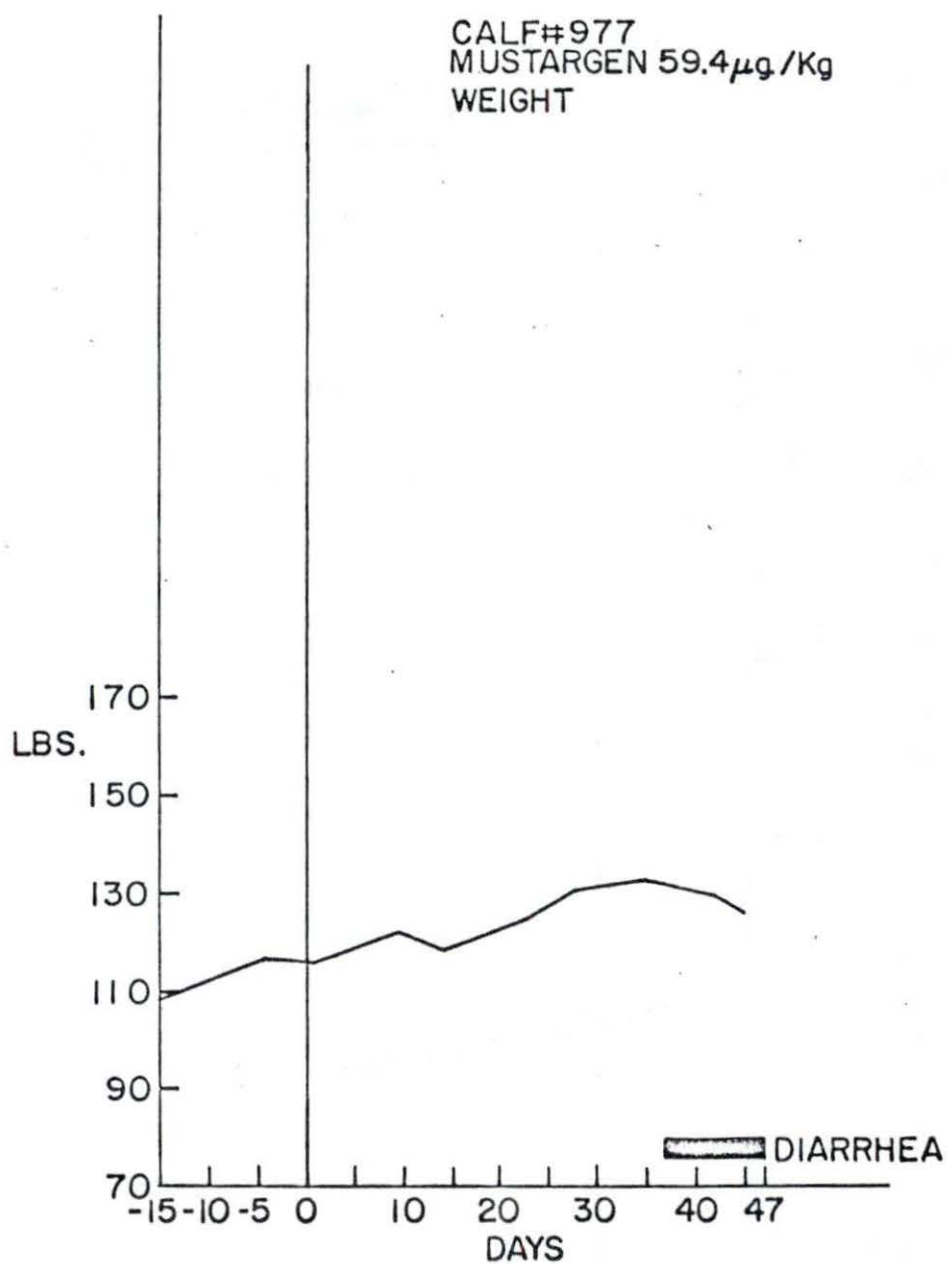


Figure 14. Calf 977. Weight and clinical data

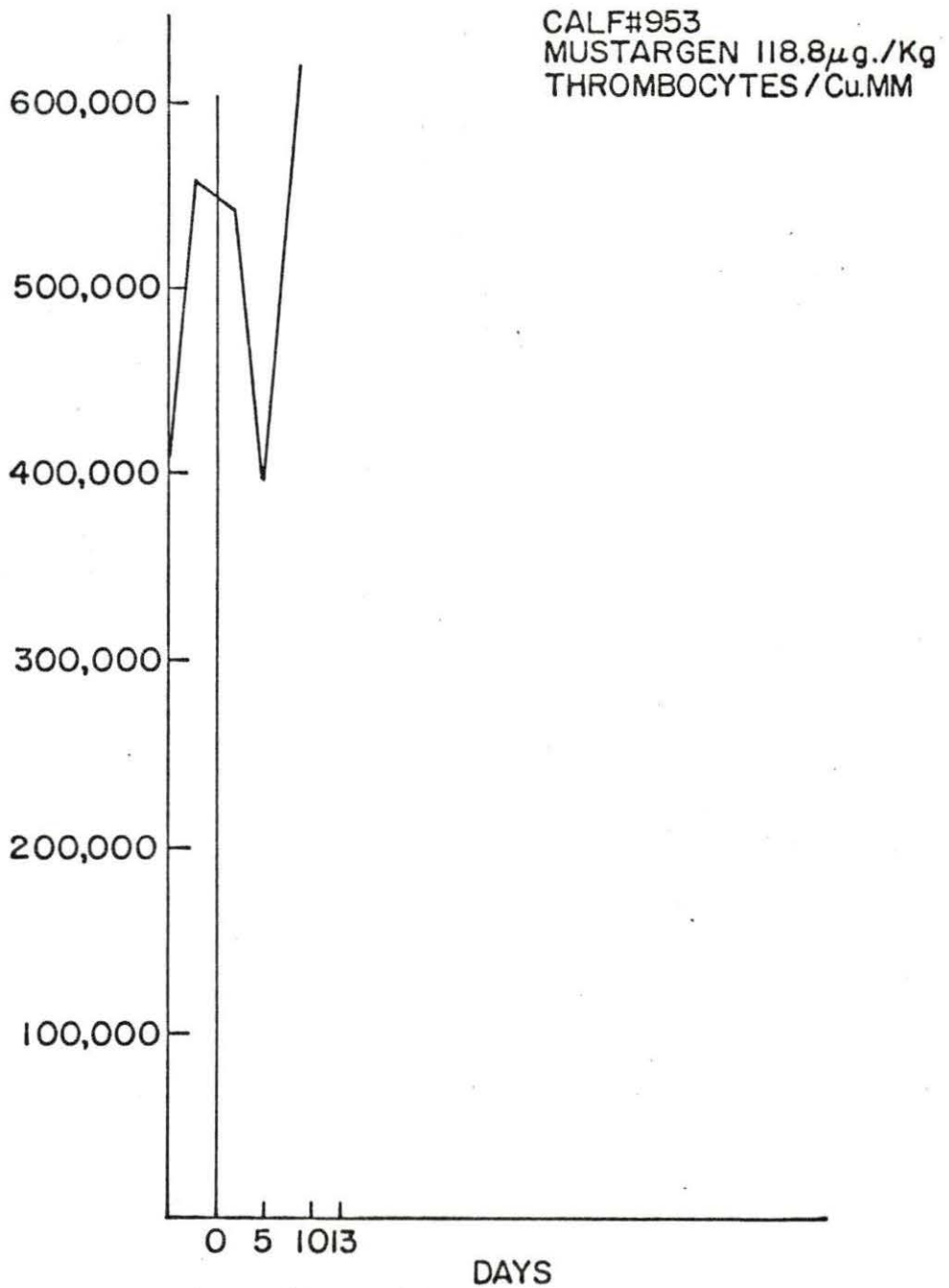


Figure 15. Calf 953. Thrombocyte counts

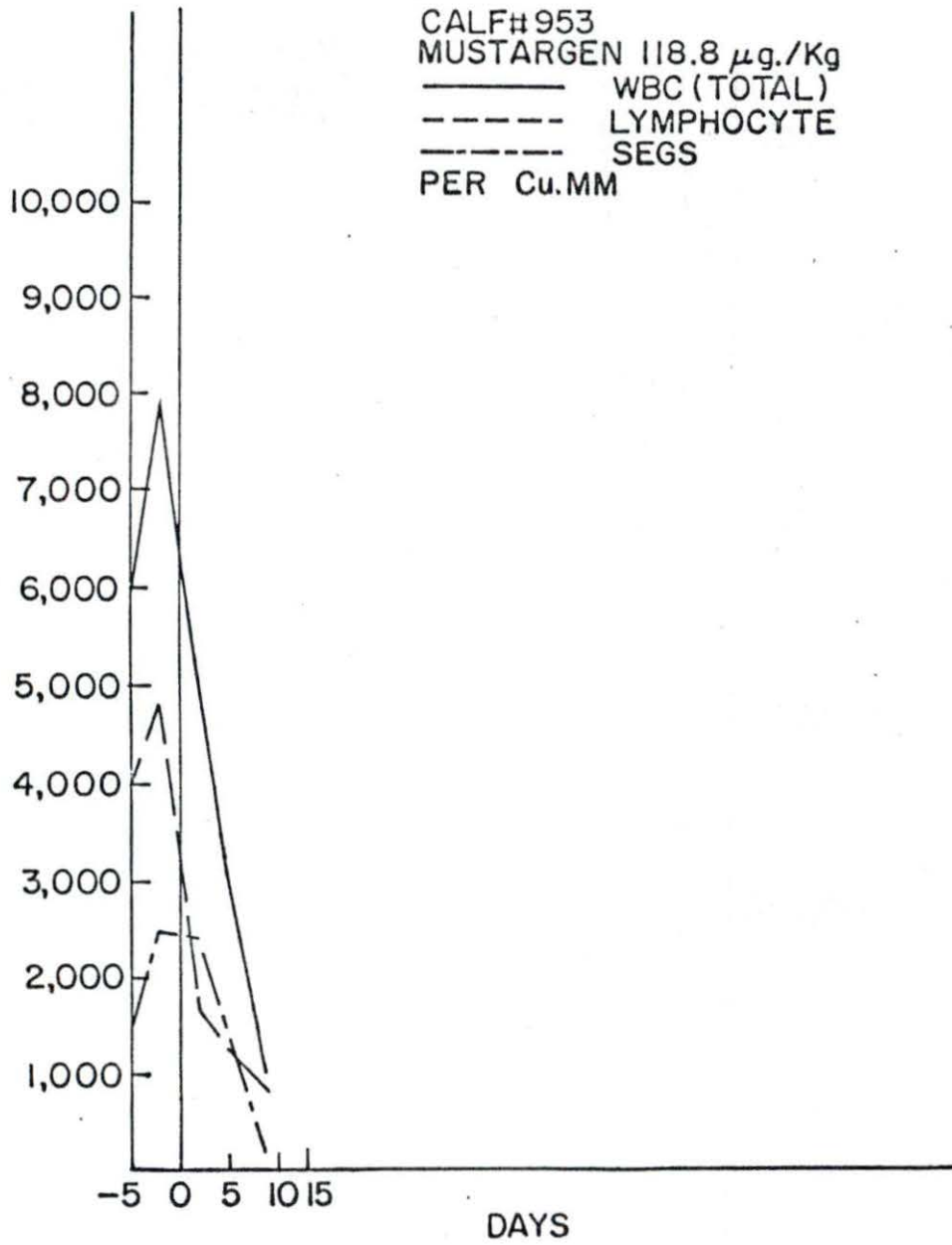


Figure 16. Calf 953. Leukocyte counts

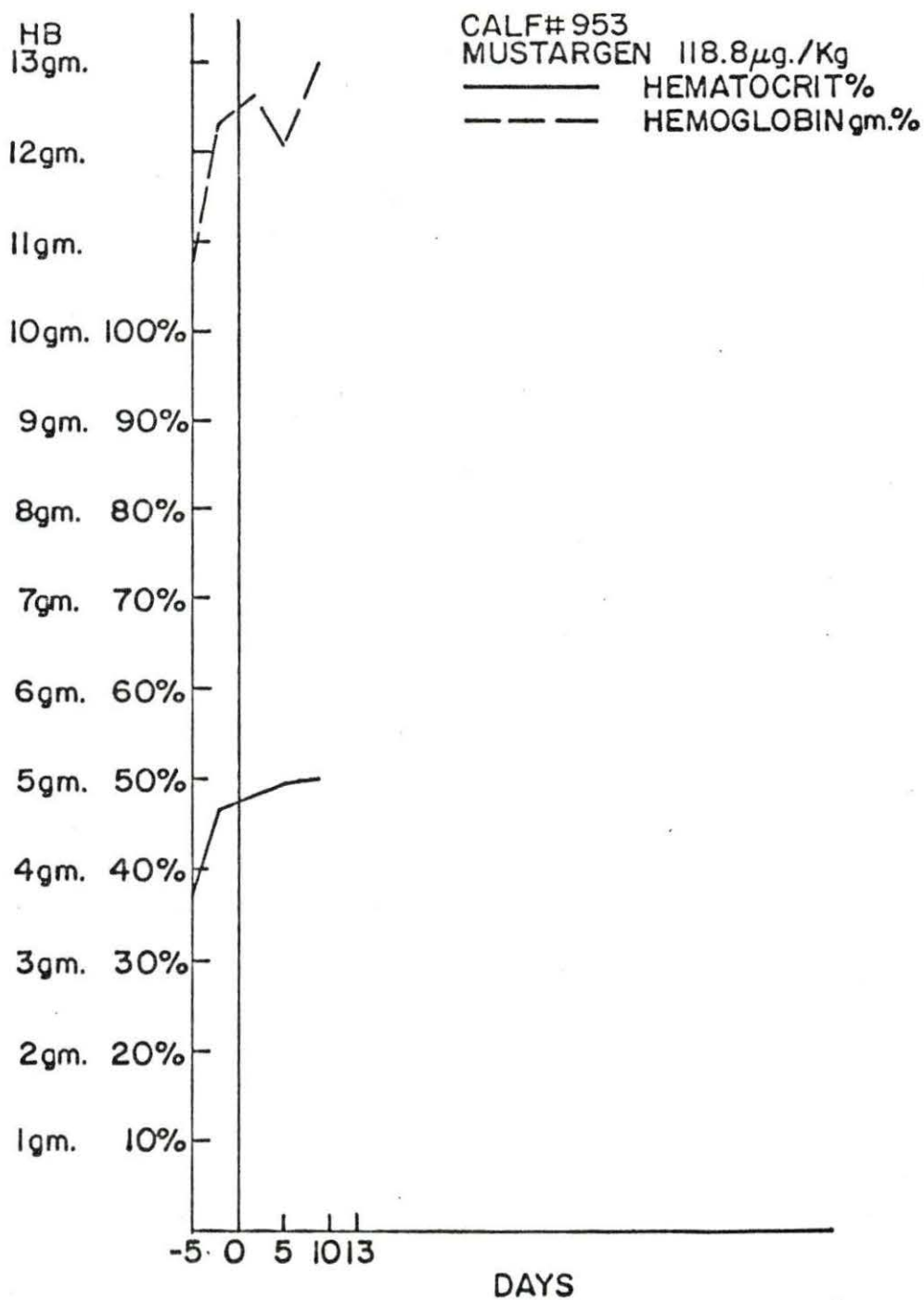


Figure 17. Calf 953. Hematocrit and hemoglobin

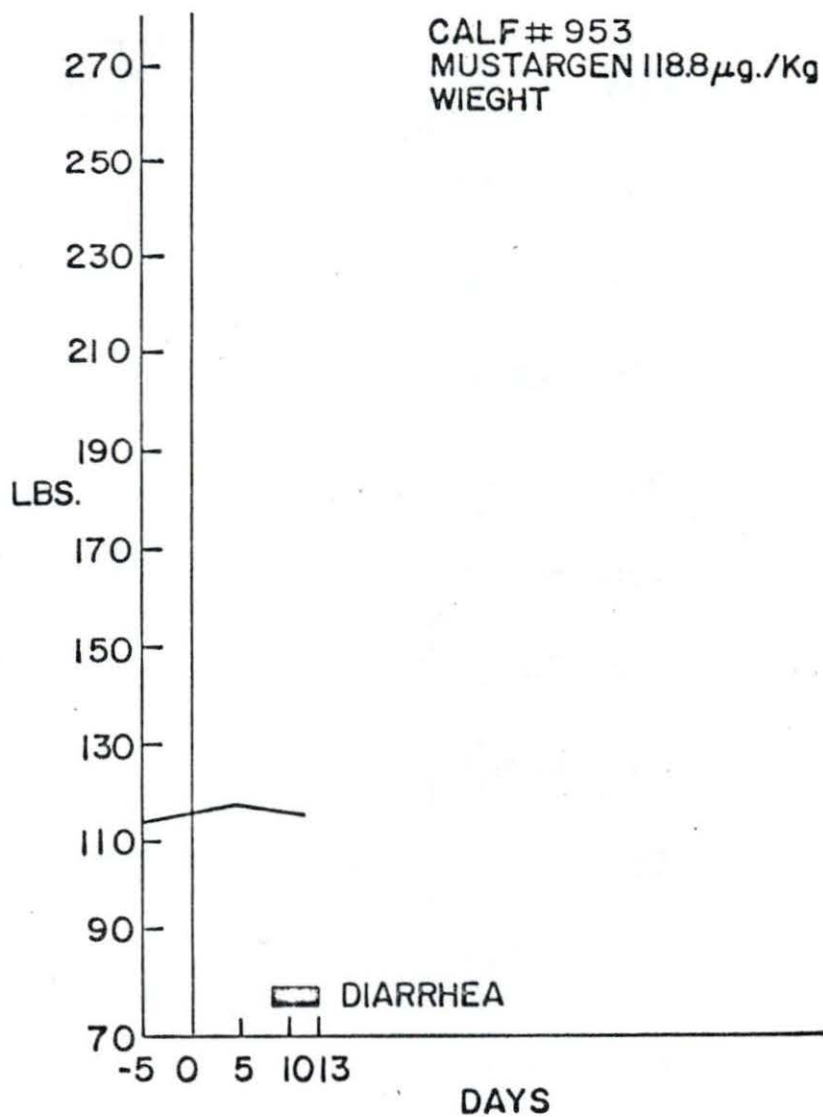


Figure 18. Calf 953. Weight and clinical data

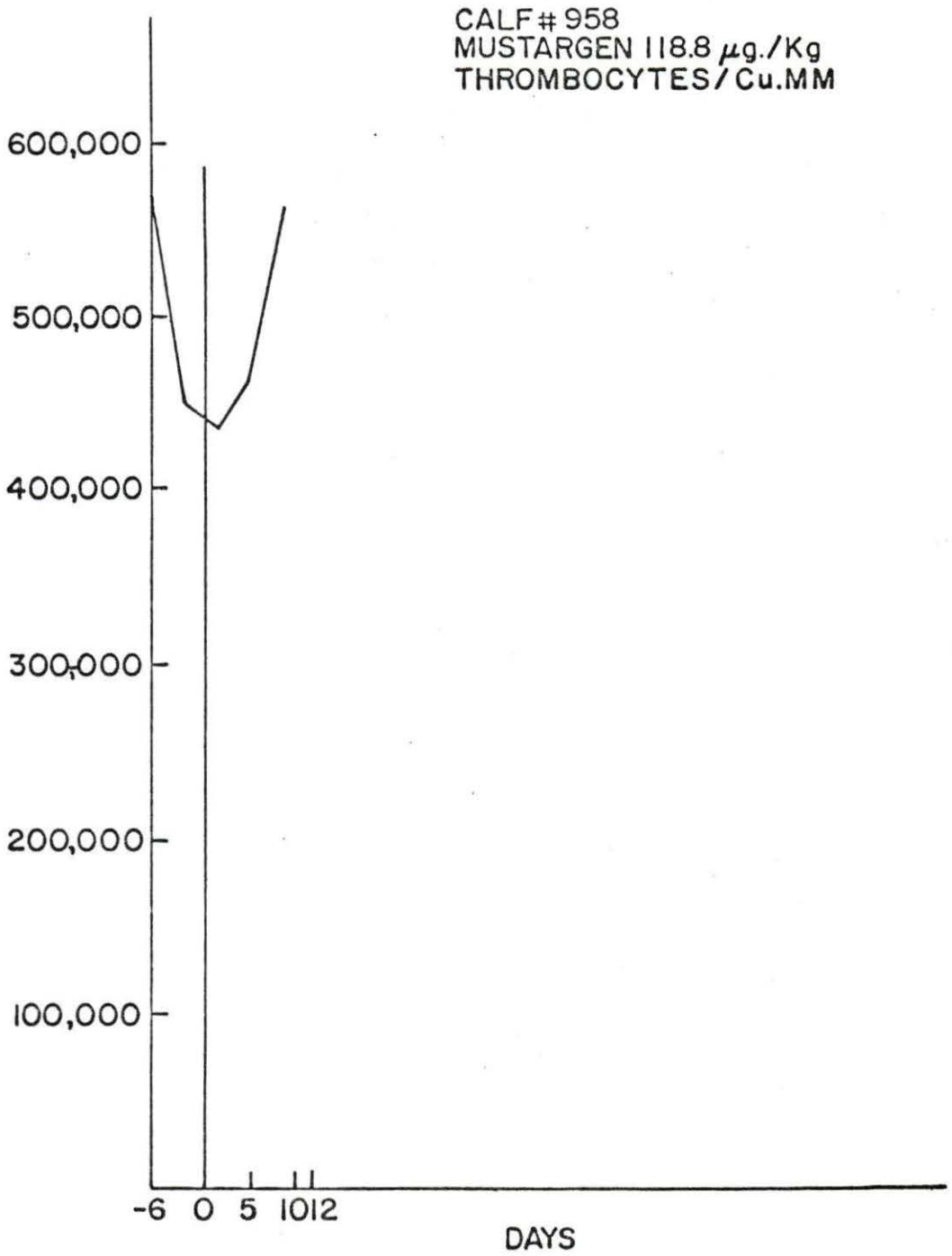


Figure 19. Calf 958. Thrombocyte counts

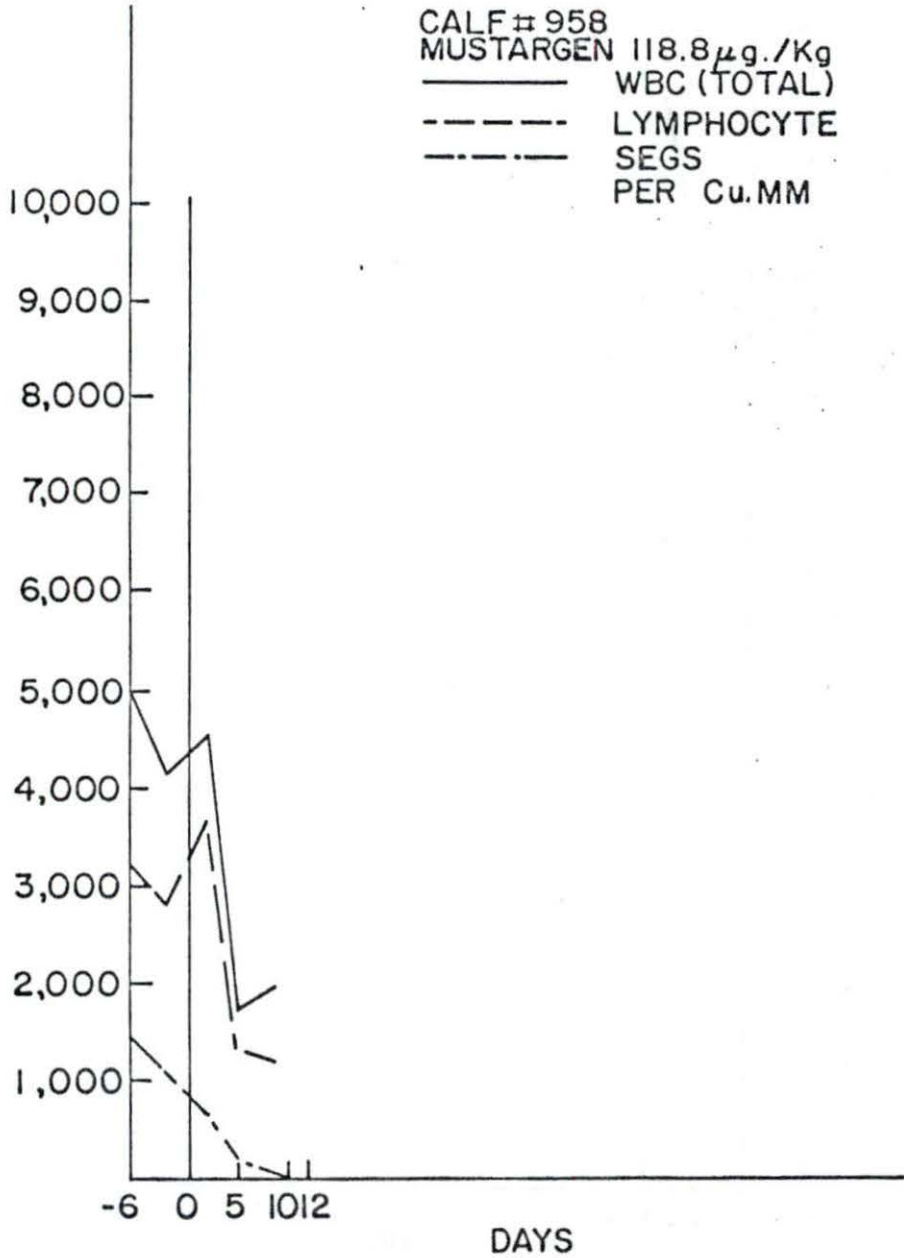


Figure 20. Calf 958. Leukocyte counts

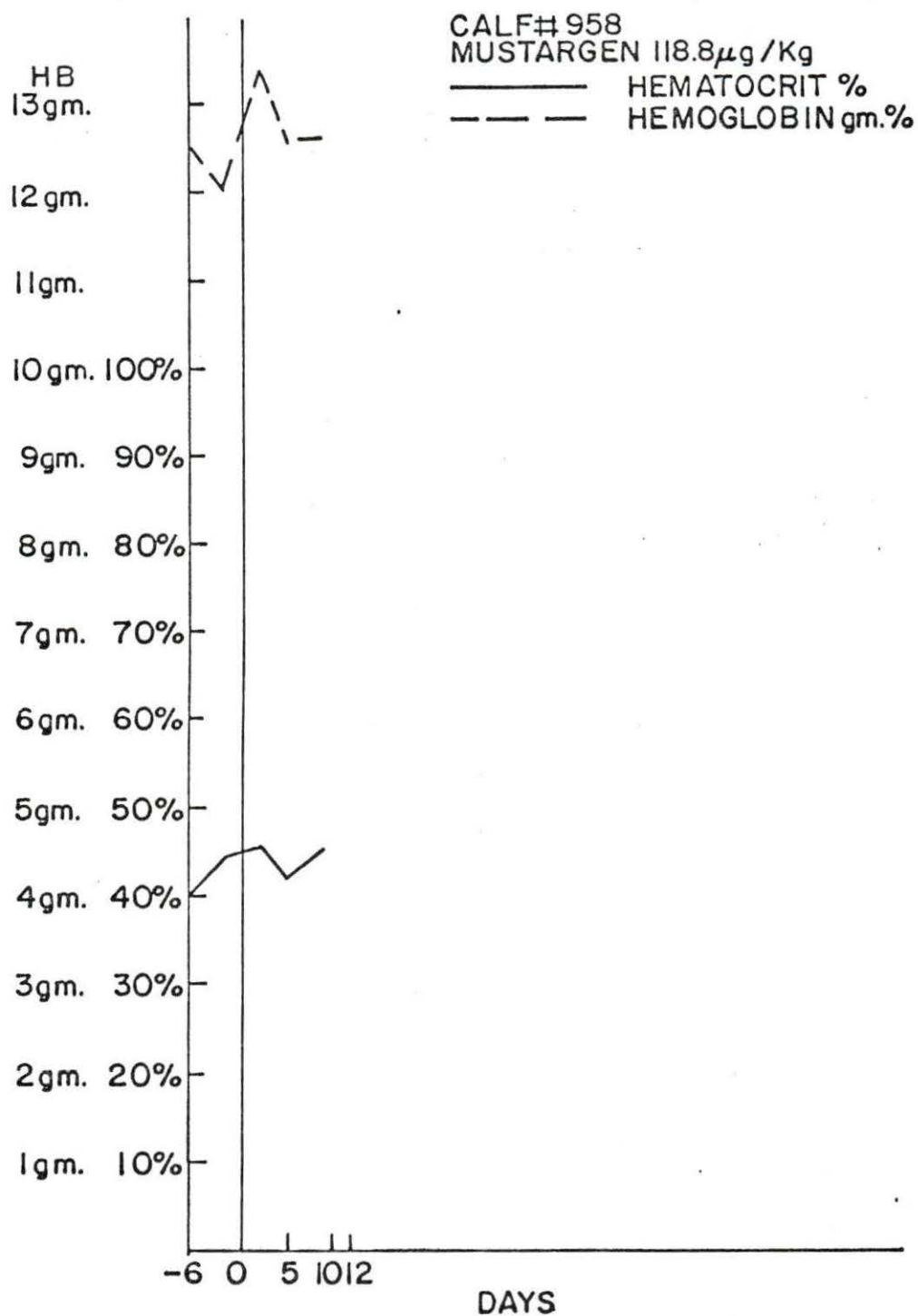


Figure 21. Calf 958. Hematocrit and hemoglobin

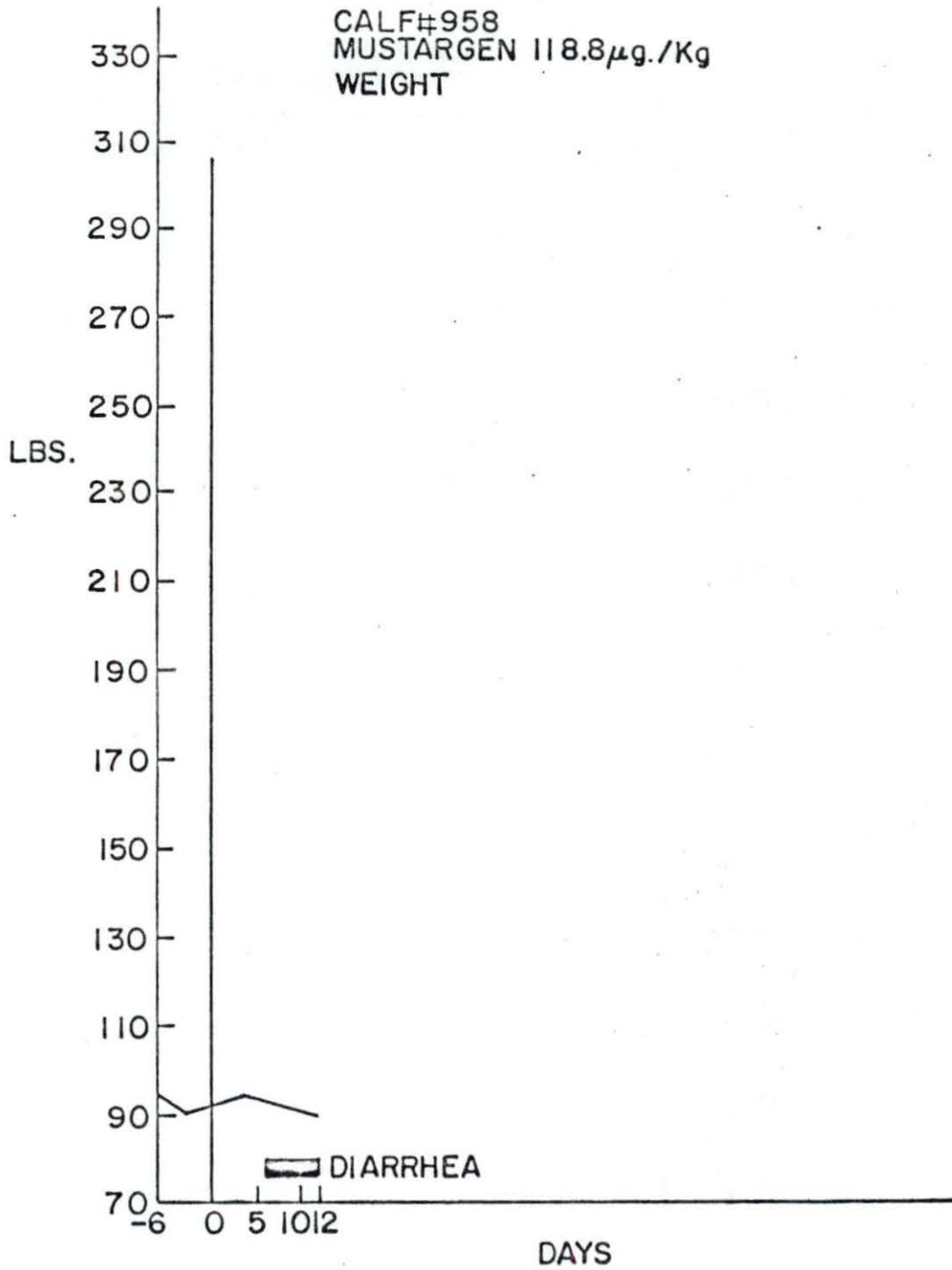


Figure 22. Calf 958. Weight and clinical data

APPENDIX III. CALF TREATED WITH DIMETHYLACETAMIDE

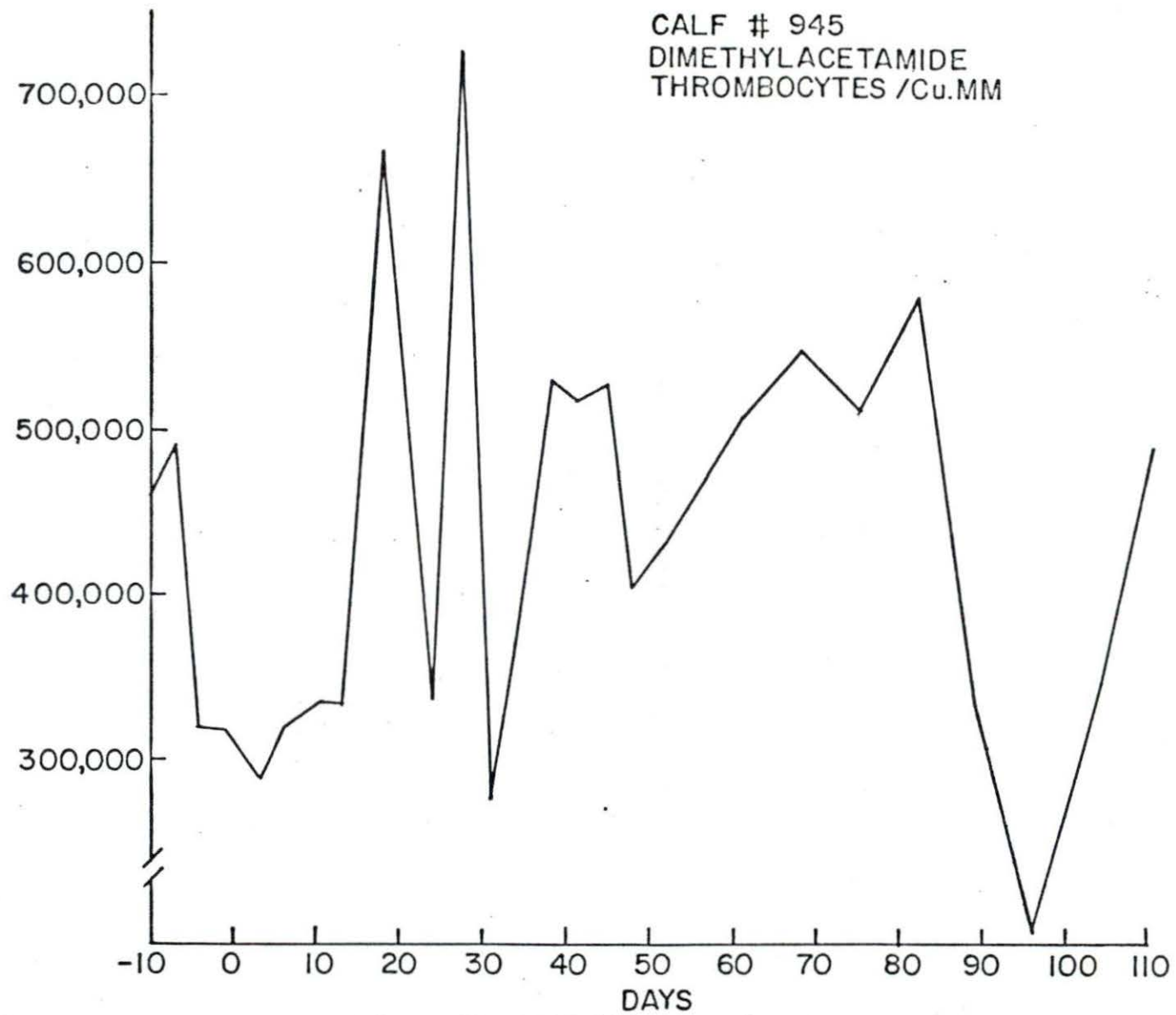


Figure 23. Calf 945. Thrombocyte counts

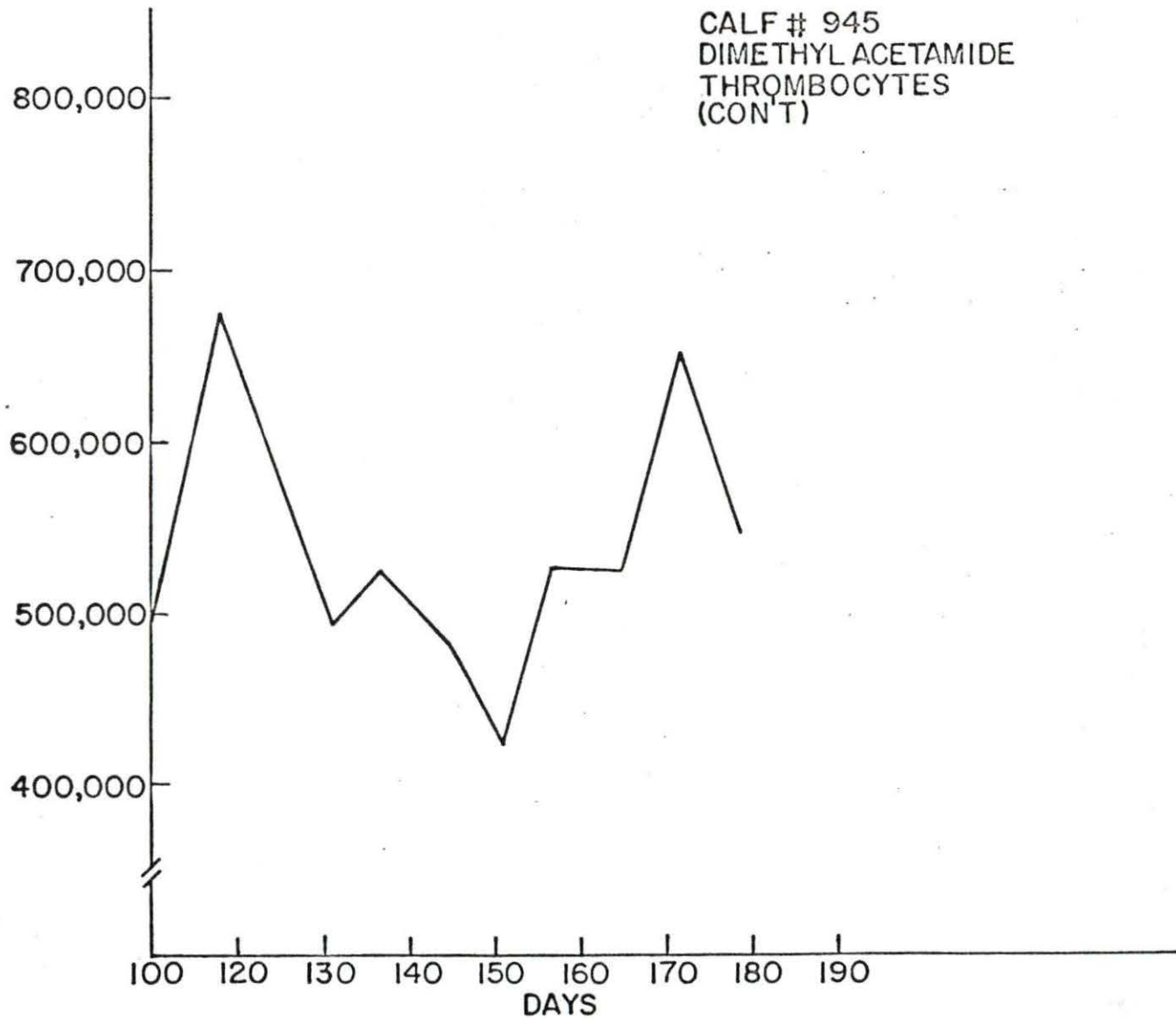


Figure 23. (Continued)

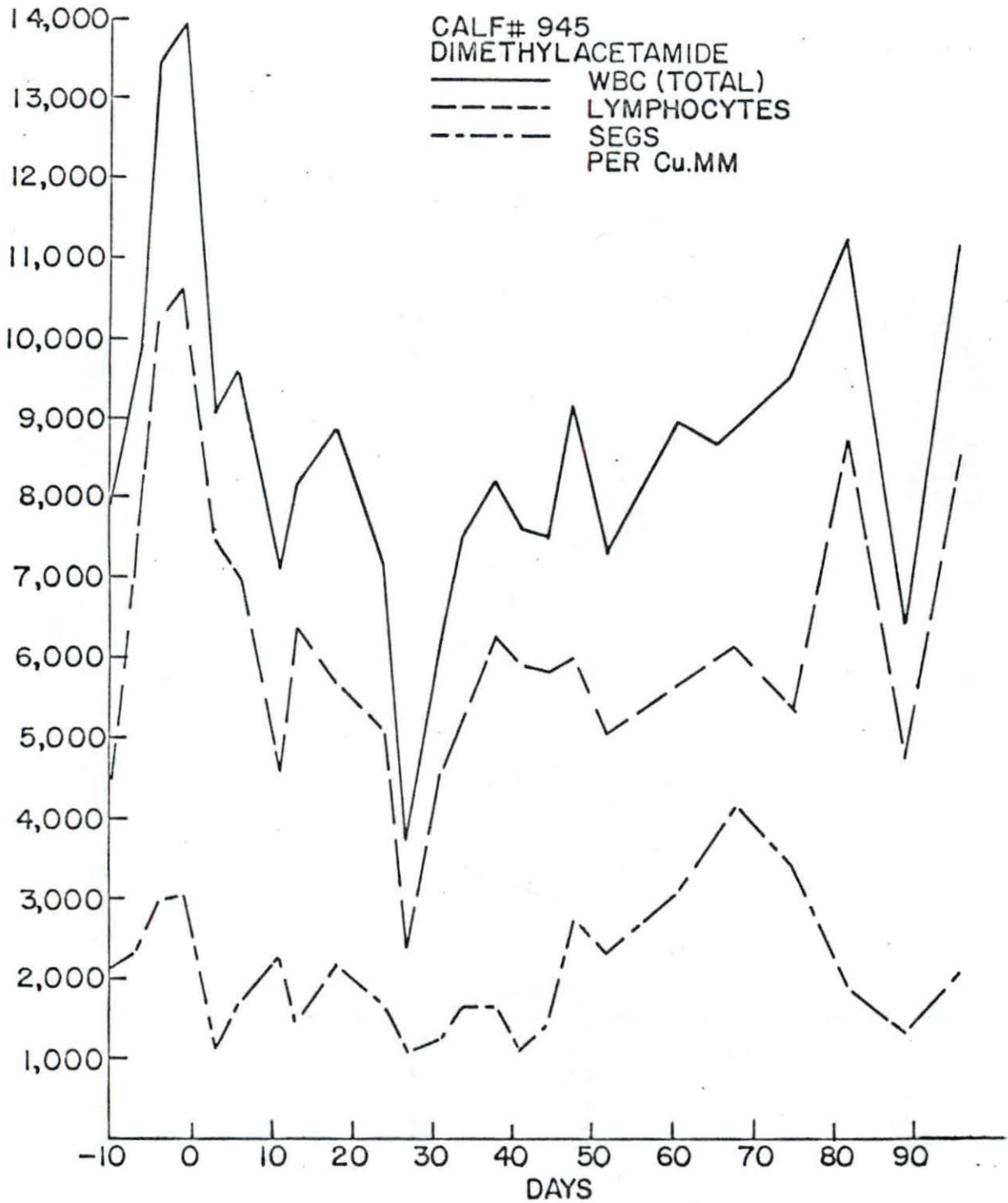


Figure 24. Calf 945. Leukocyte counts

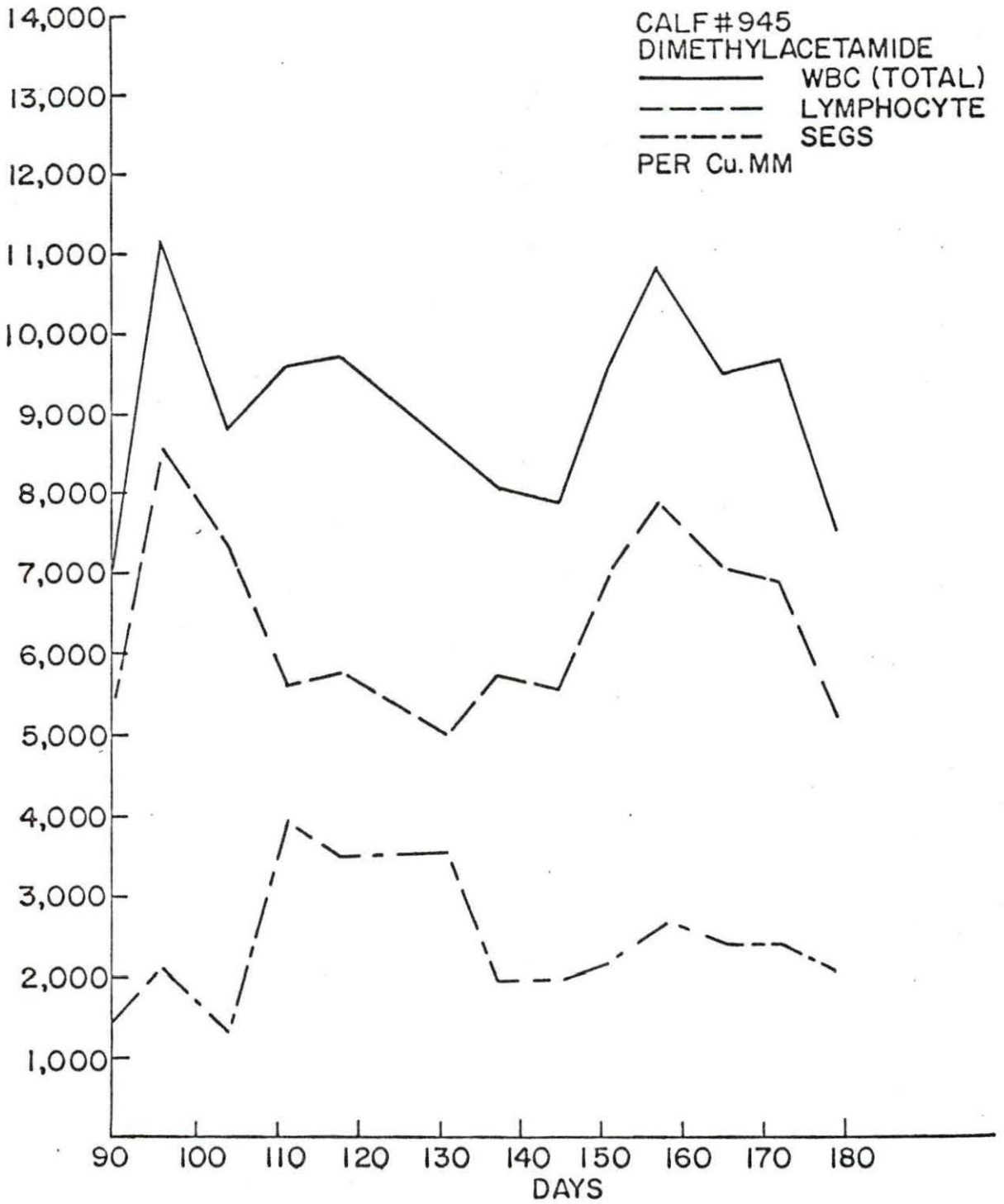


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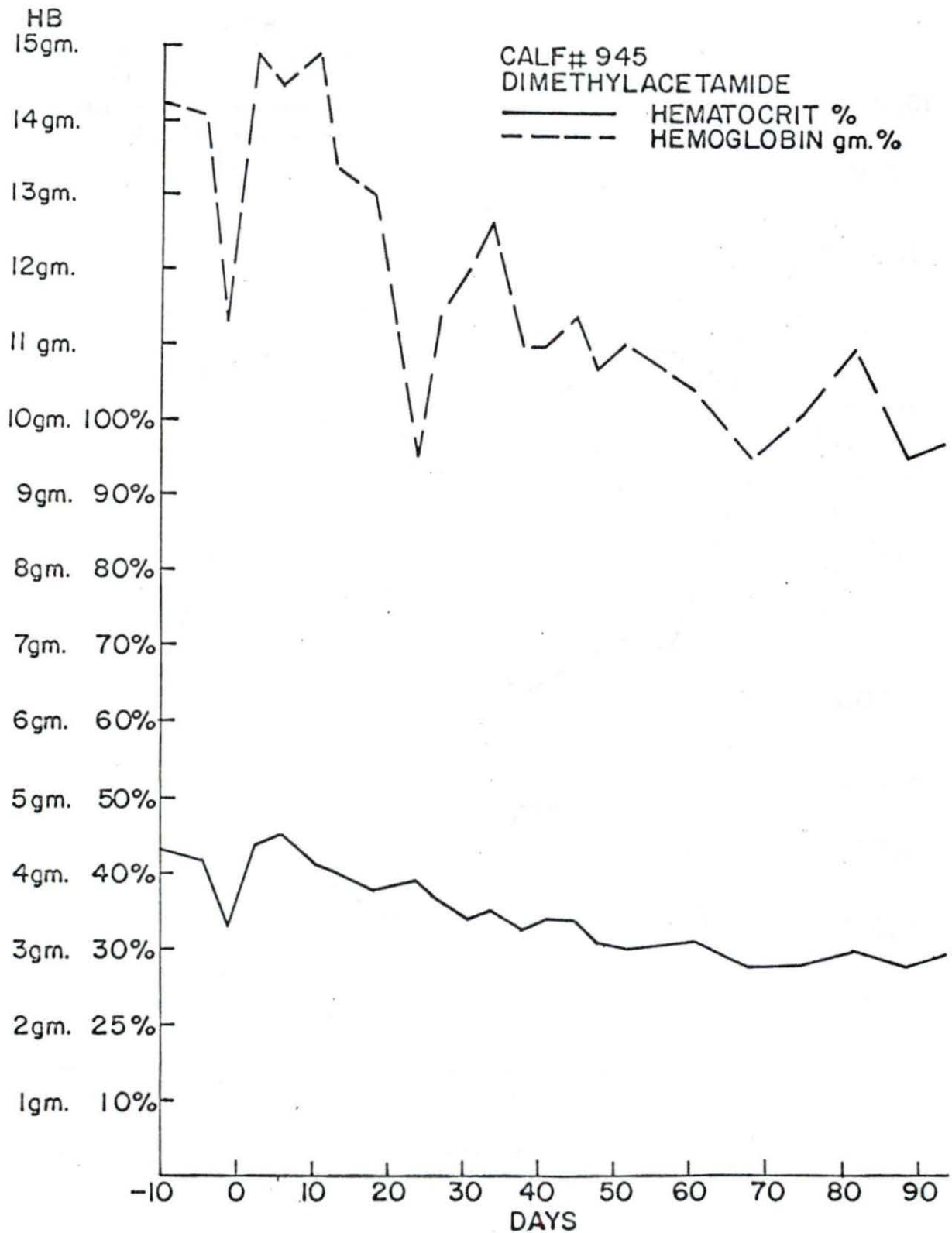


Figure 25. Calf 942. Hematocrit and hemoglobin

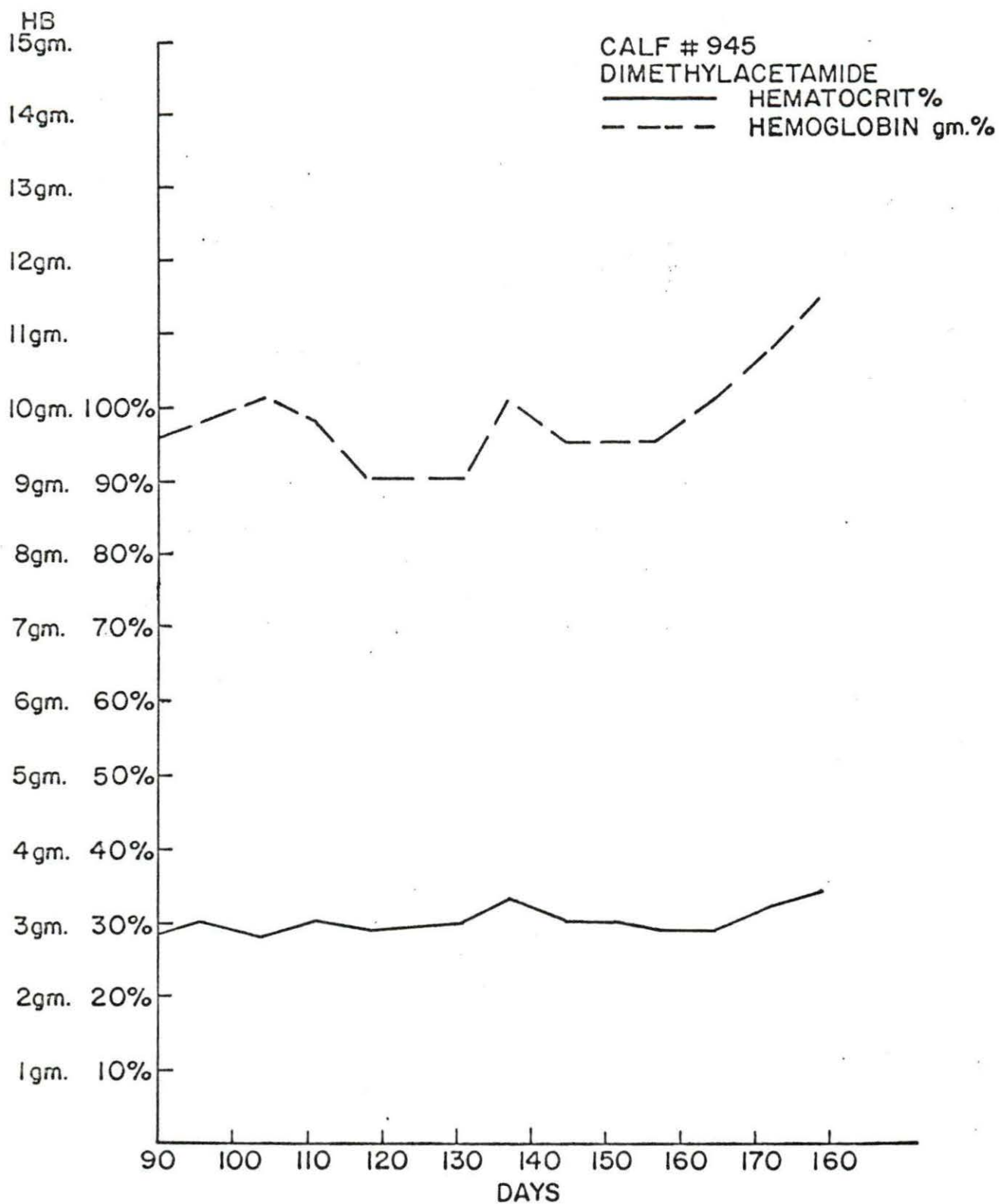


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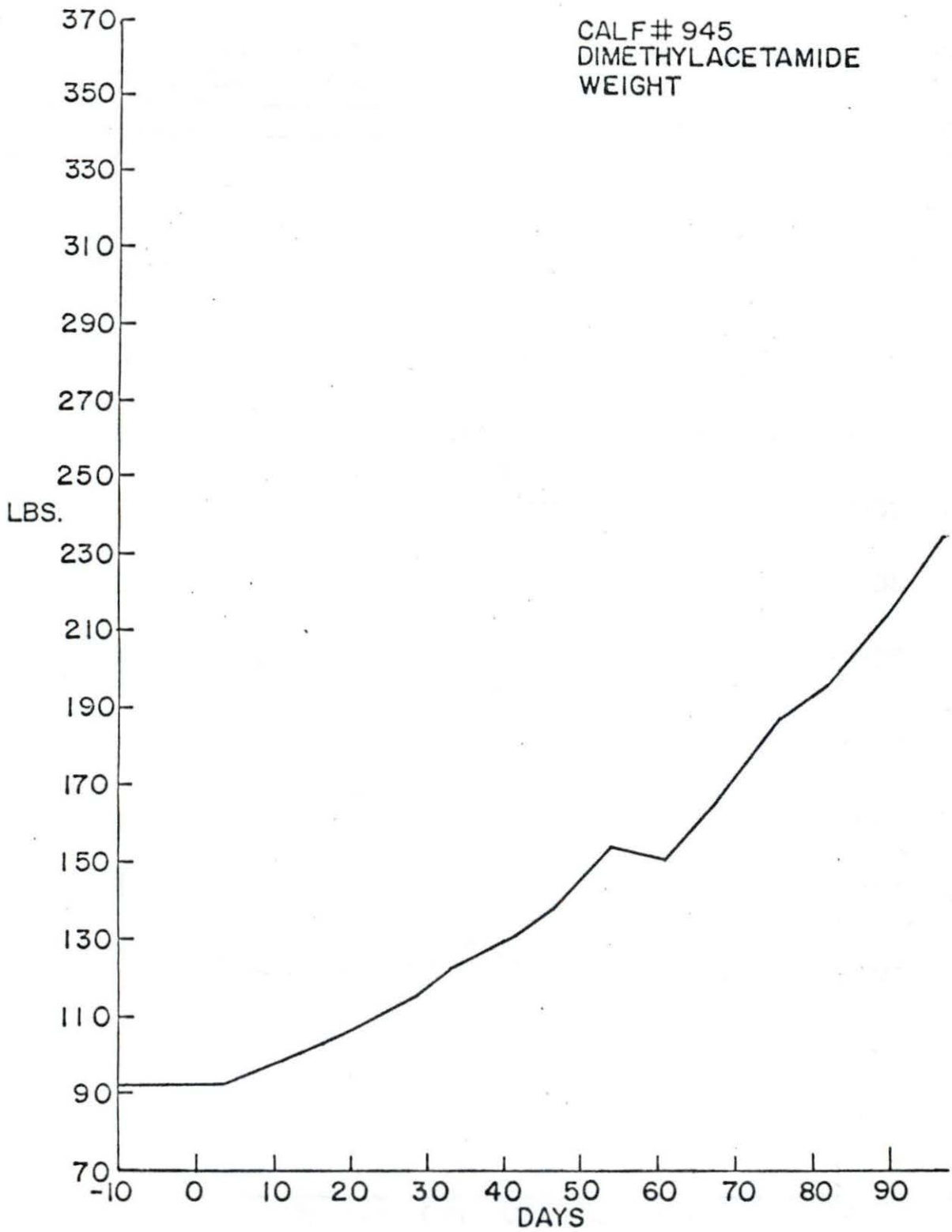


Figure 26. Calf 945. Weight and clinical data

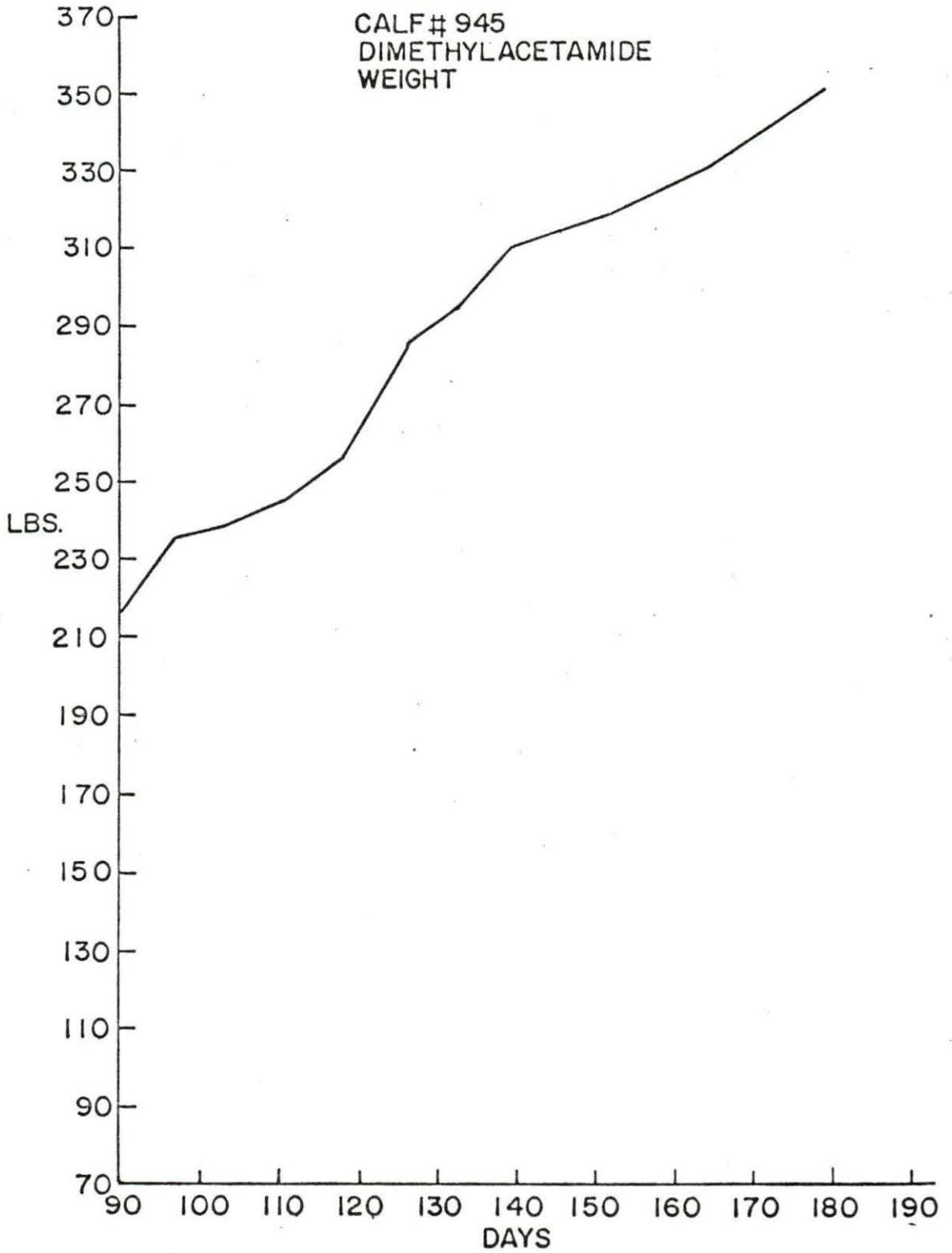


Figure 26. (Continued)

APPENDIX IV. CALVES TREATED WITH URACIL MUSTARD

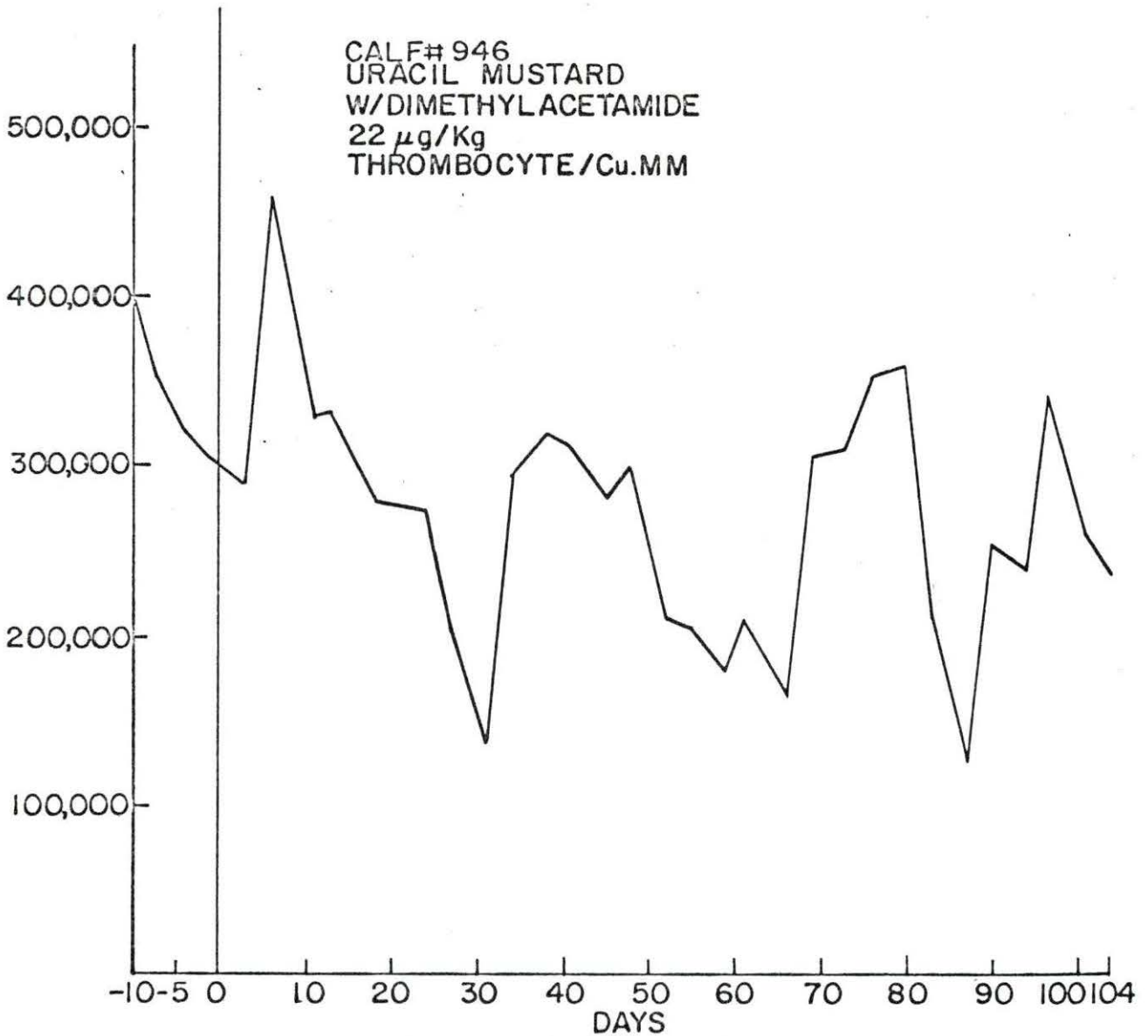


Figure 27. Calf 946. Thrombocyte counts

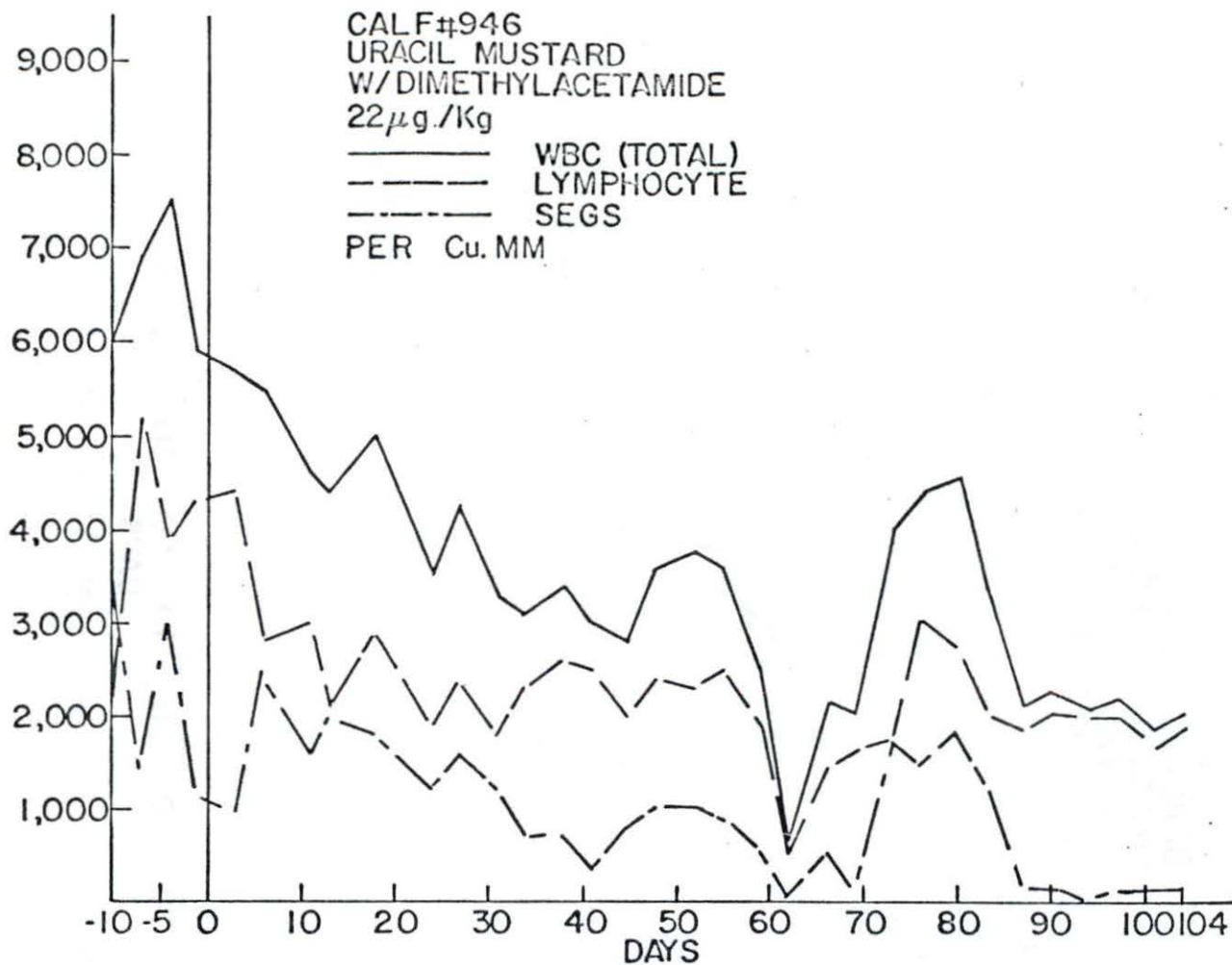


Figure 28. Calf 946. Leukocyte counts

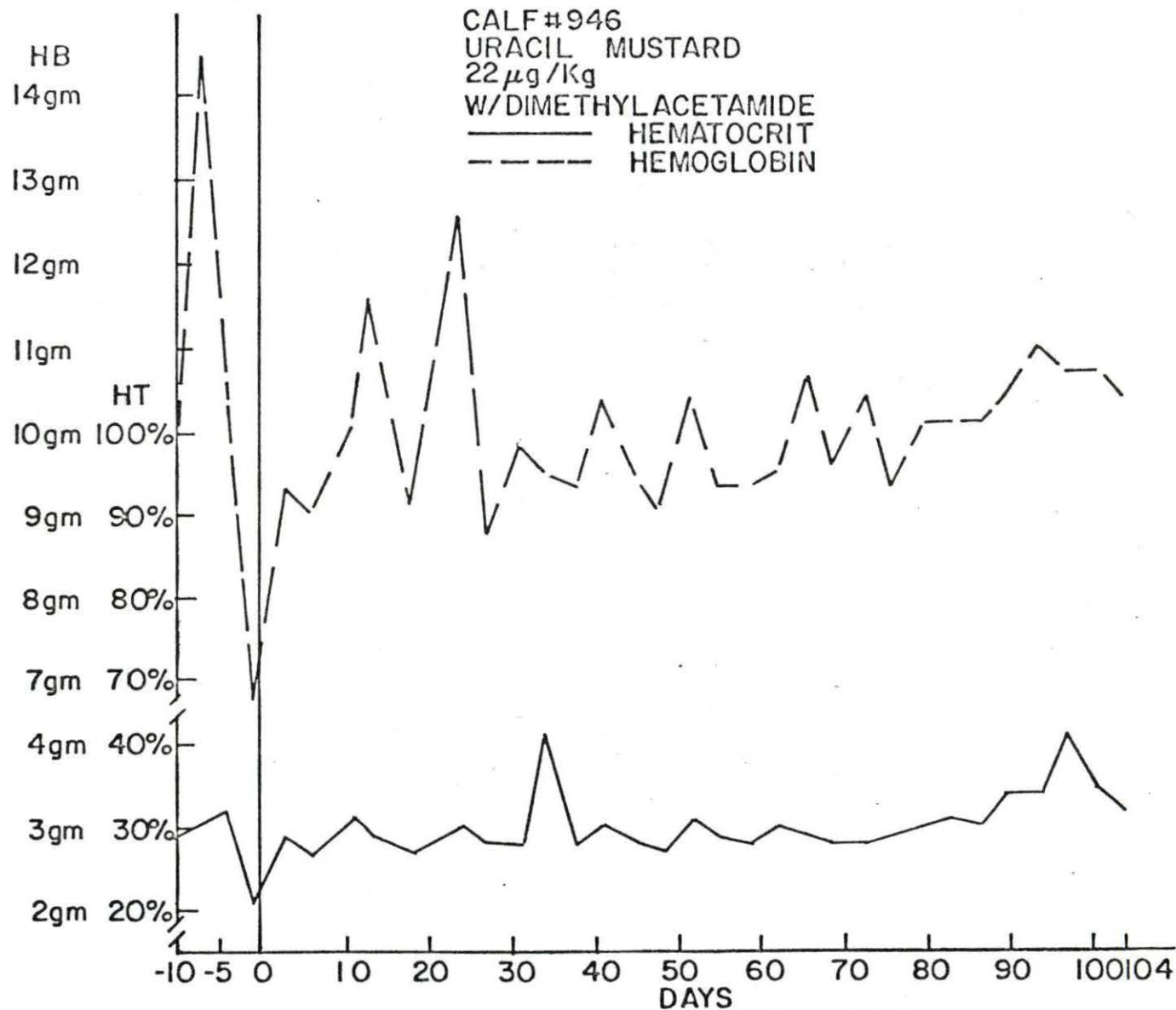


Figure 29. Calf 946. Hematocrit and hemoglobin

CALF # 946
URACIL MUSTARD
W/DIMETHYLACETAMIDE
22 μ g/Kg
WEIGHT

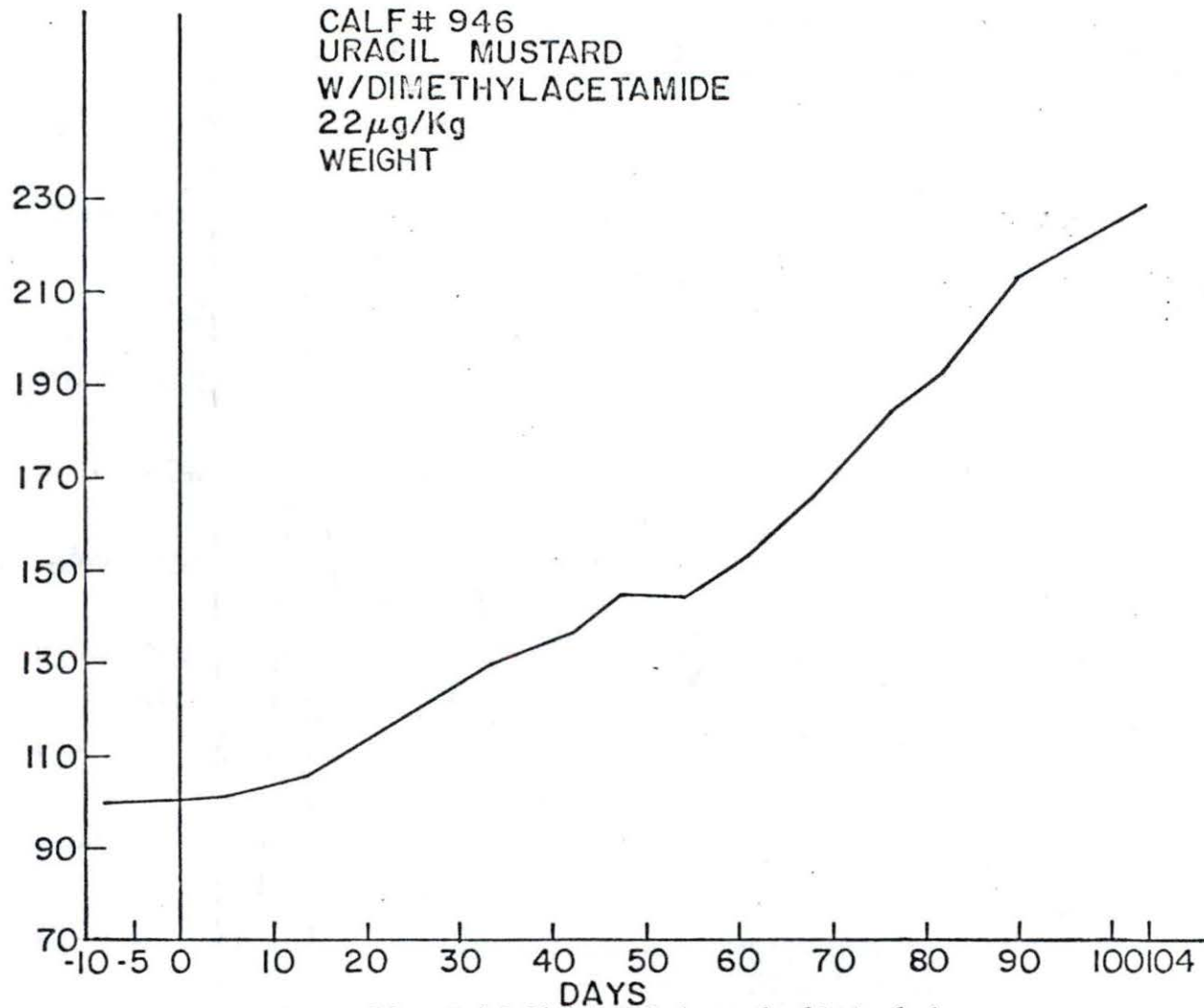


Figure 30. Calf 946. Weight and clinical data

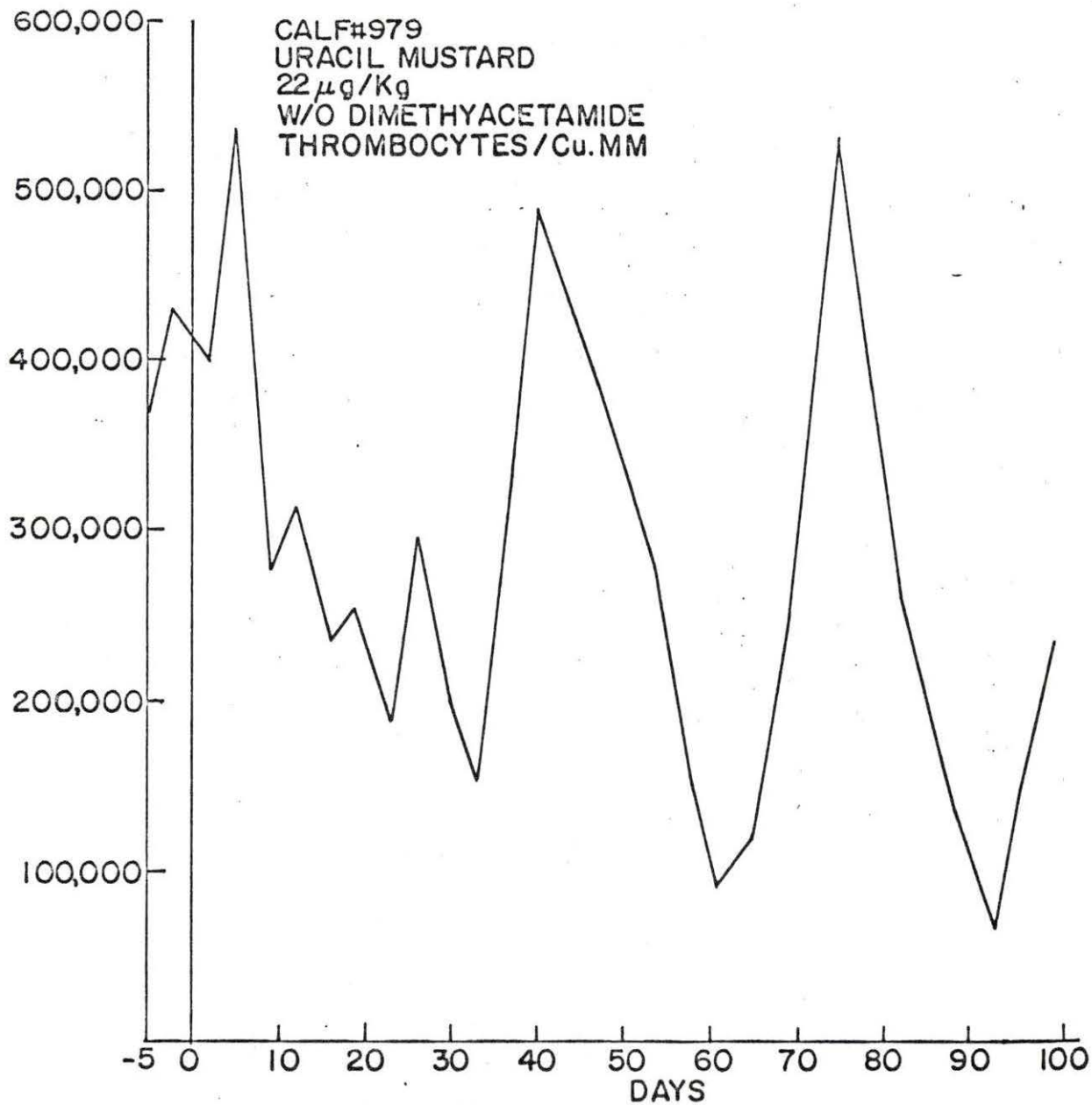


Figure 31. Calf 979. Thrombocyte counts

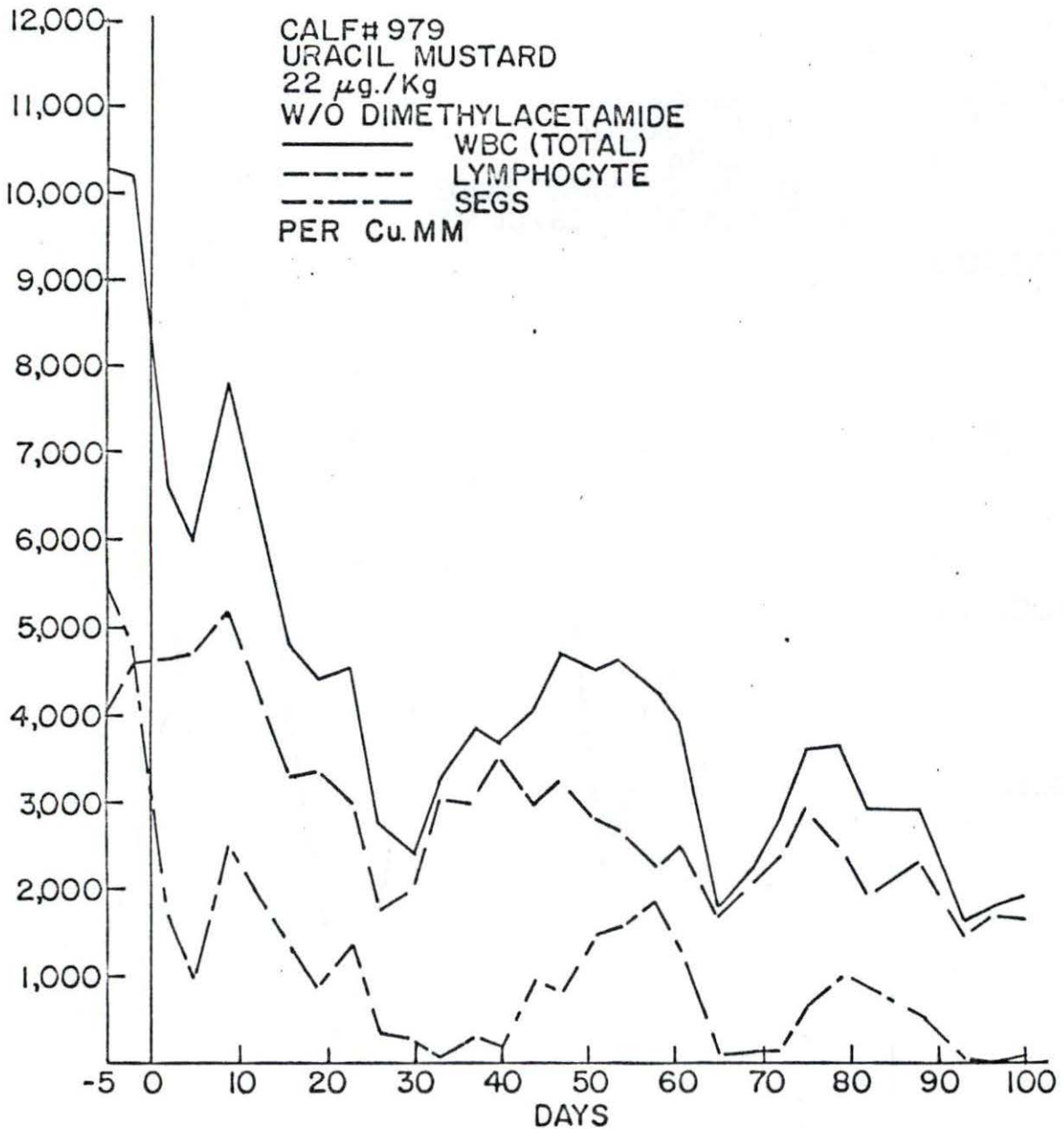


Figure 32. Calf 979. Leukocyte counts

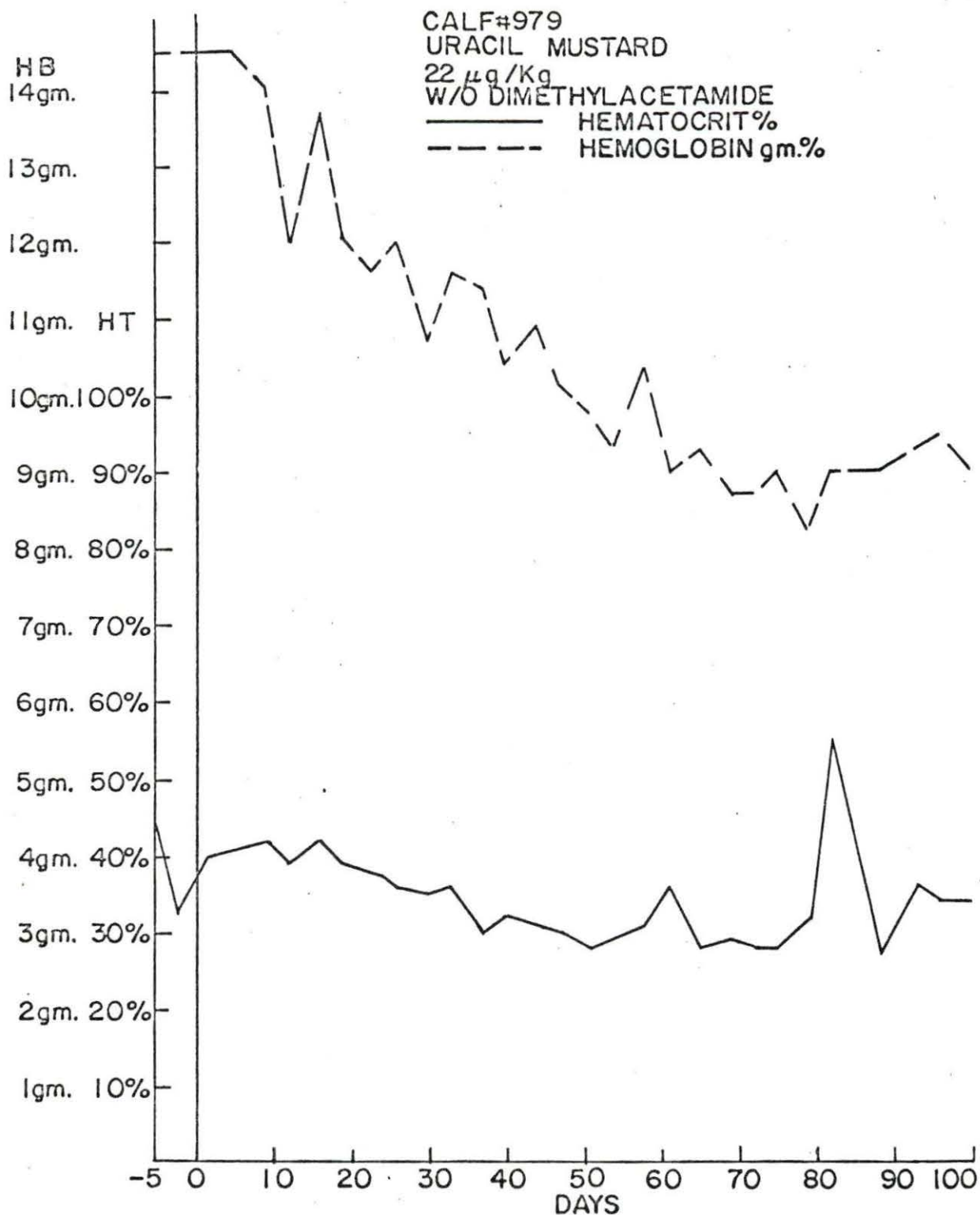


Figure 33. Calf 979. Hematocrit and hemoglobin

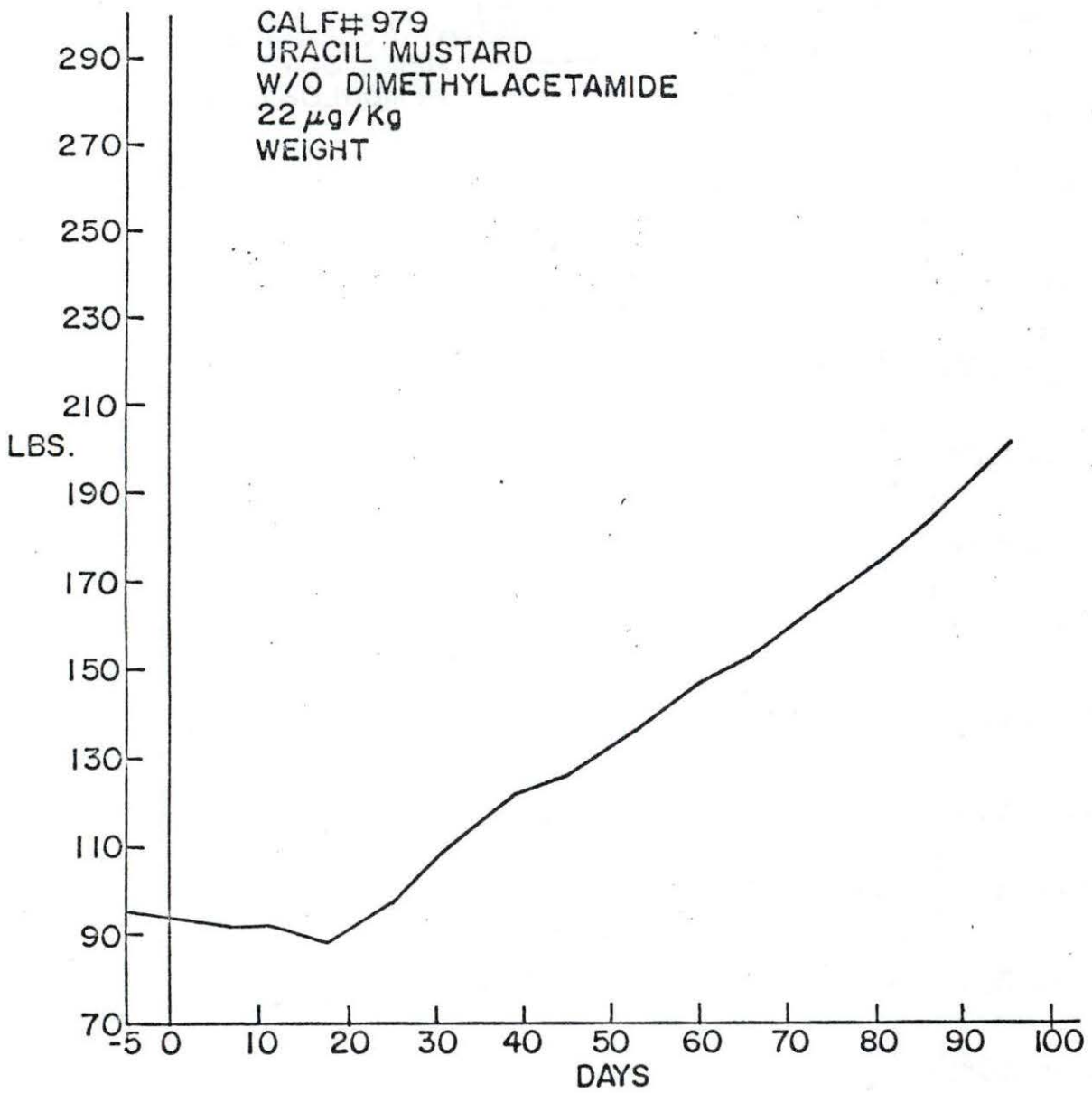


Figure 34. Calf 979. Weight and clinical data

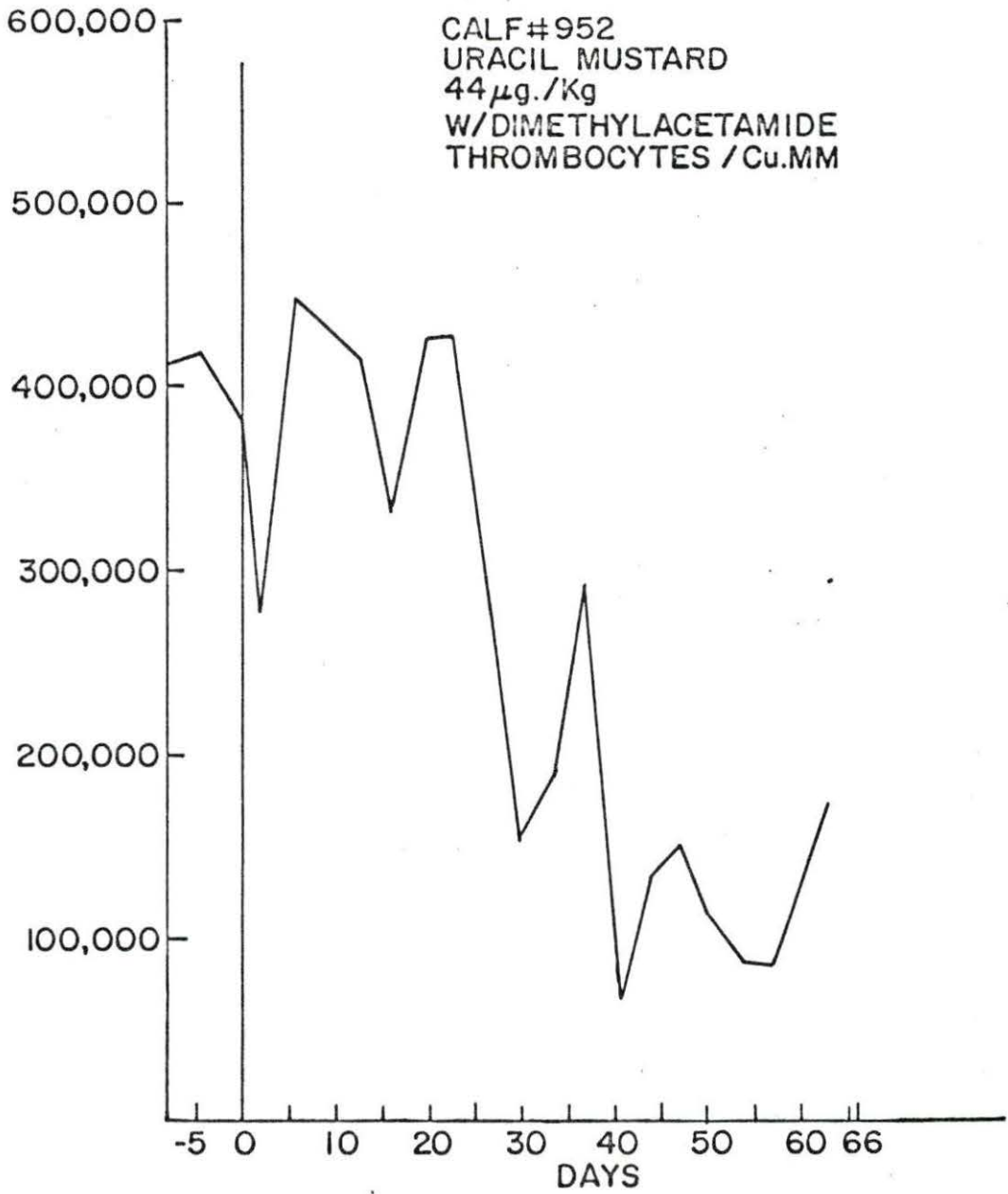


Figure 35. Calf 952. Thrombocyte counts

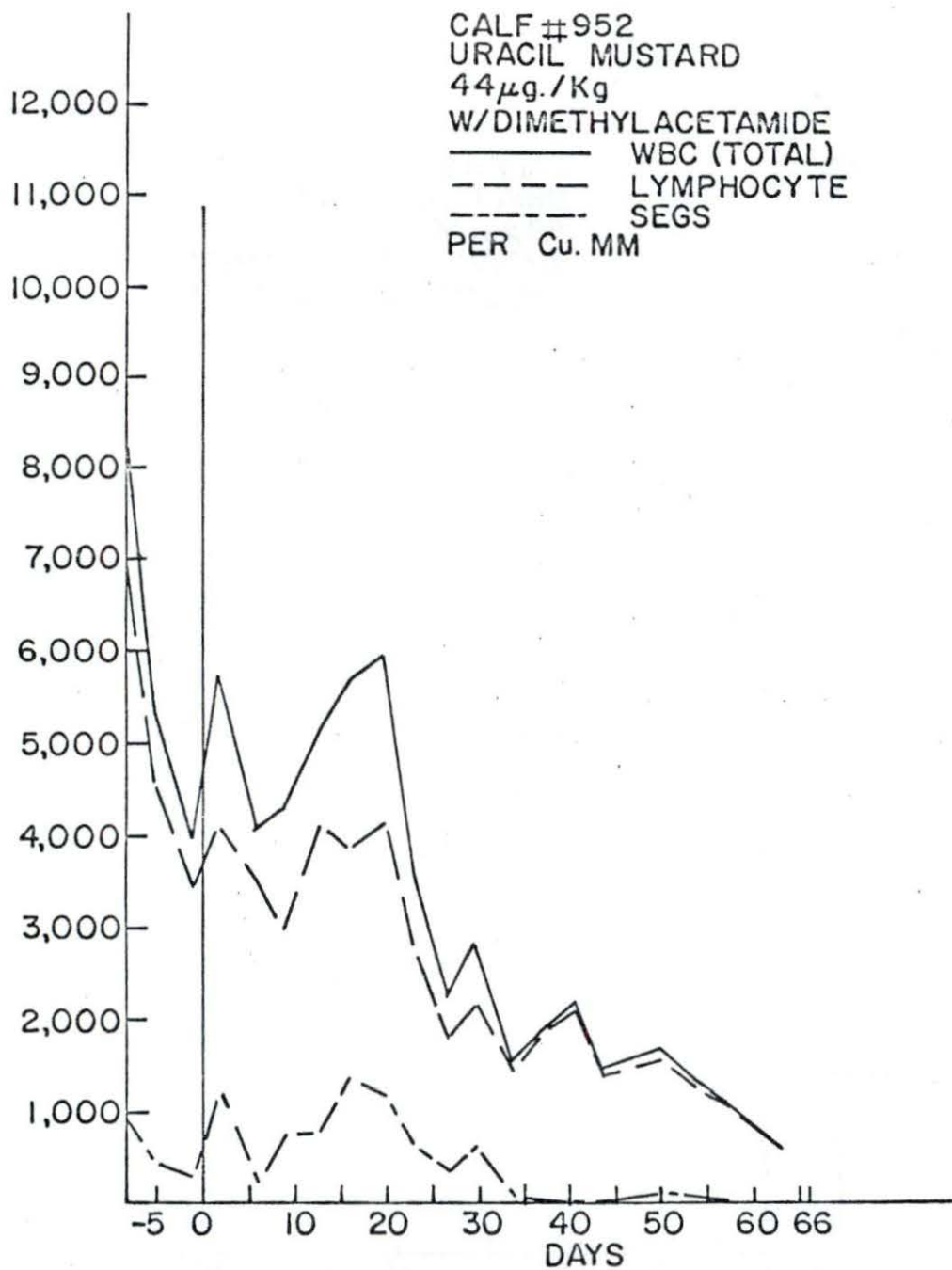


Figure 36. Calf 952. Leukocyte counts

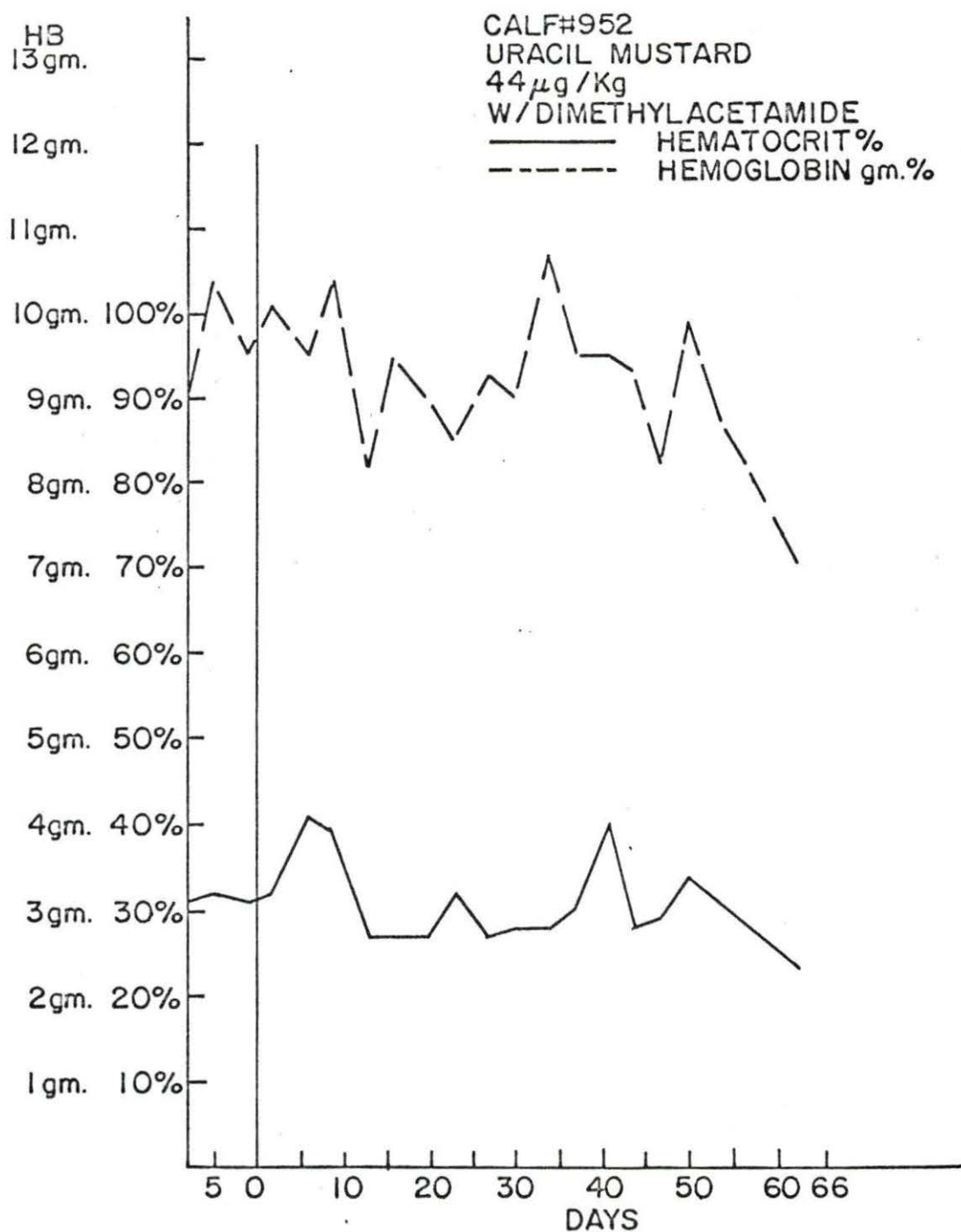


Figure 37. Calf 952. Hematocrit and hemoglobin

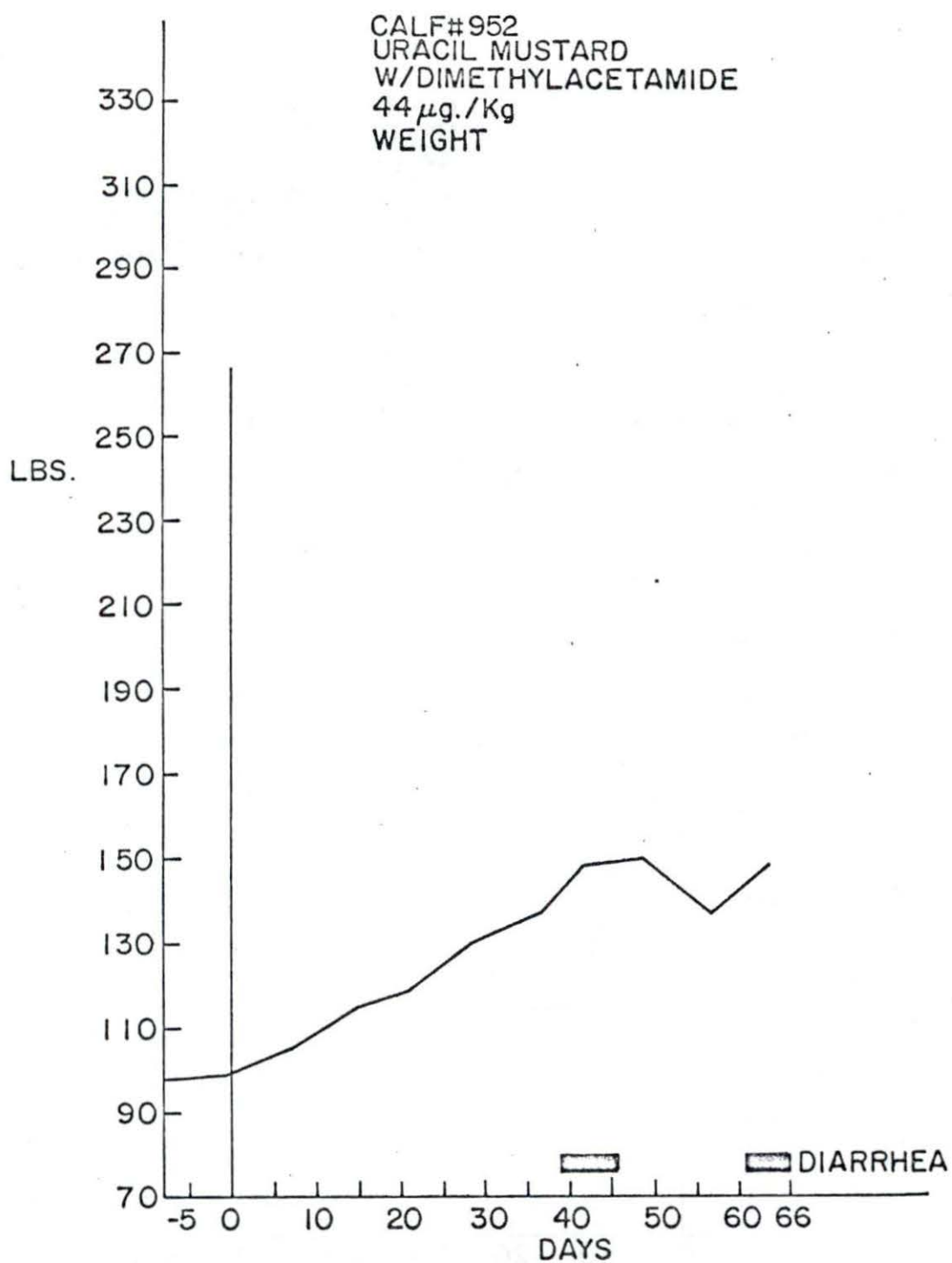


Figure 38. Calf 952. Weight and clinical data

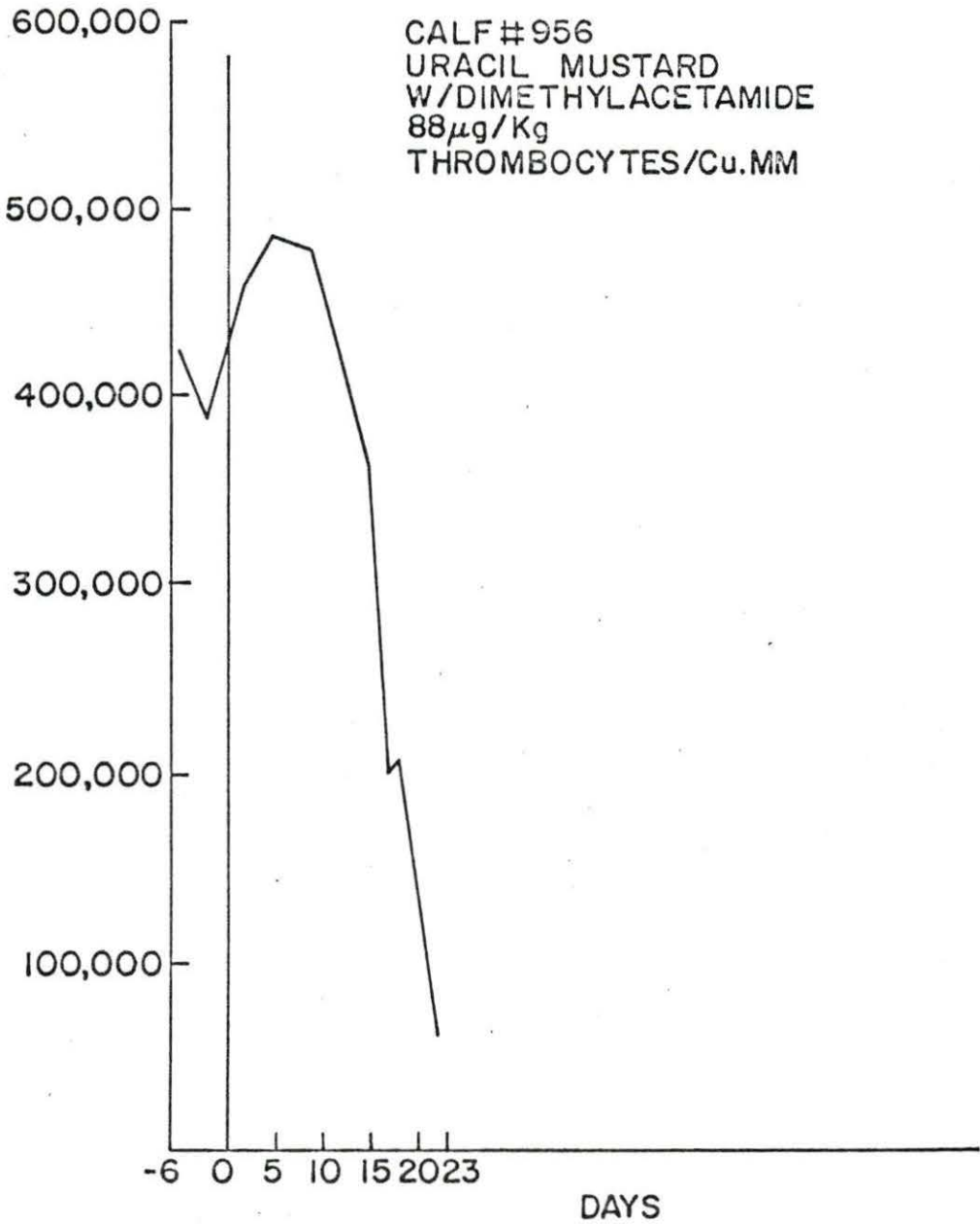


Figure 39. Calf 956. Thrombocyte counts

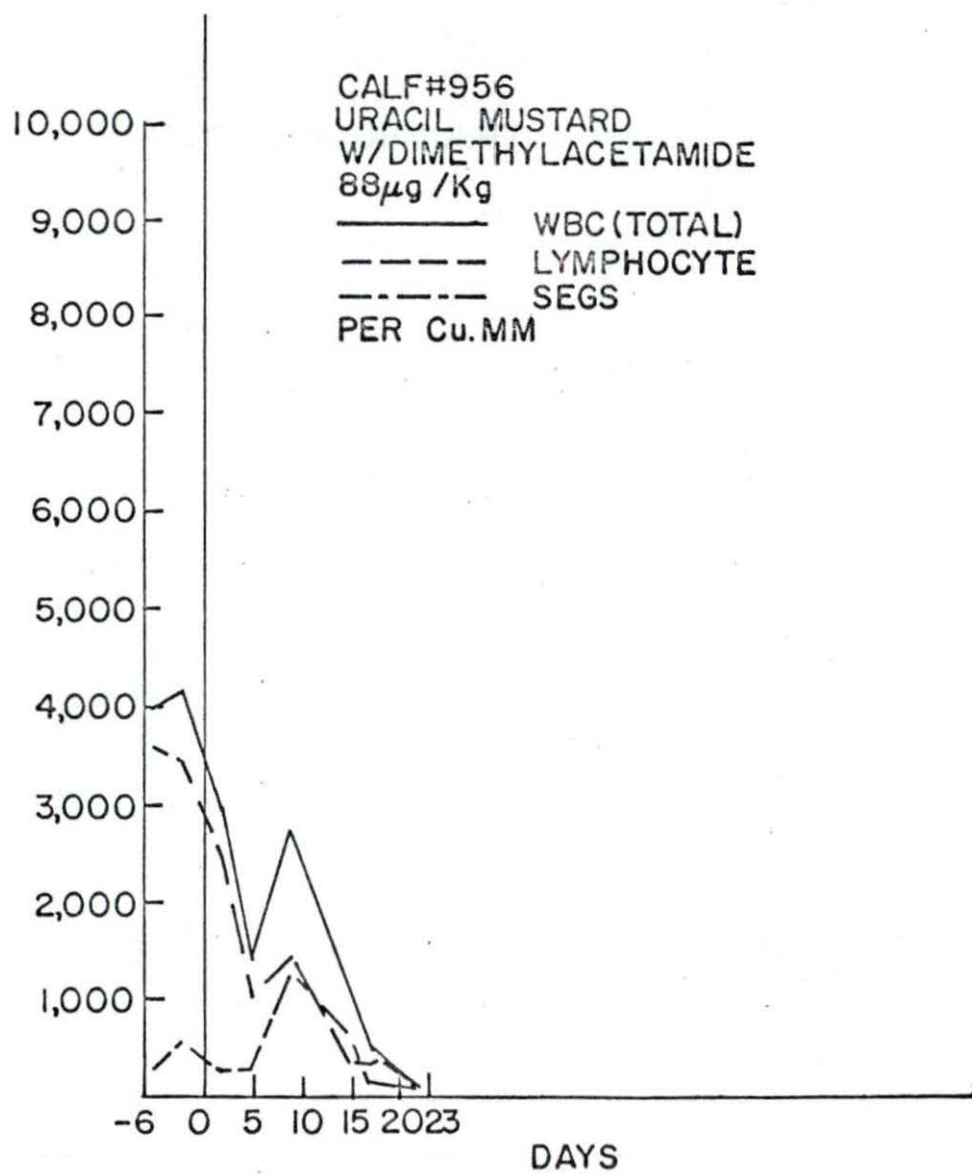


Figure 40. Calf 956. Leukocyte counts

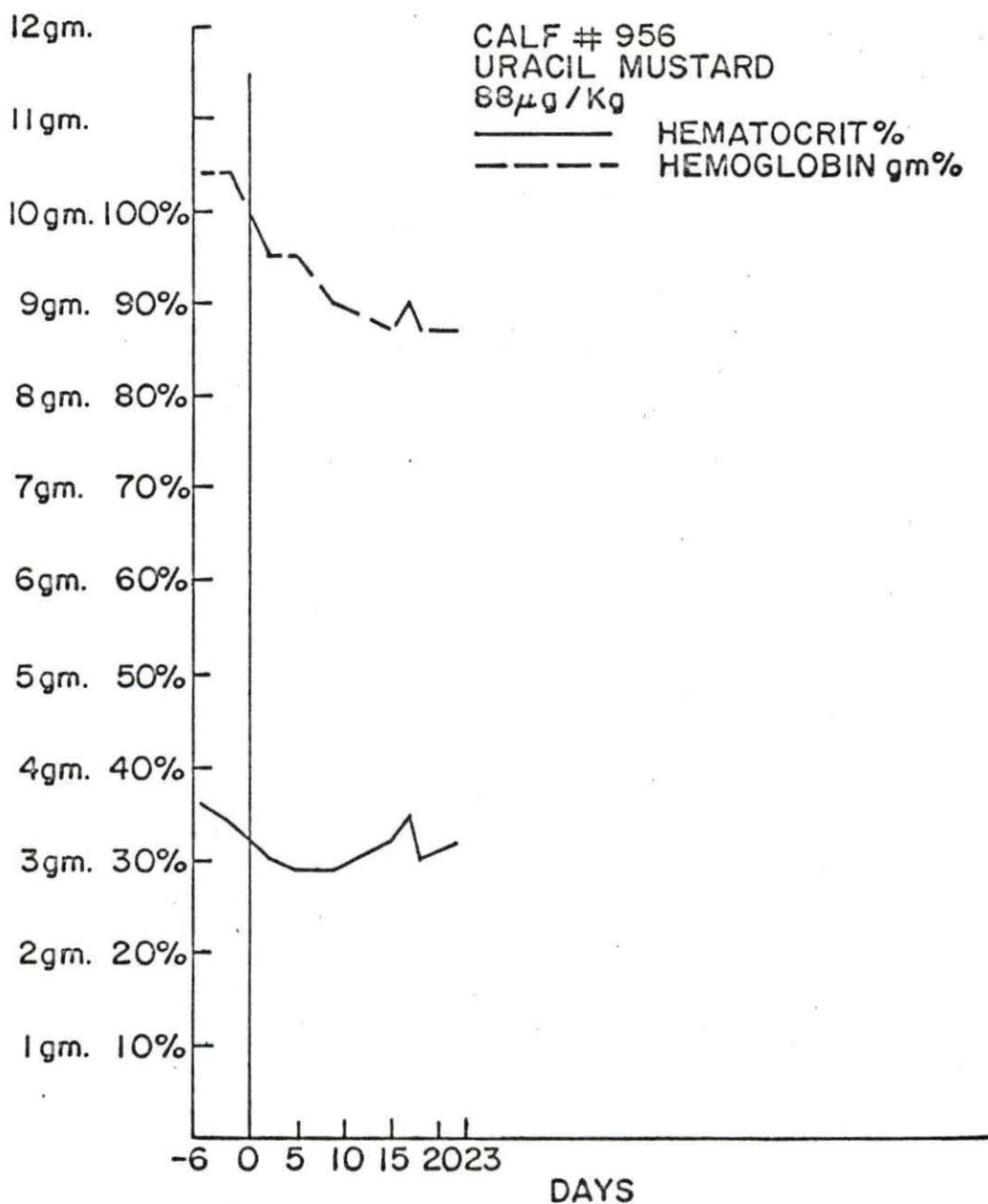


Figure 41. Calf 956. Hematocrit and hemoglobin

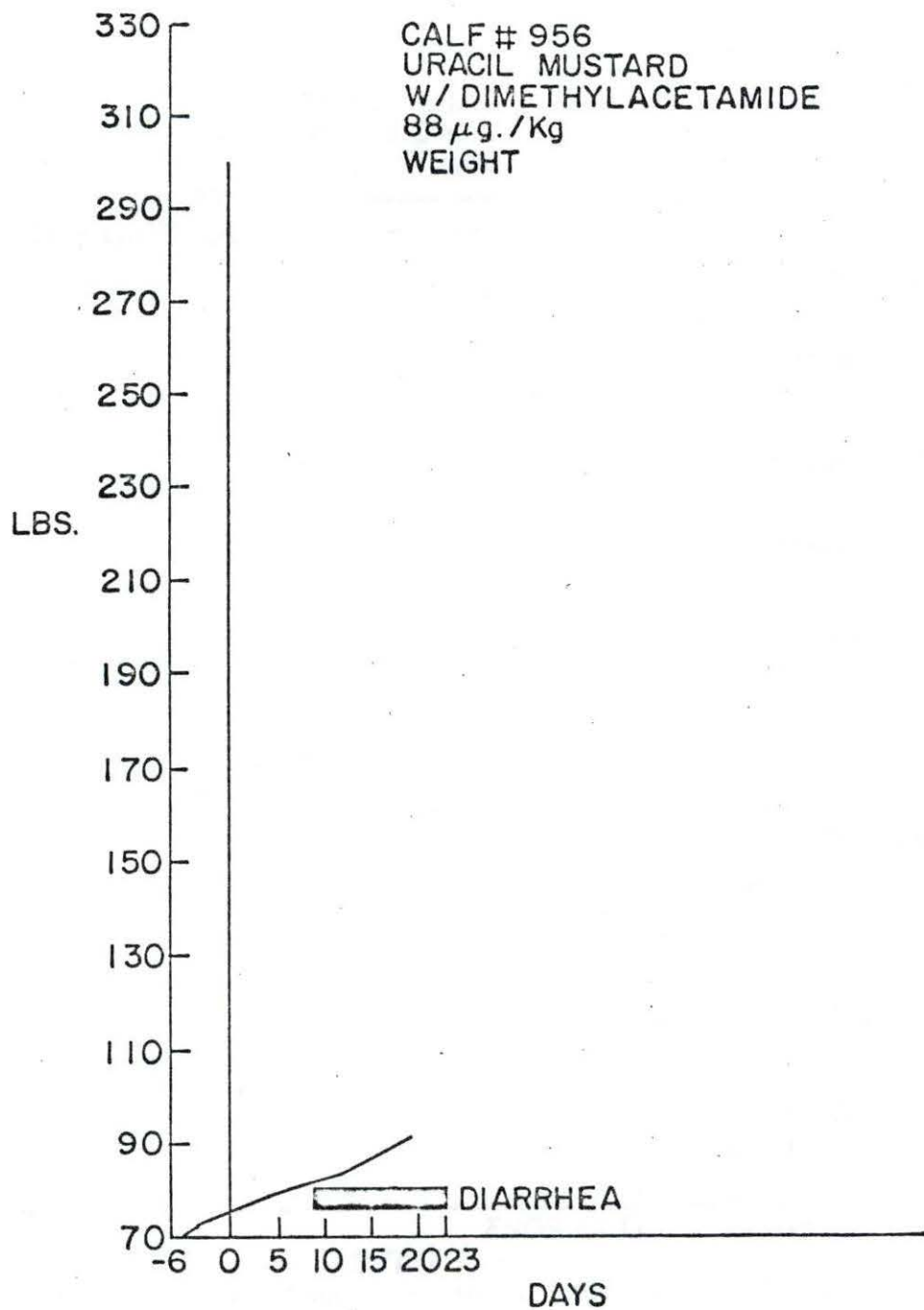


Figure 42. Calf 956. Weight and clinical data

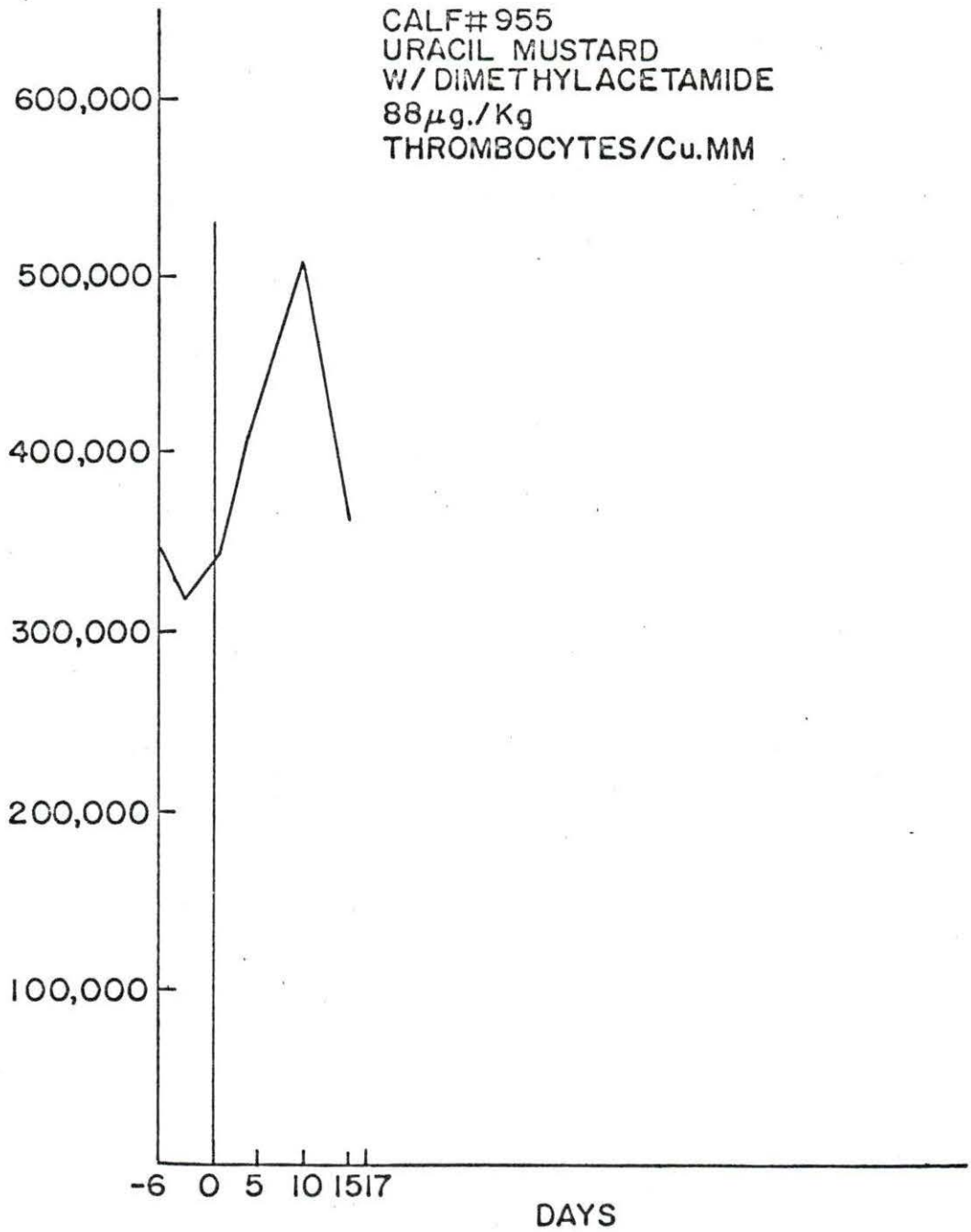


Figure 43. Calf 955. Thrombocyte counts

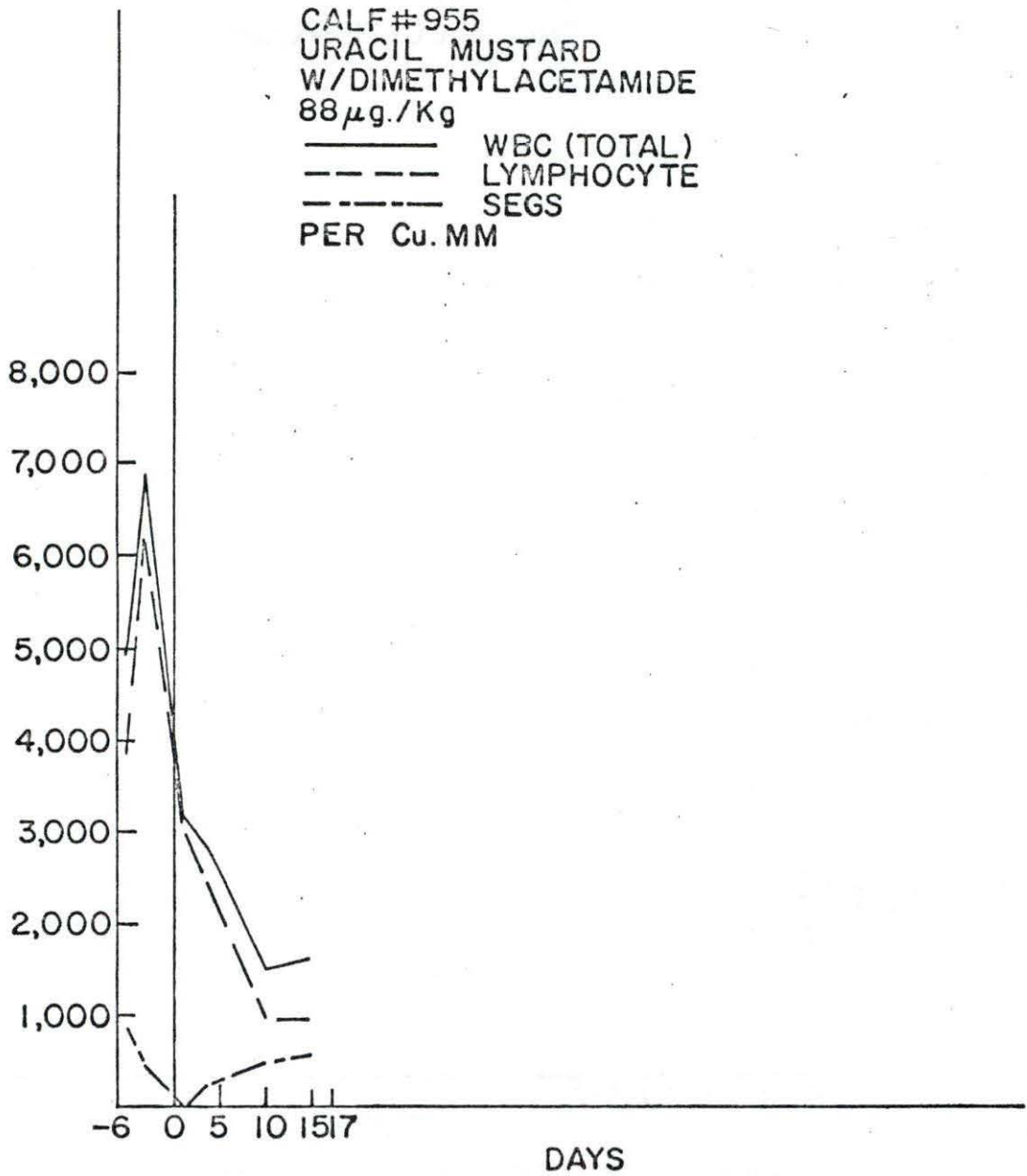


Figure 44. Calf 955. Leukocyte counts

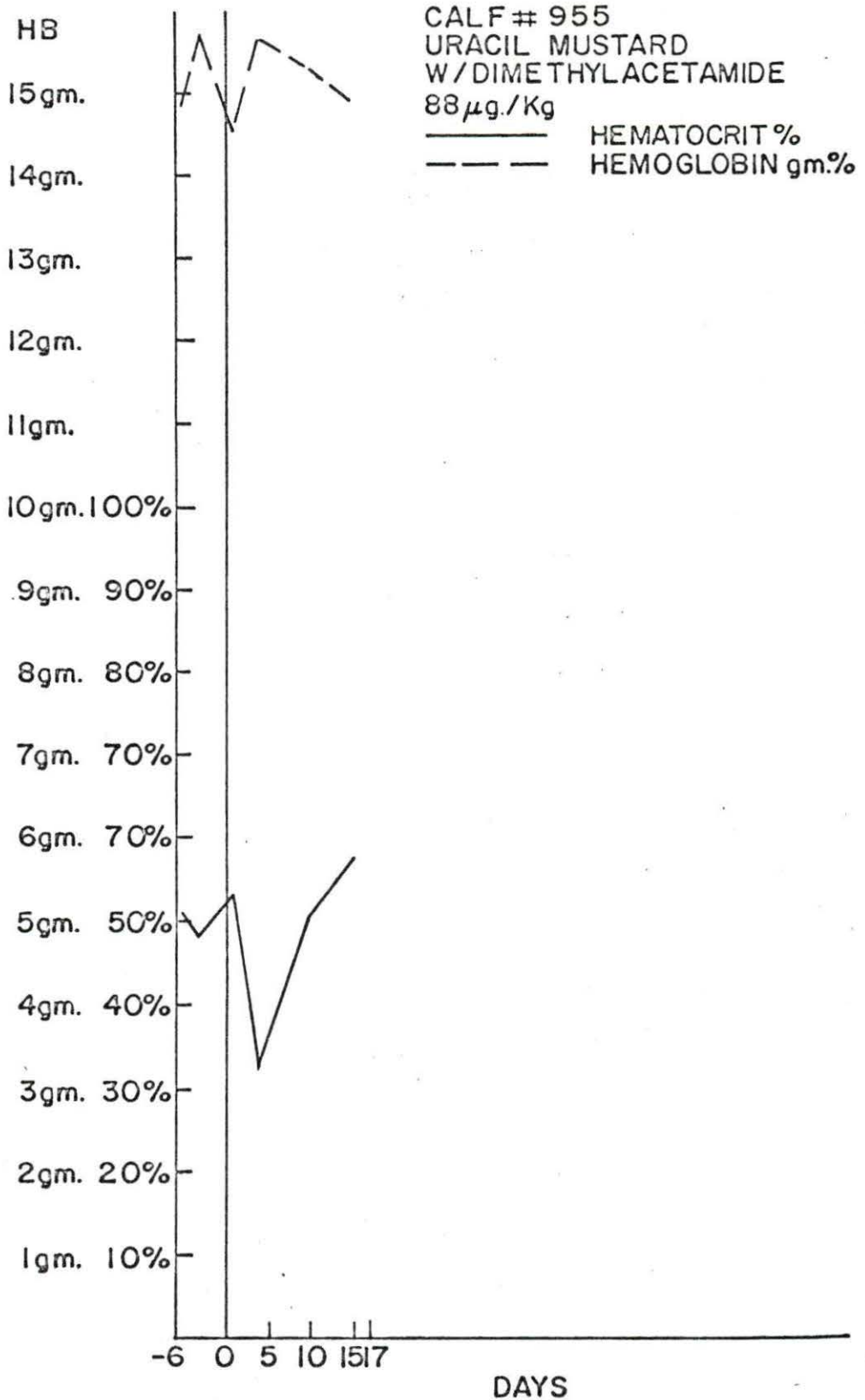


Figure 45. Calf 955. Hematocrit and hemoglobin

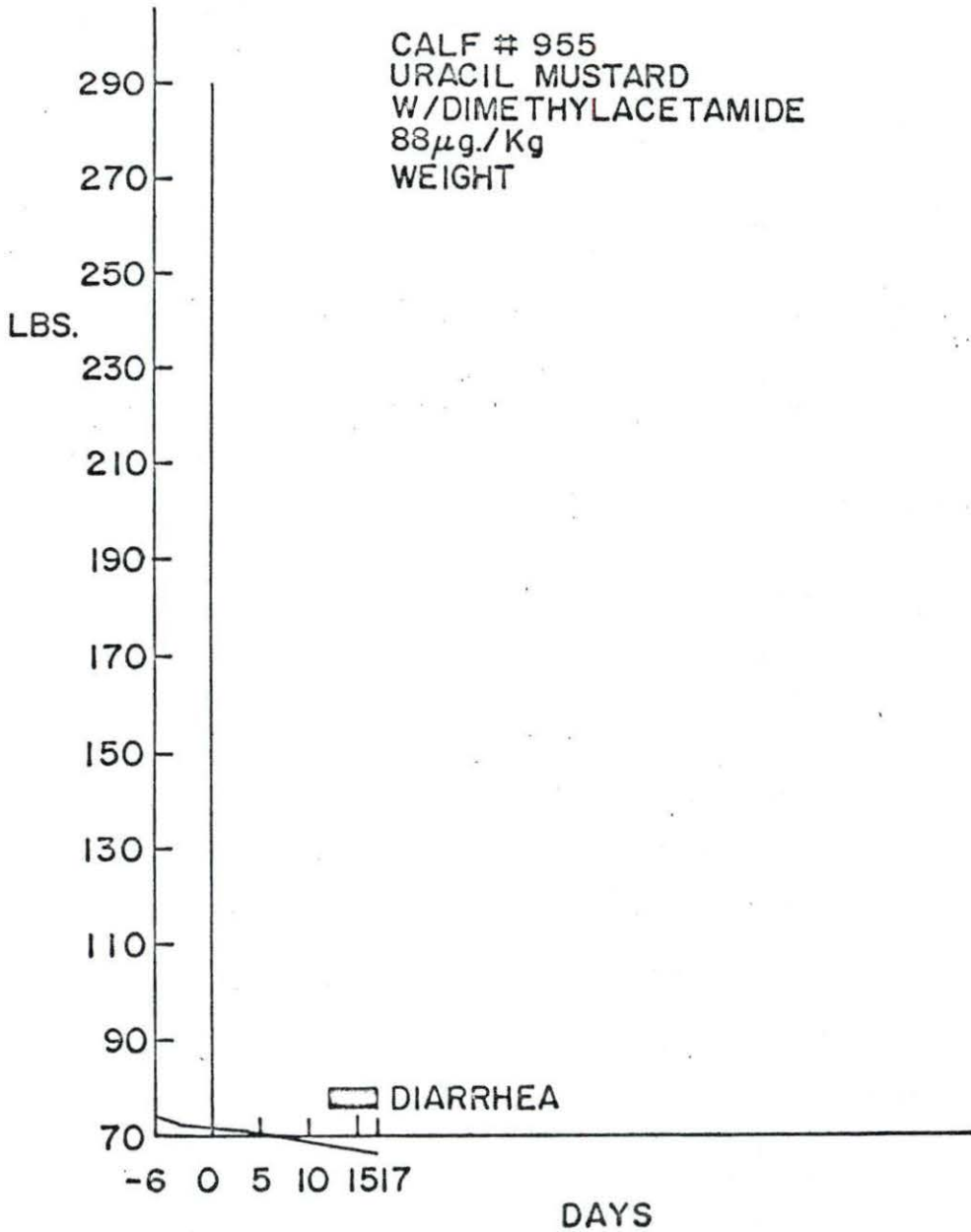


Figure 46. Calf 955. Height and clinical data

APPENDIX V. CALVES TREATED WITH 5-FLUORO URACIL

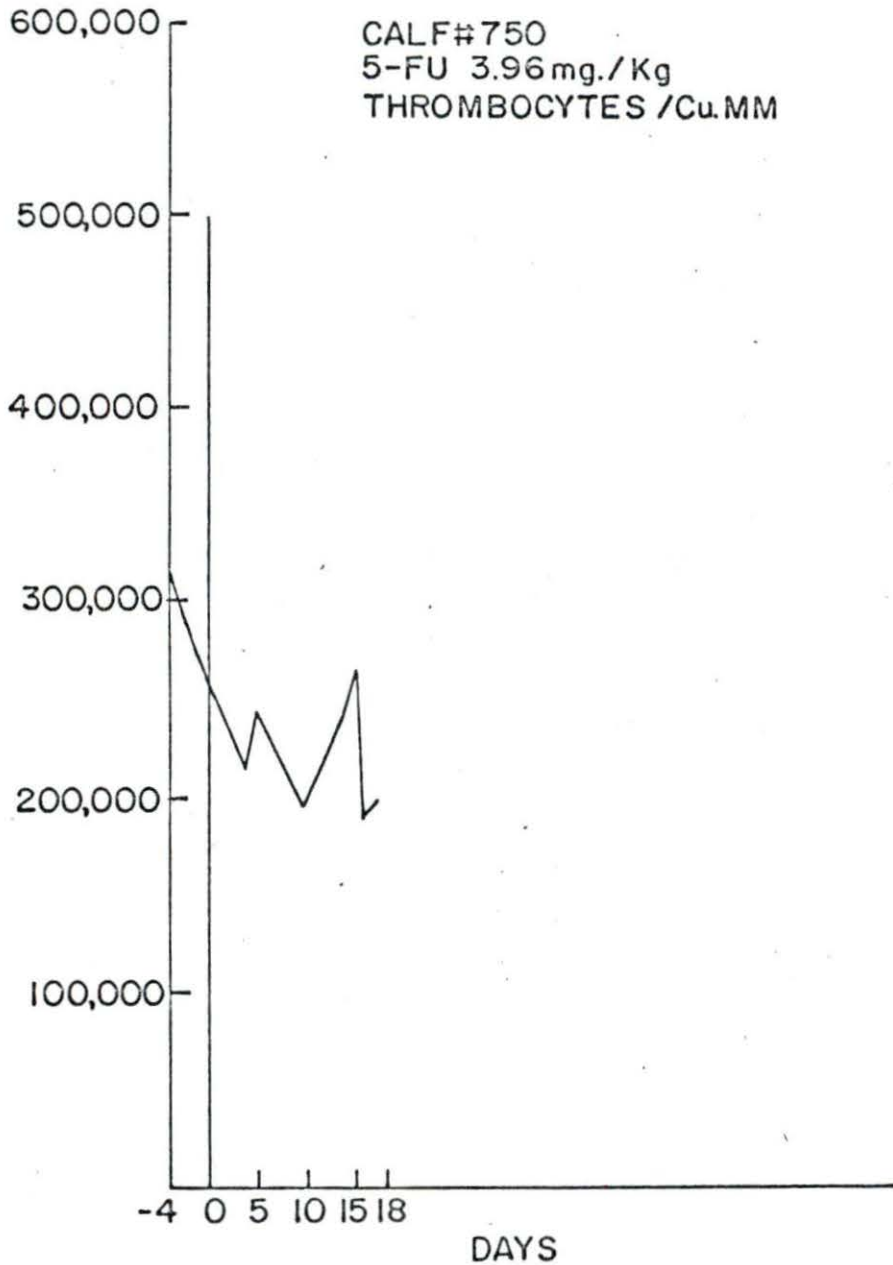


Figure 47. Calf 750. Thrombocyte counts

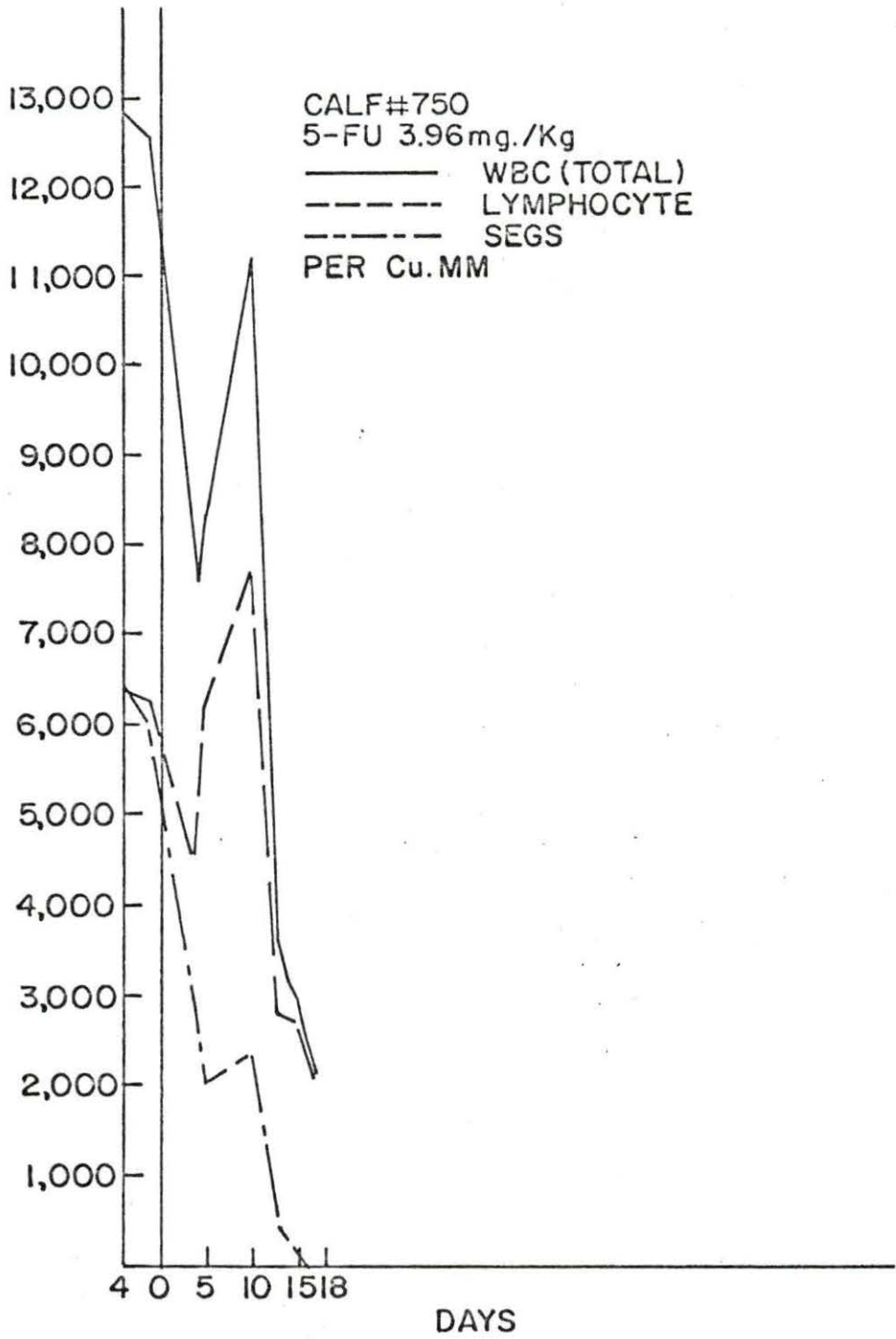


Figure 48. Calf 750. Leukocyte counts

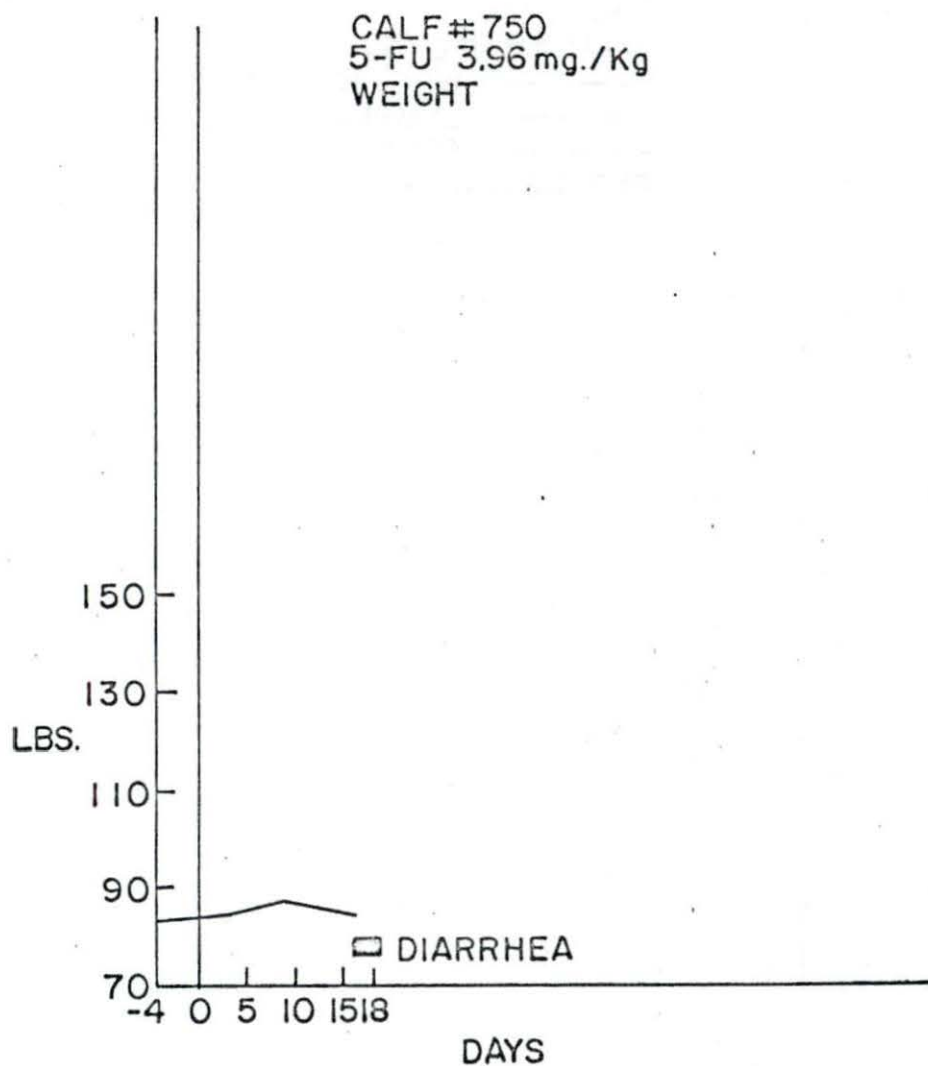


Figure 49. Calf 750. Weight and clinical data

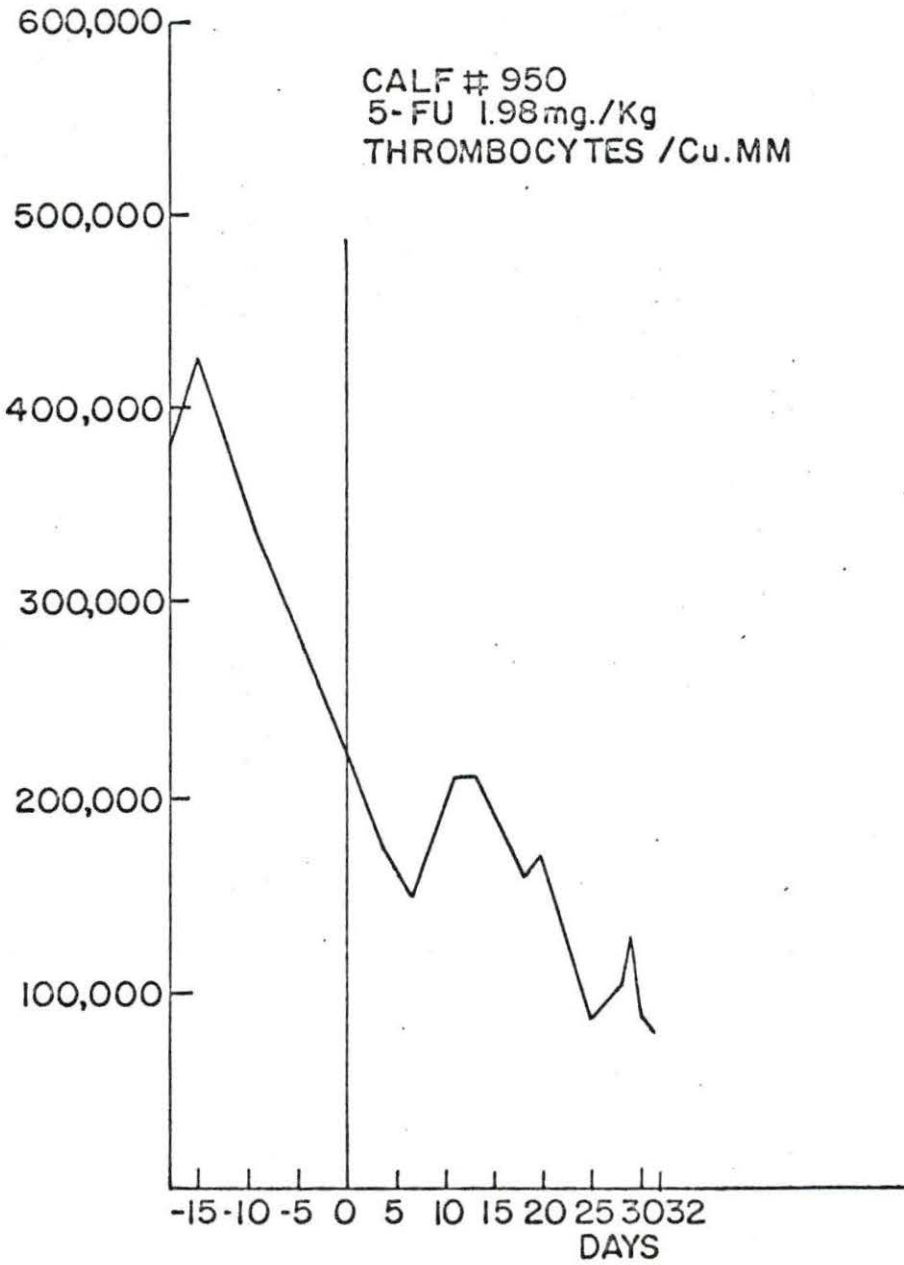


Figure 50. Calf 950. Thrombocyte counts

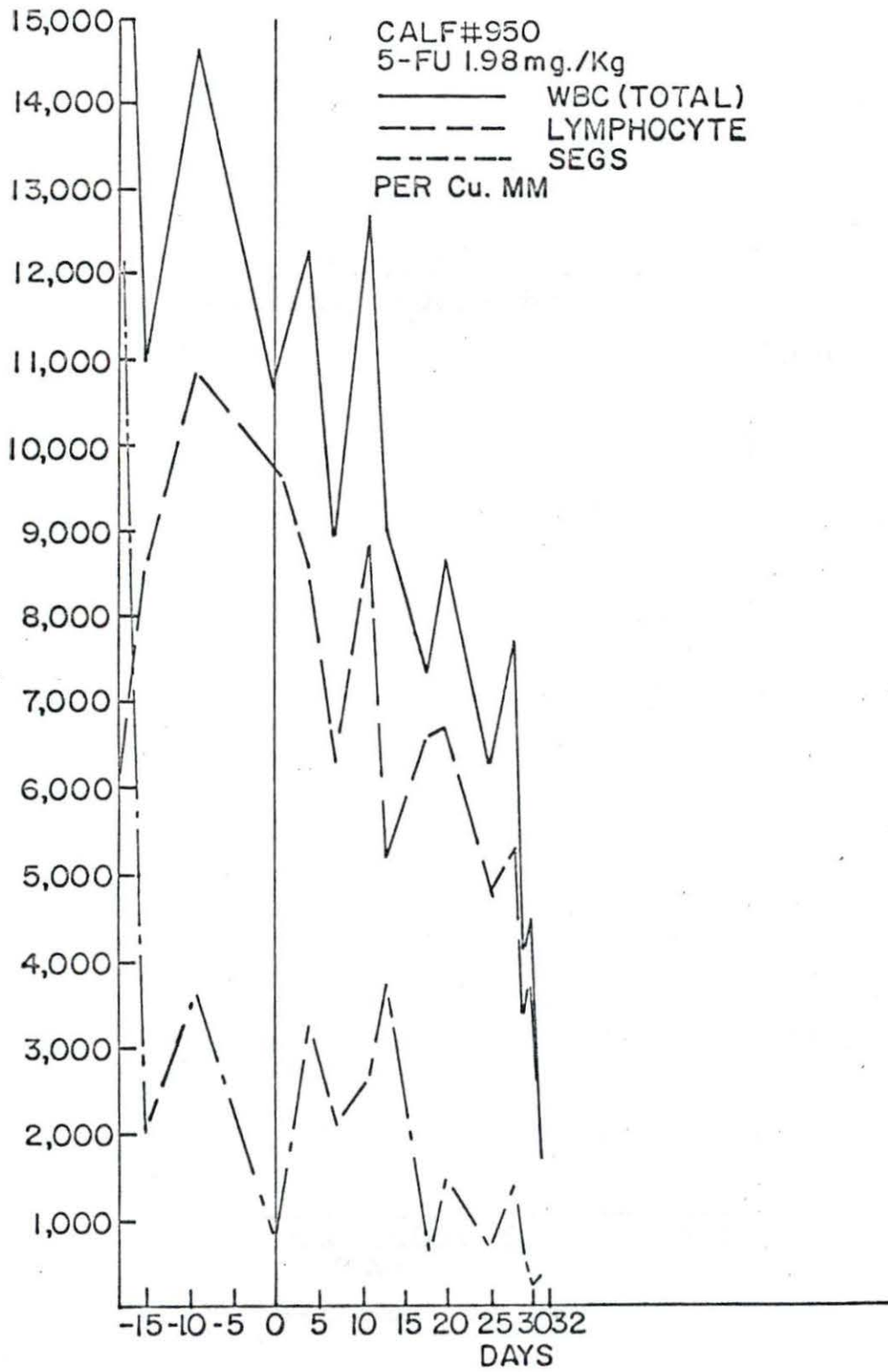


Figure 51. Calf 950. Leukocyte counts

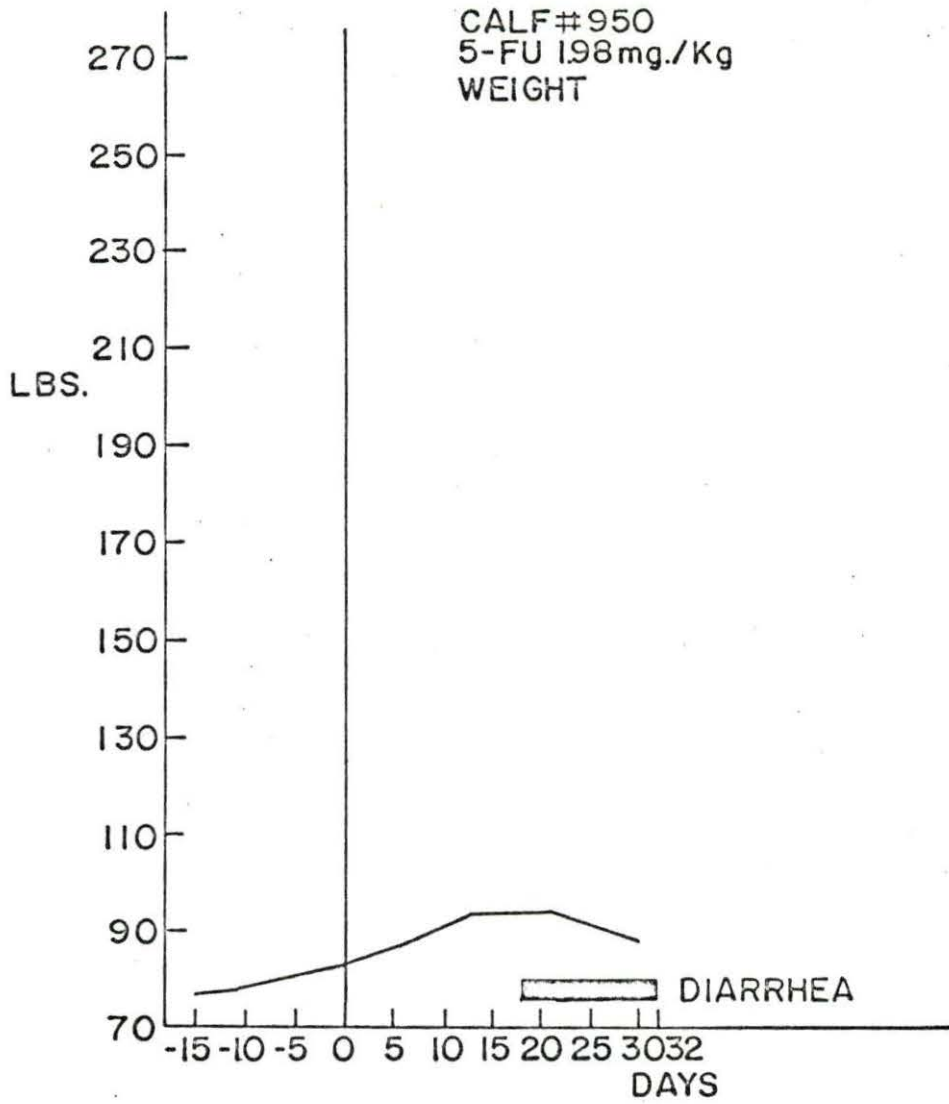


Figure 52. Calf 950. Weight and clinical data

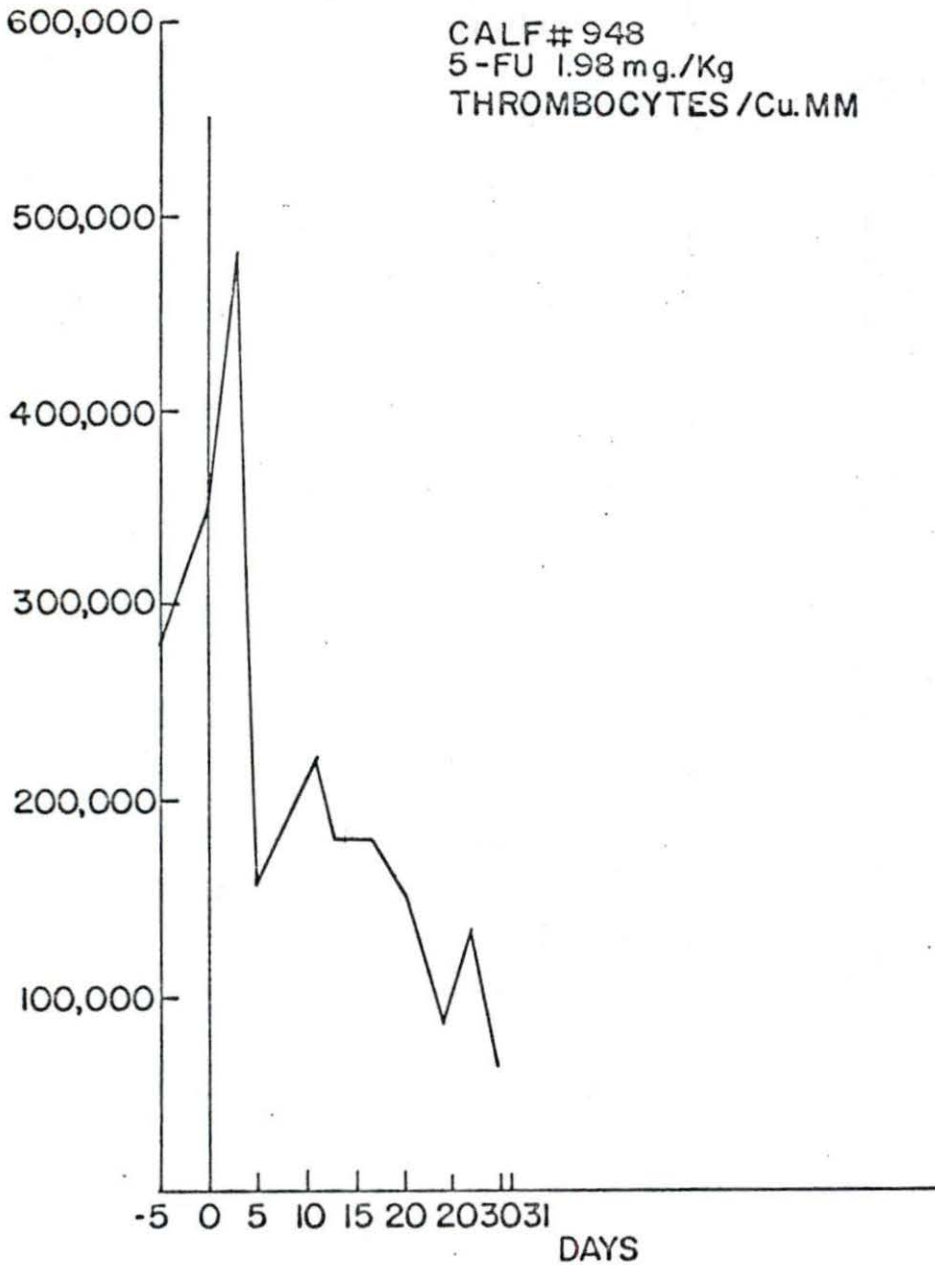


Figure 53. Calf 948. Thrombocyte counts

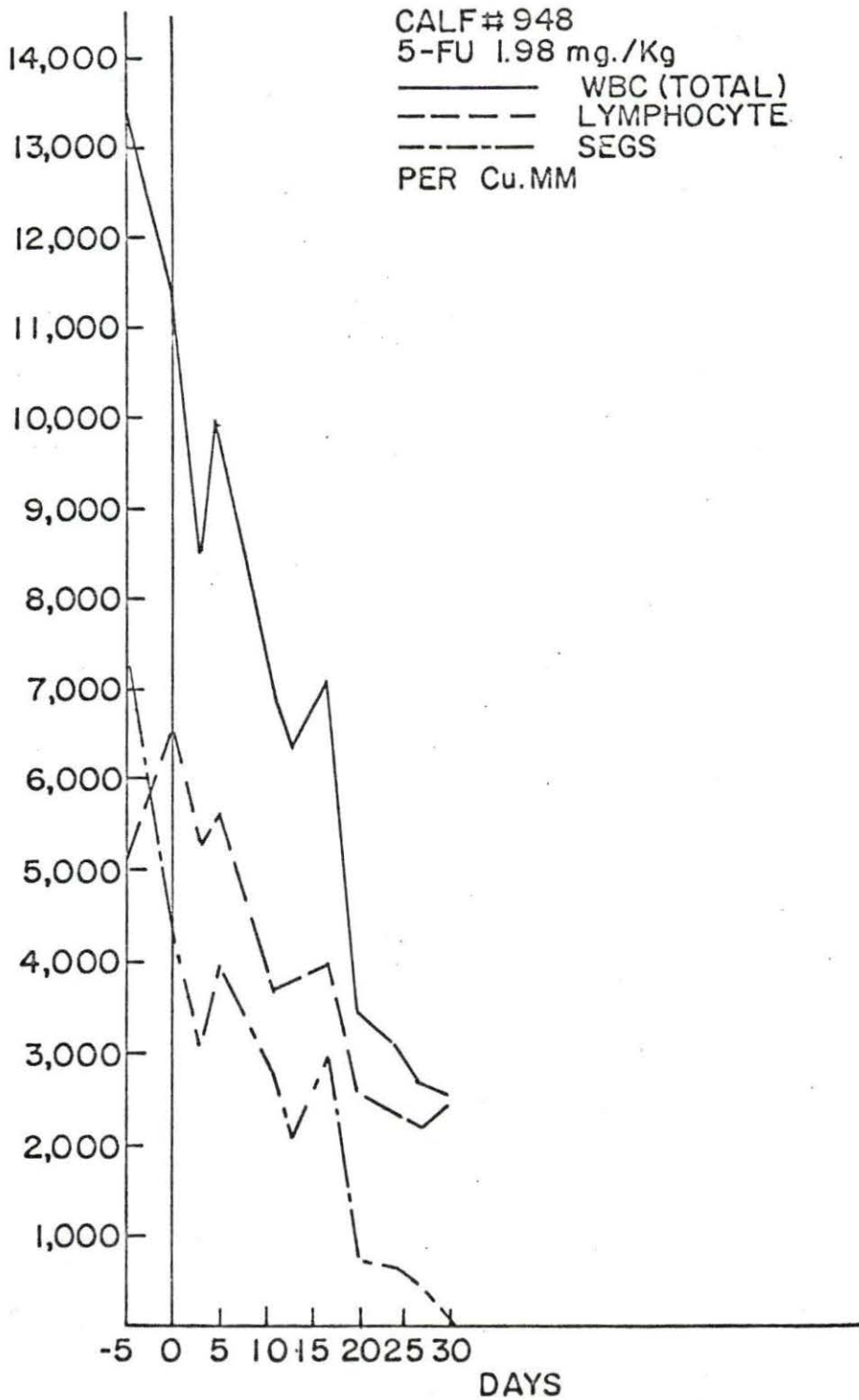


Figure 54. Calf 948. Leukocyte counts

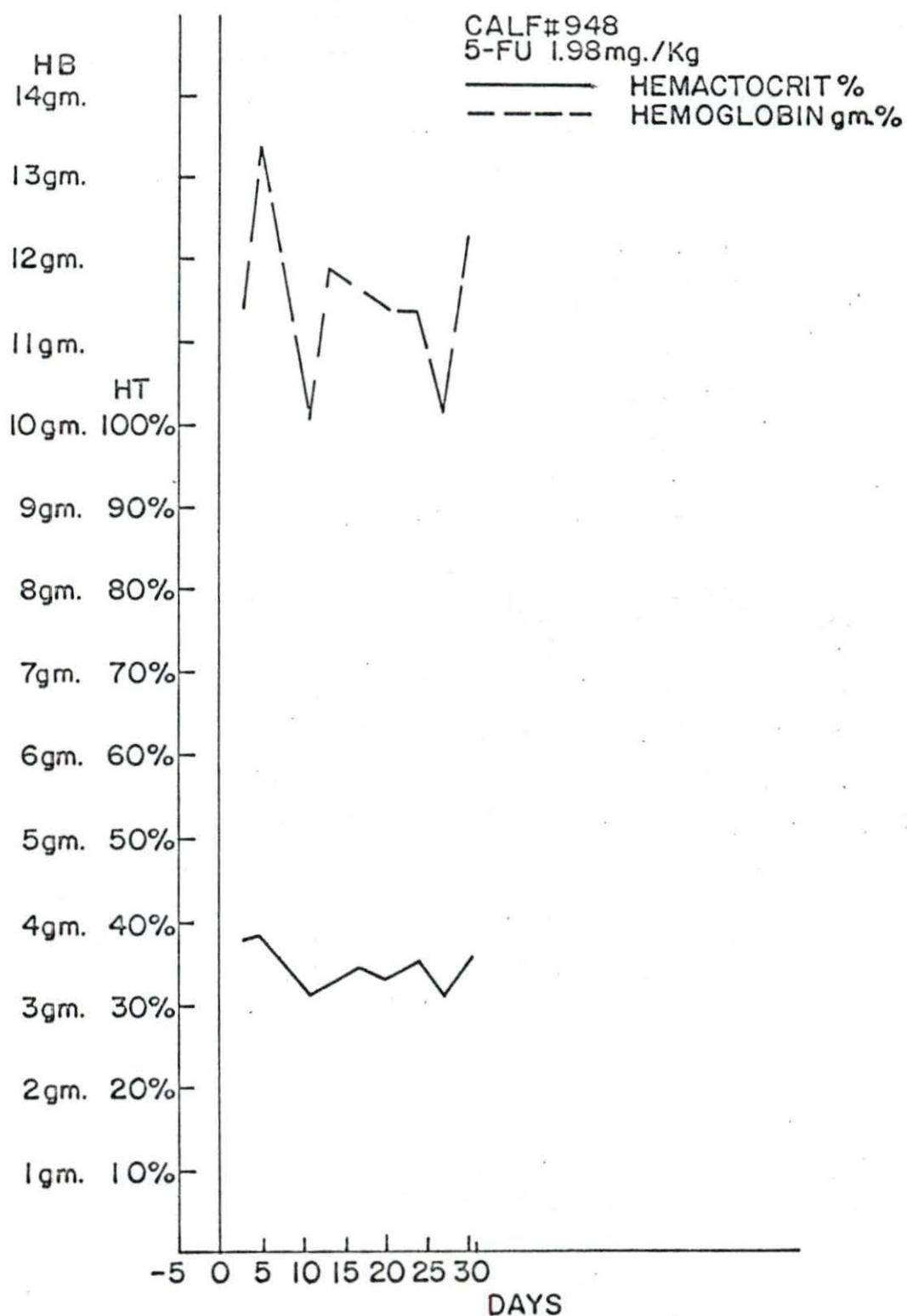


Figure 55. Calf 948. Hematocrit and hemoglobin

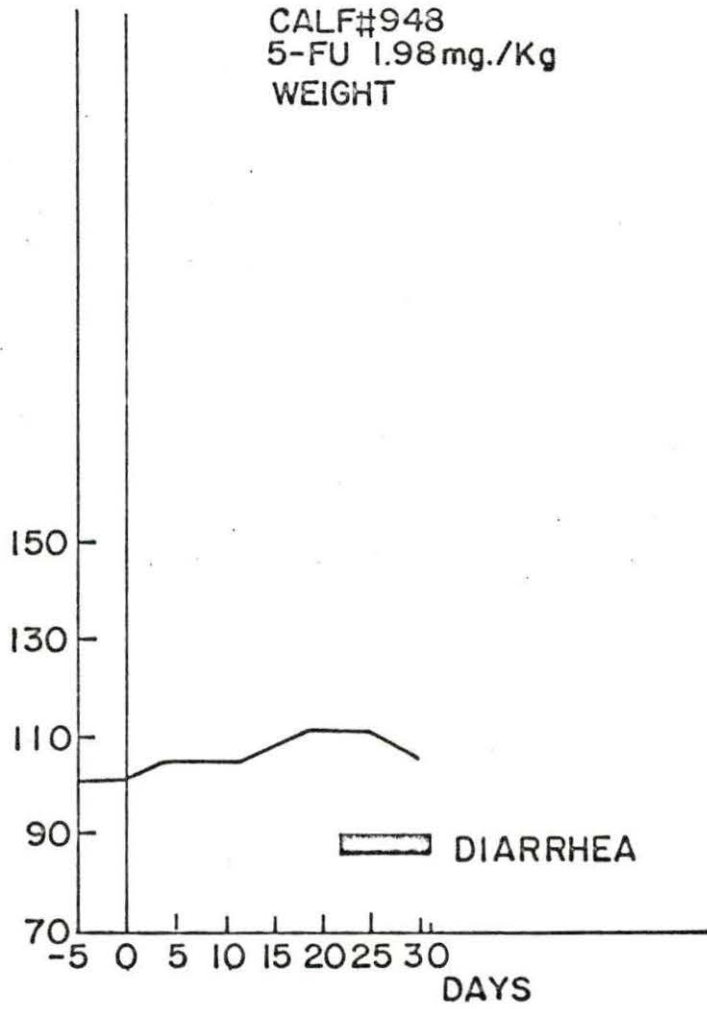


Figure 56. Calf 948. Weight and clinical data

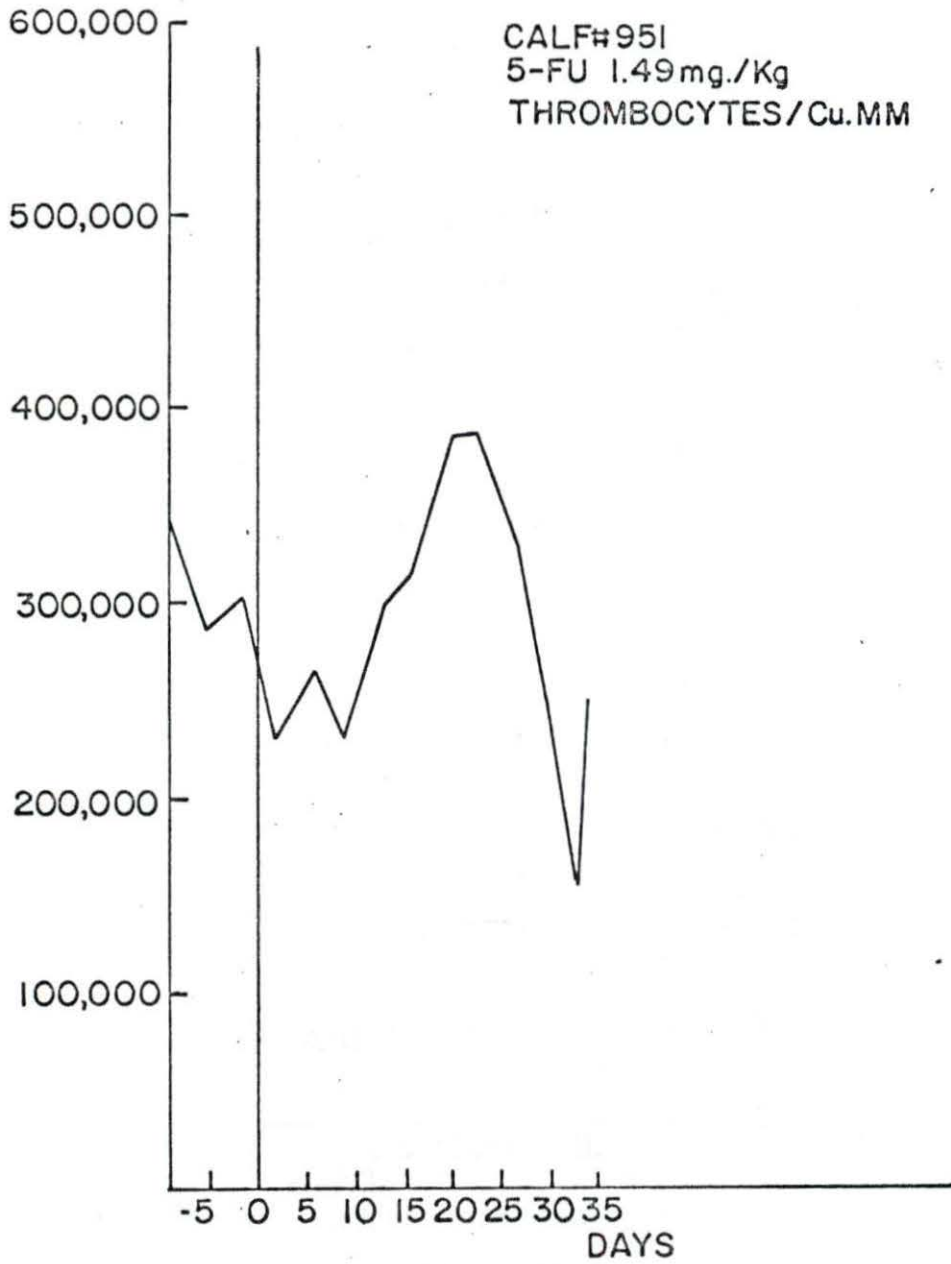


Figure 57. Calf 951. Thrombocyte counts

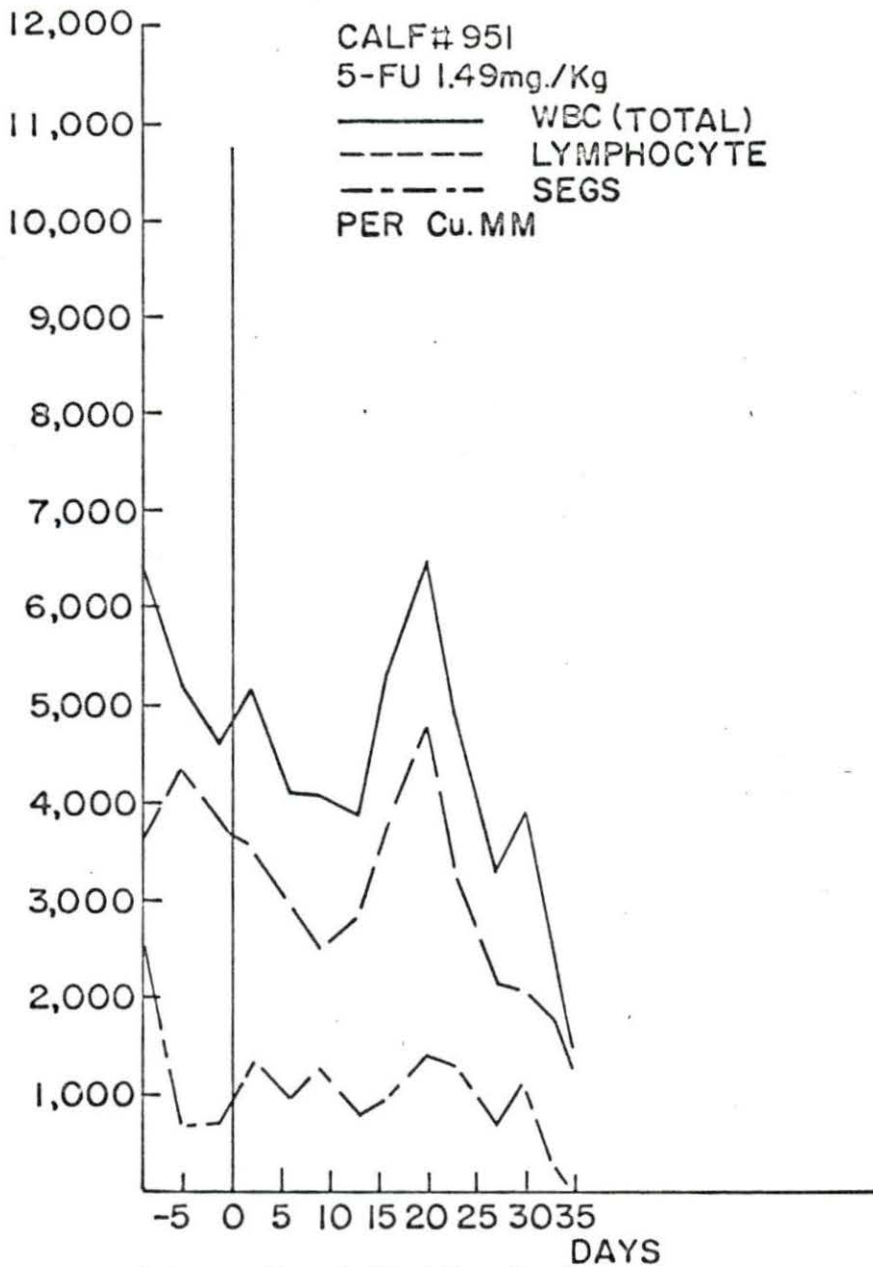


Figure 58. Calf 951. Leukocyte counts

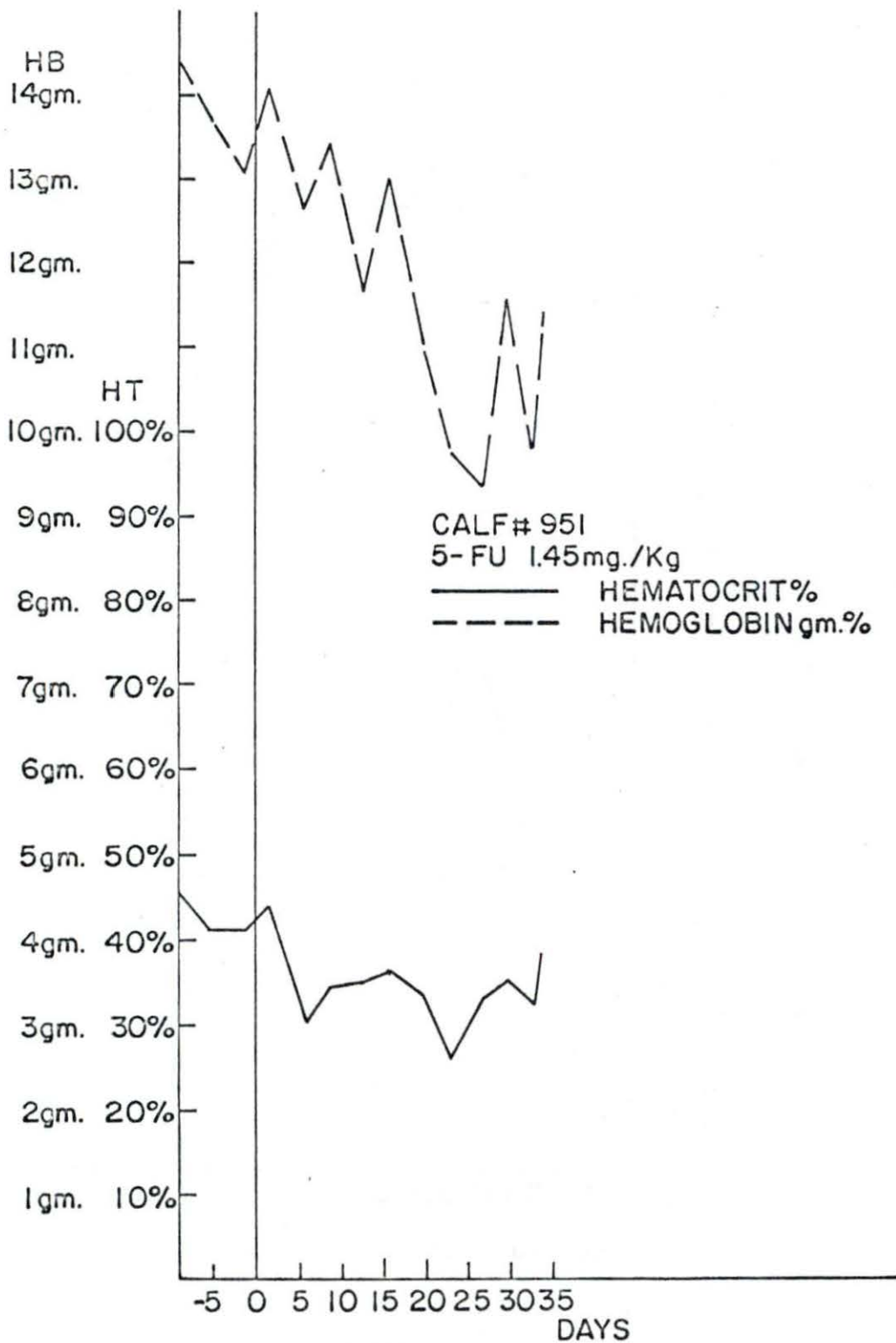


Figure 59. Calf 951. Hematocrit and hemoglobin

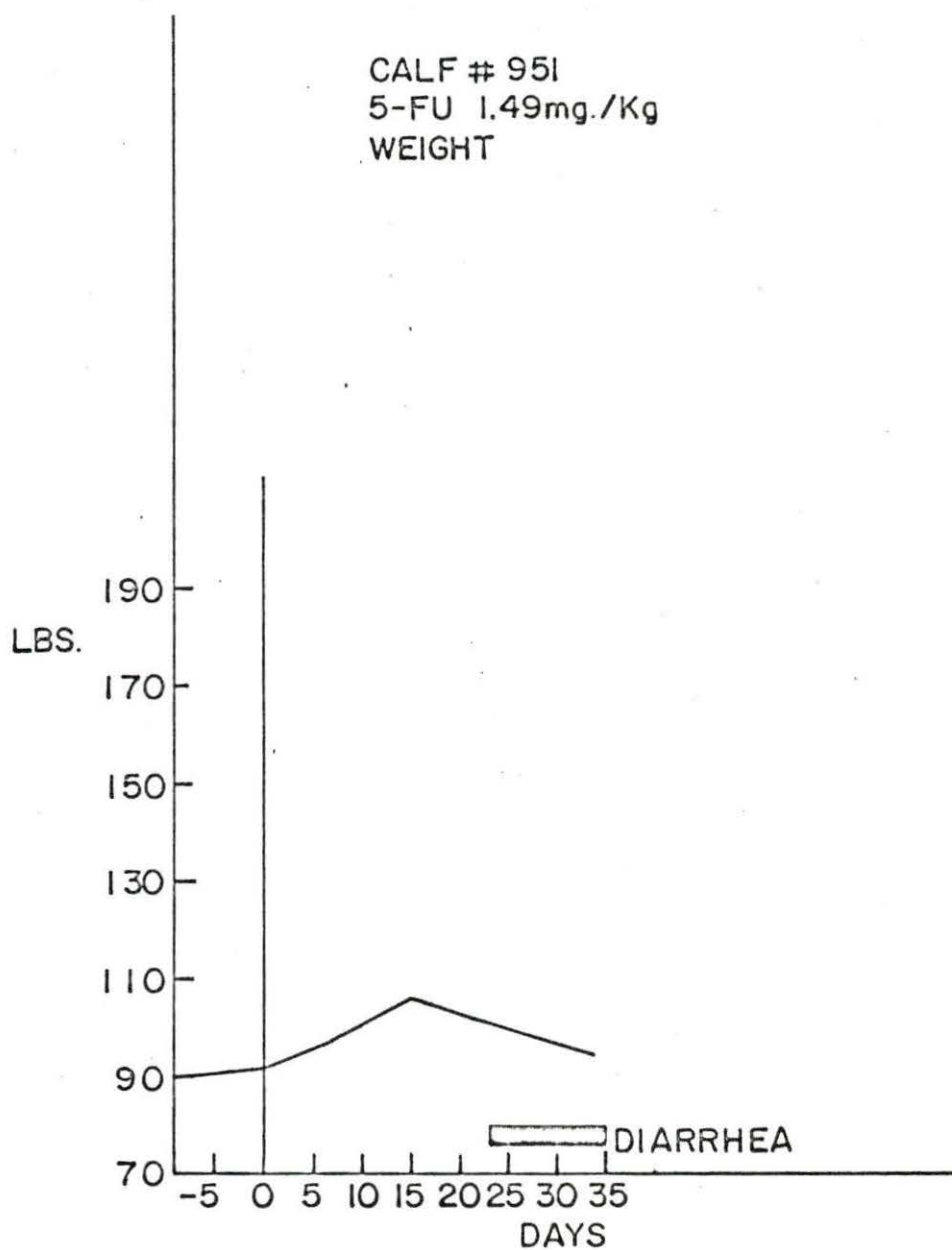


Figure 60. Calf 951. Weight and clinical data

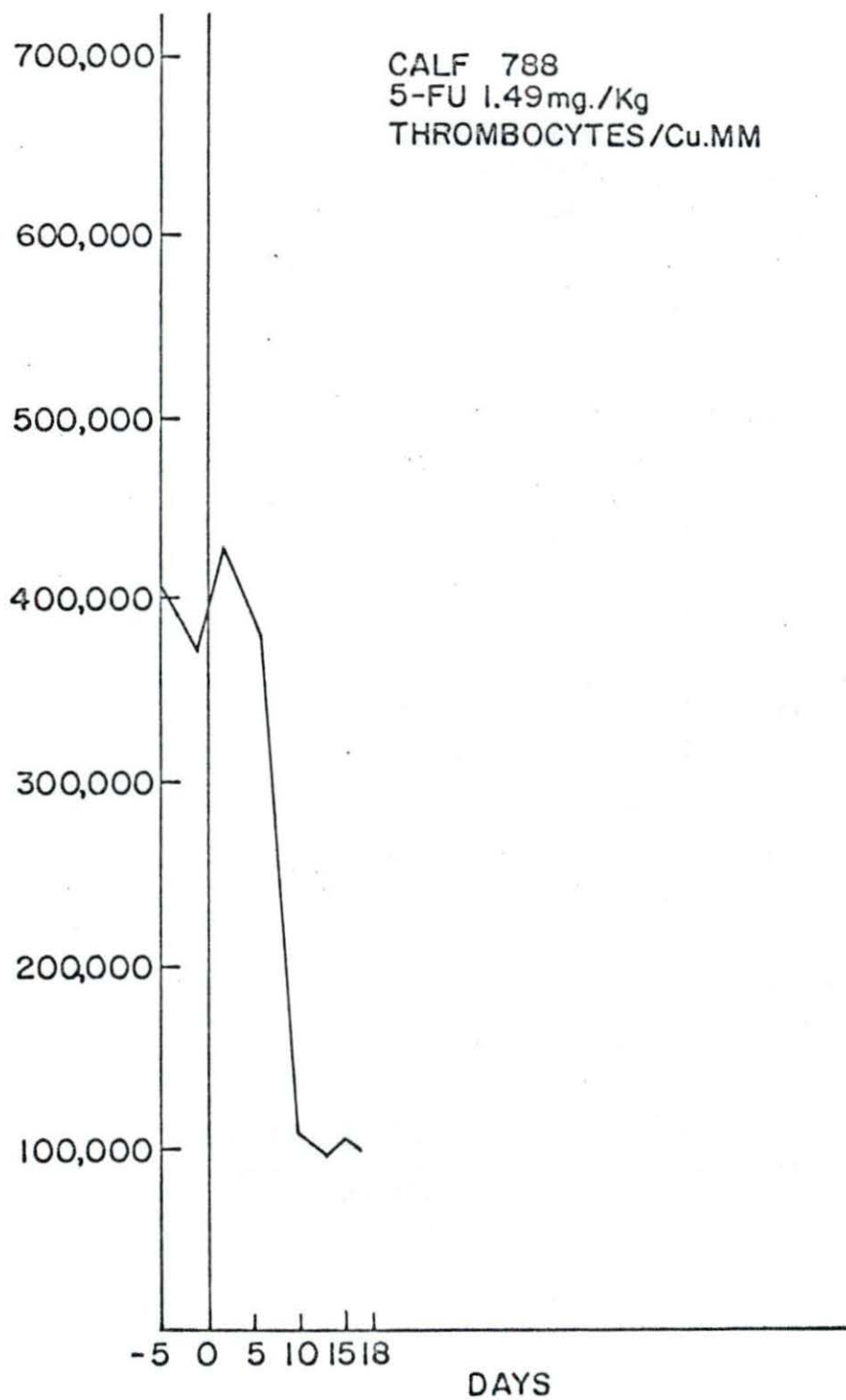


Figure 61. Calf 788. Thrombocyte counts

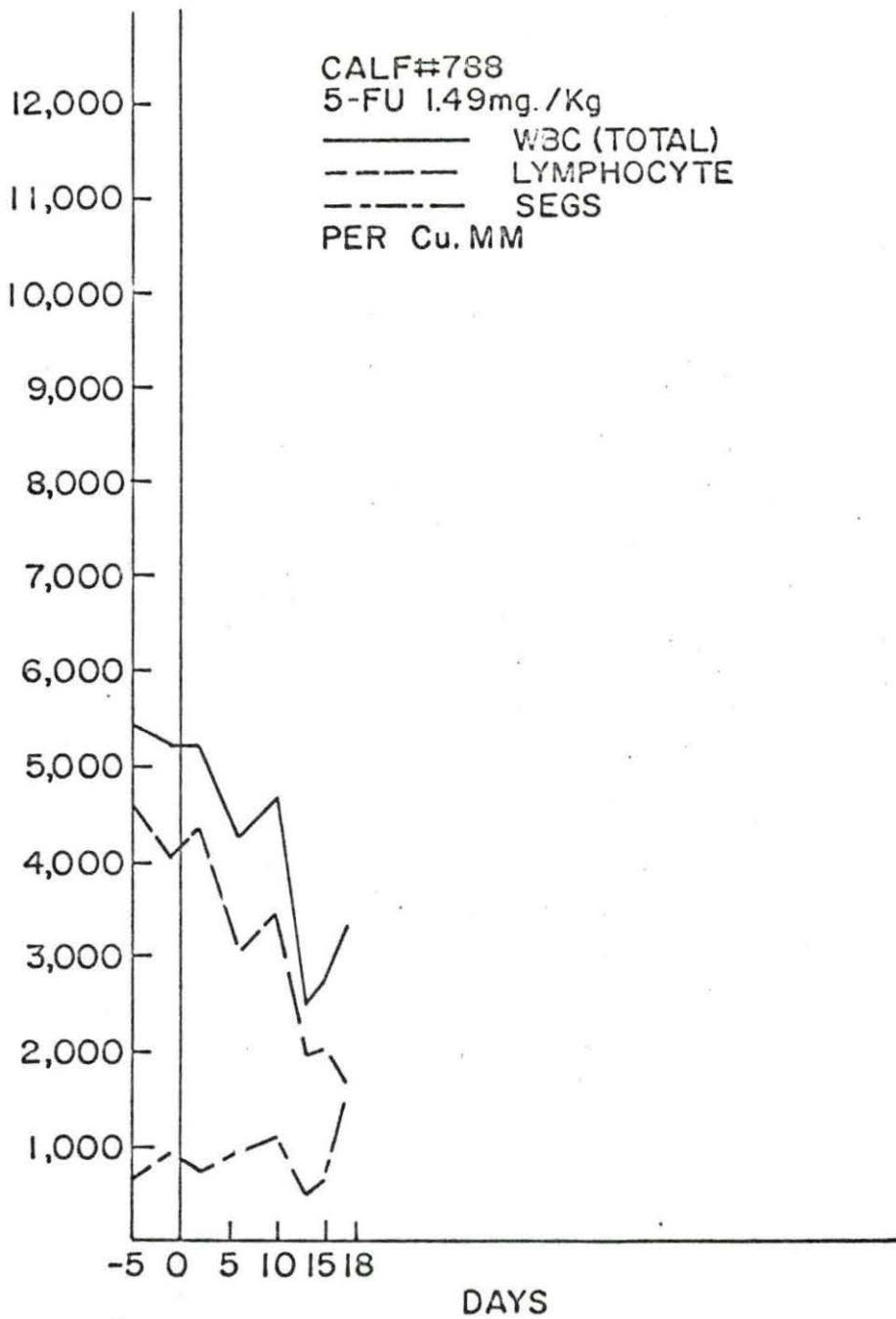


Figure 62. Calf 788. Leukocyte counts

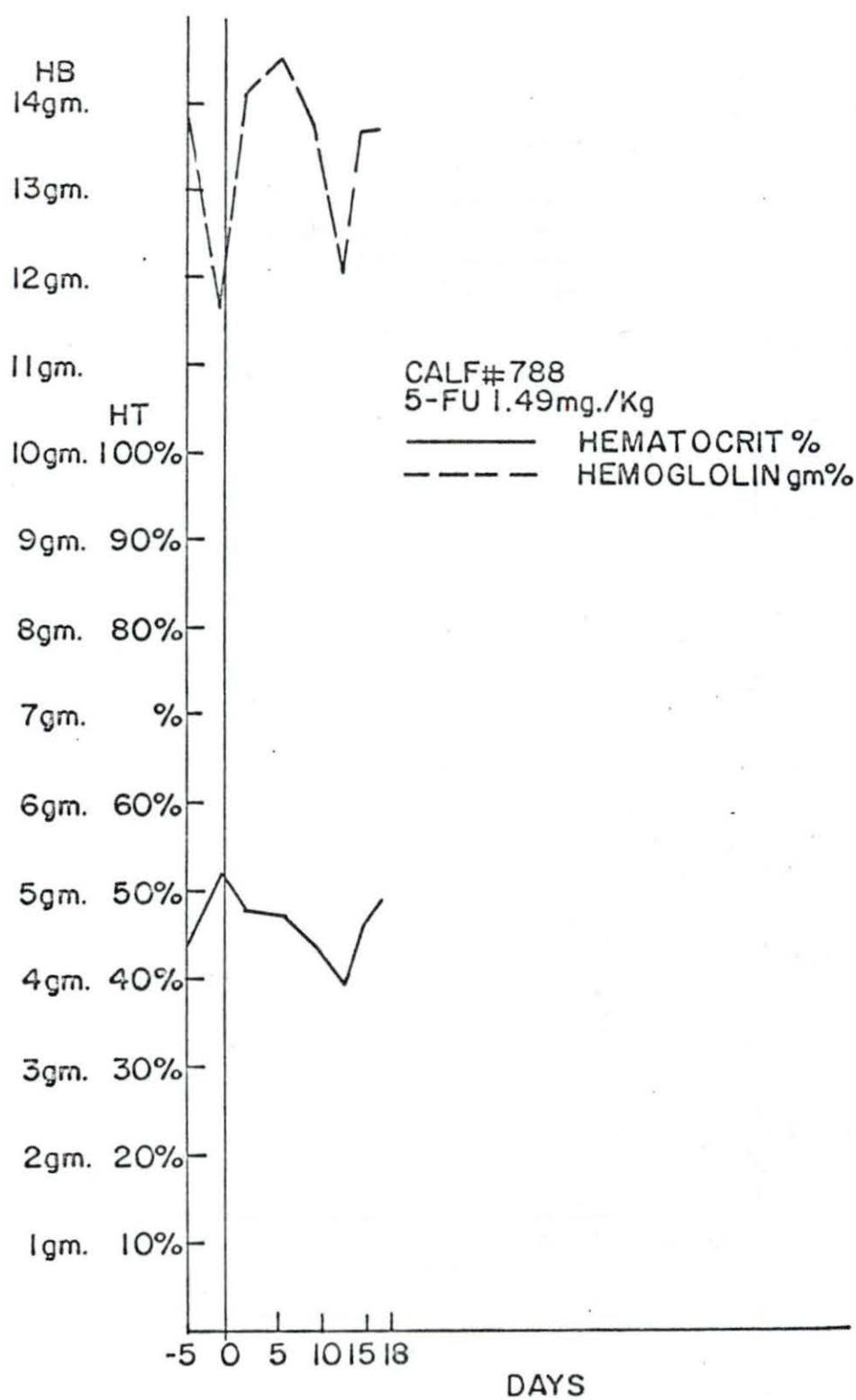


Figure 63. Calf 788. Hematocrit and hemoglobin

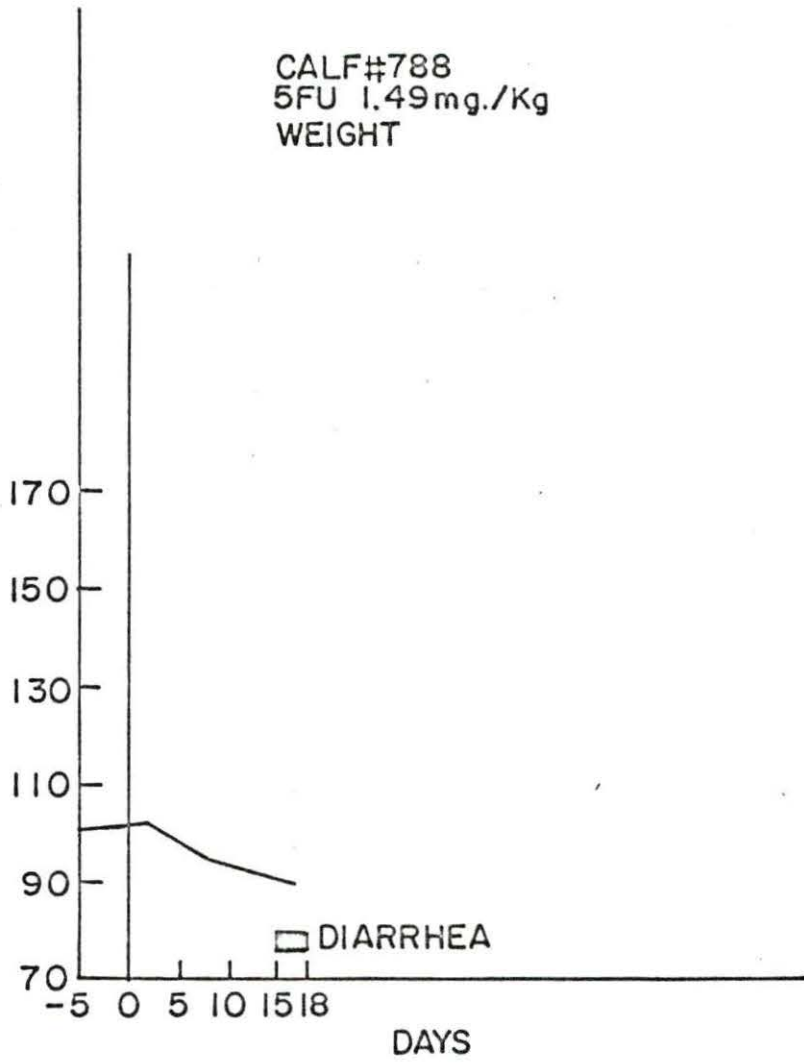


Figure 64. Calf 788. Weight and clinical data

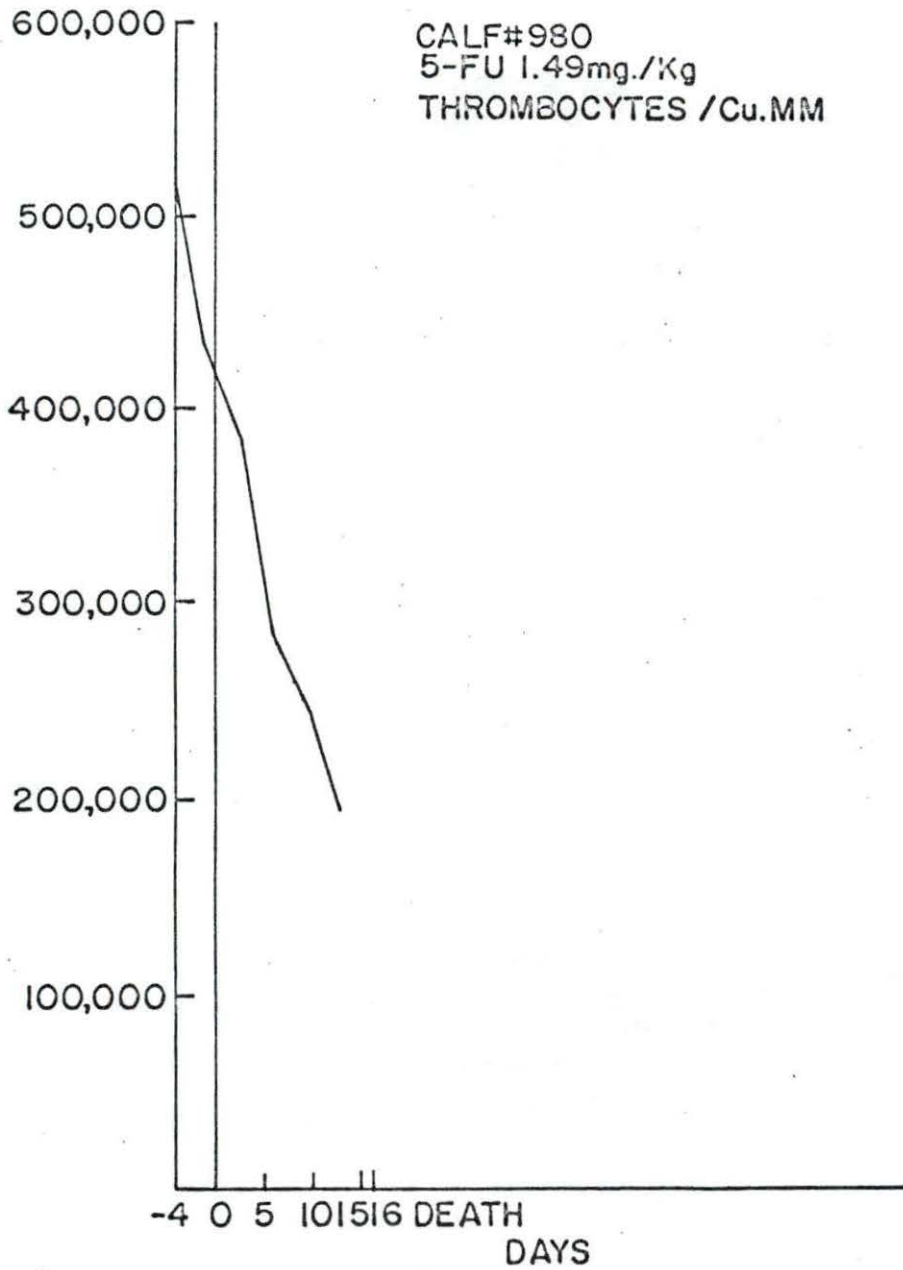


Figure 65. Calf 980. Thrombocyte counts

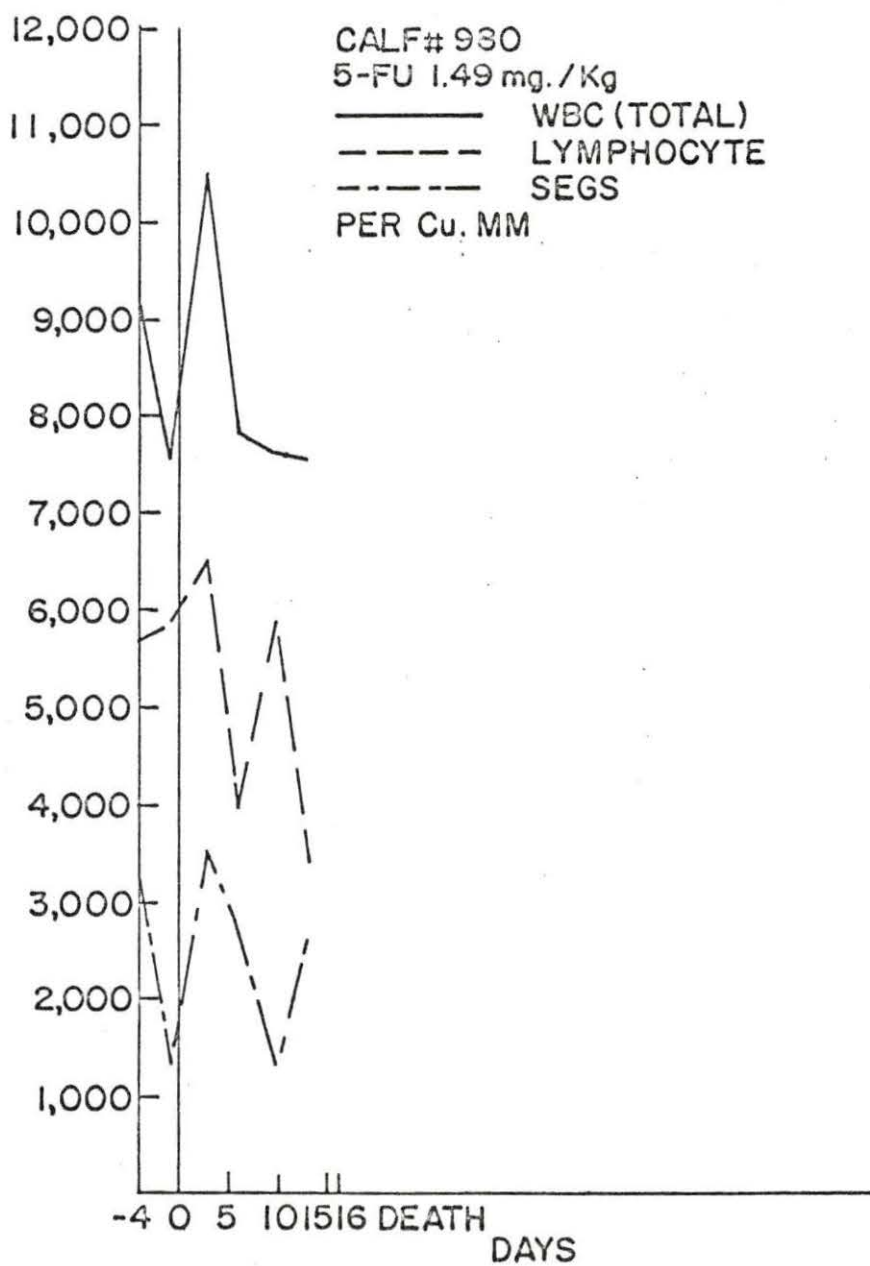


Figure 66. Calf 980. Leukocyte counts

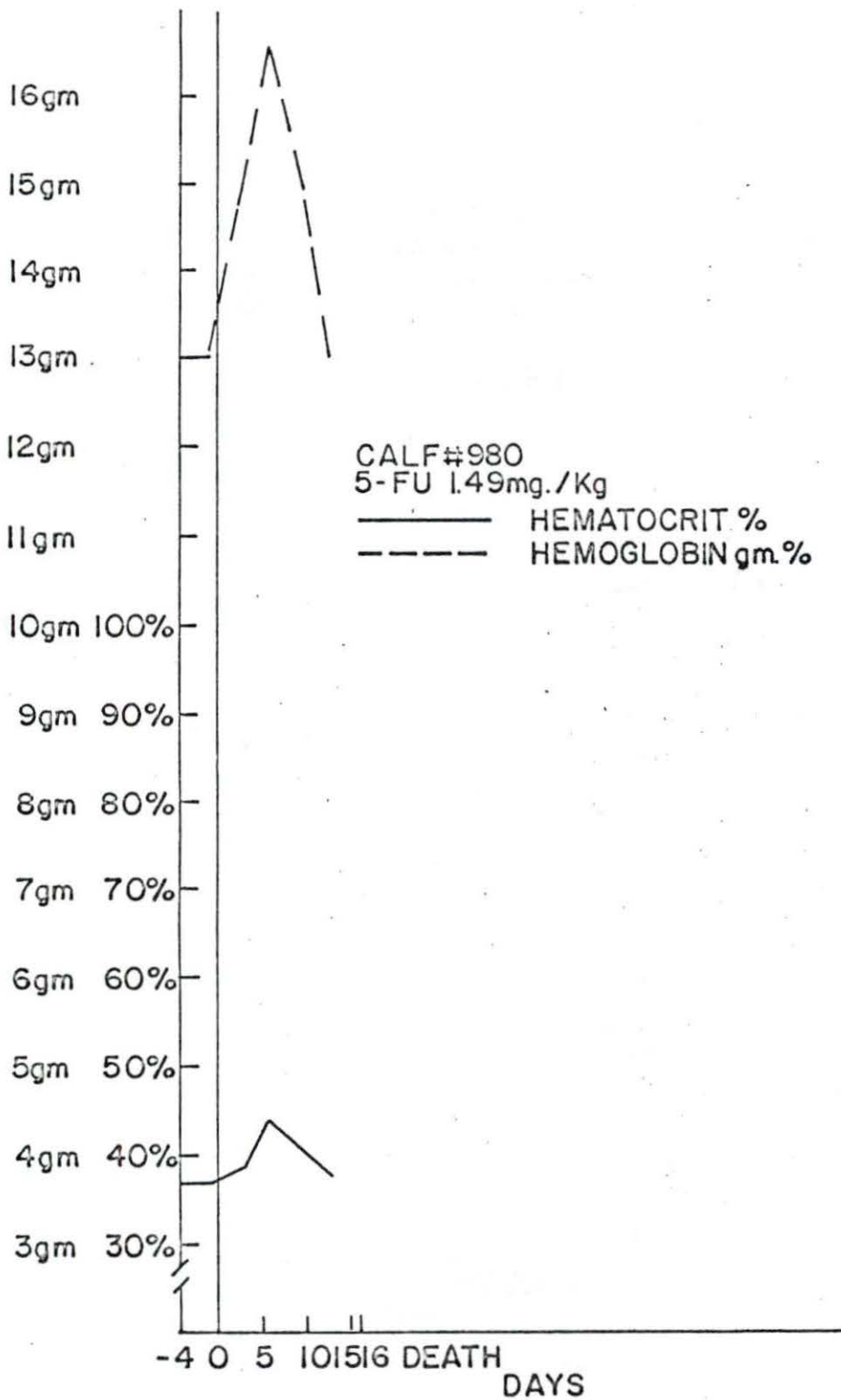


Figure 67. Calf 980. Hematocrit and hemoglobin

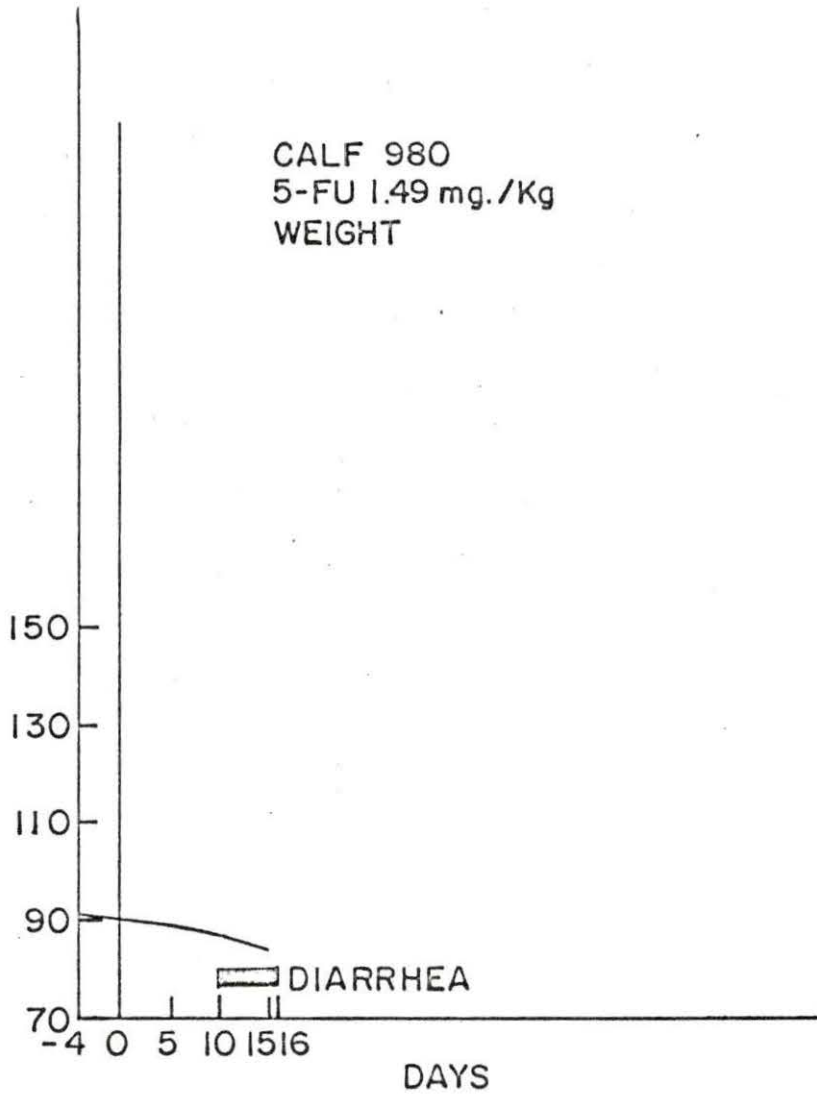


Figure 68. Calf 980. Weight and clinical data

APPENDIX VI. CALVES TREATED WITH AMETHOPTERIN

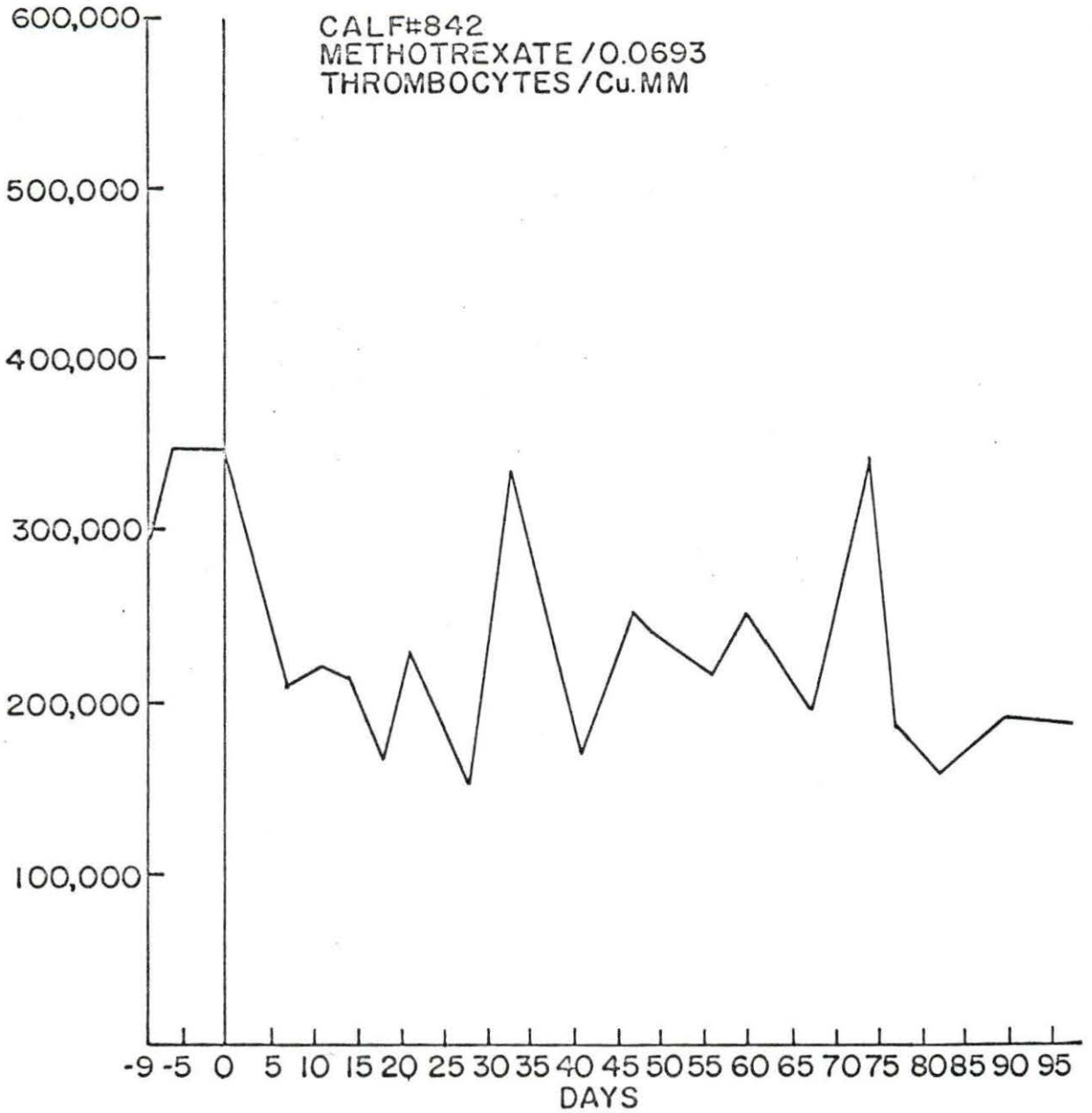


Figure 69. Calf 842. Thrombocyte counts

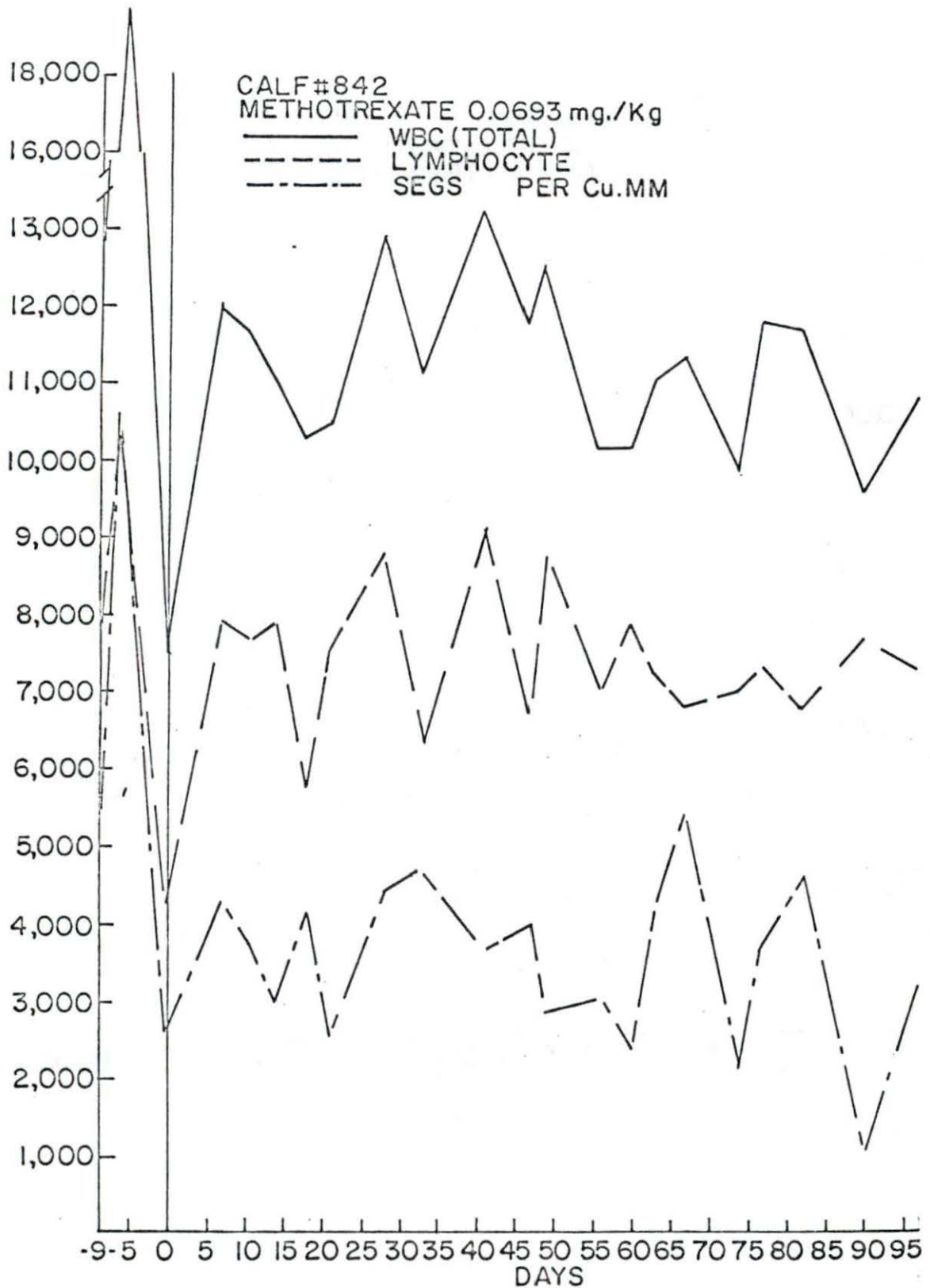


Figure 70. Calf 842. Leukocyte counts

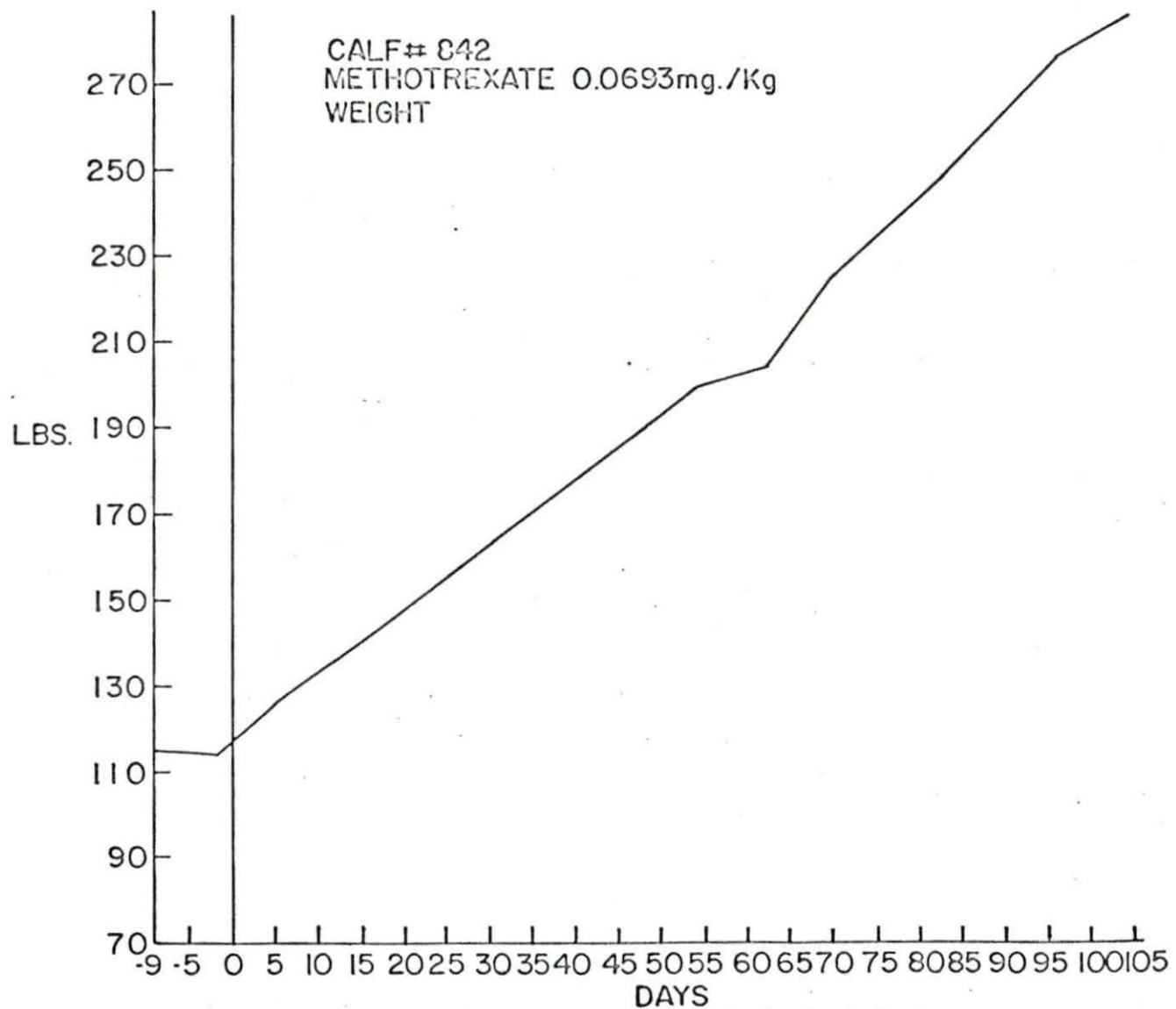


Figure 71. Calf 842. Weight and clinical data

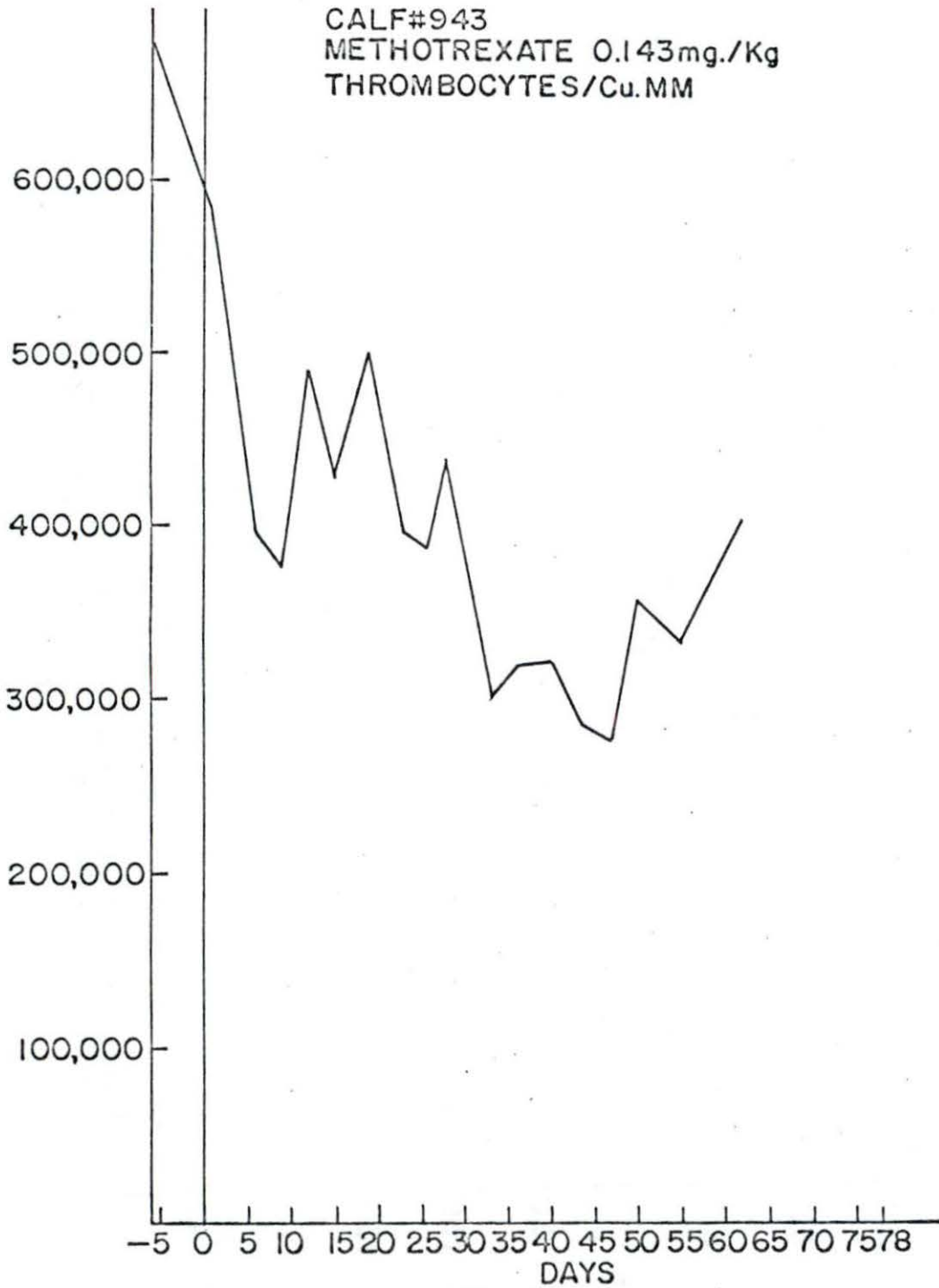


Figure 72, Calf 943. Thrombocyte counts

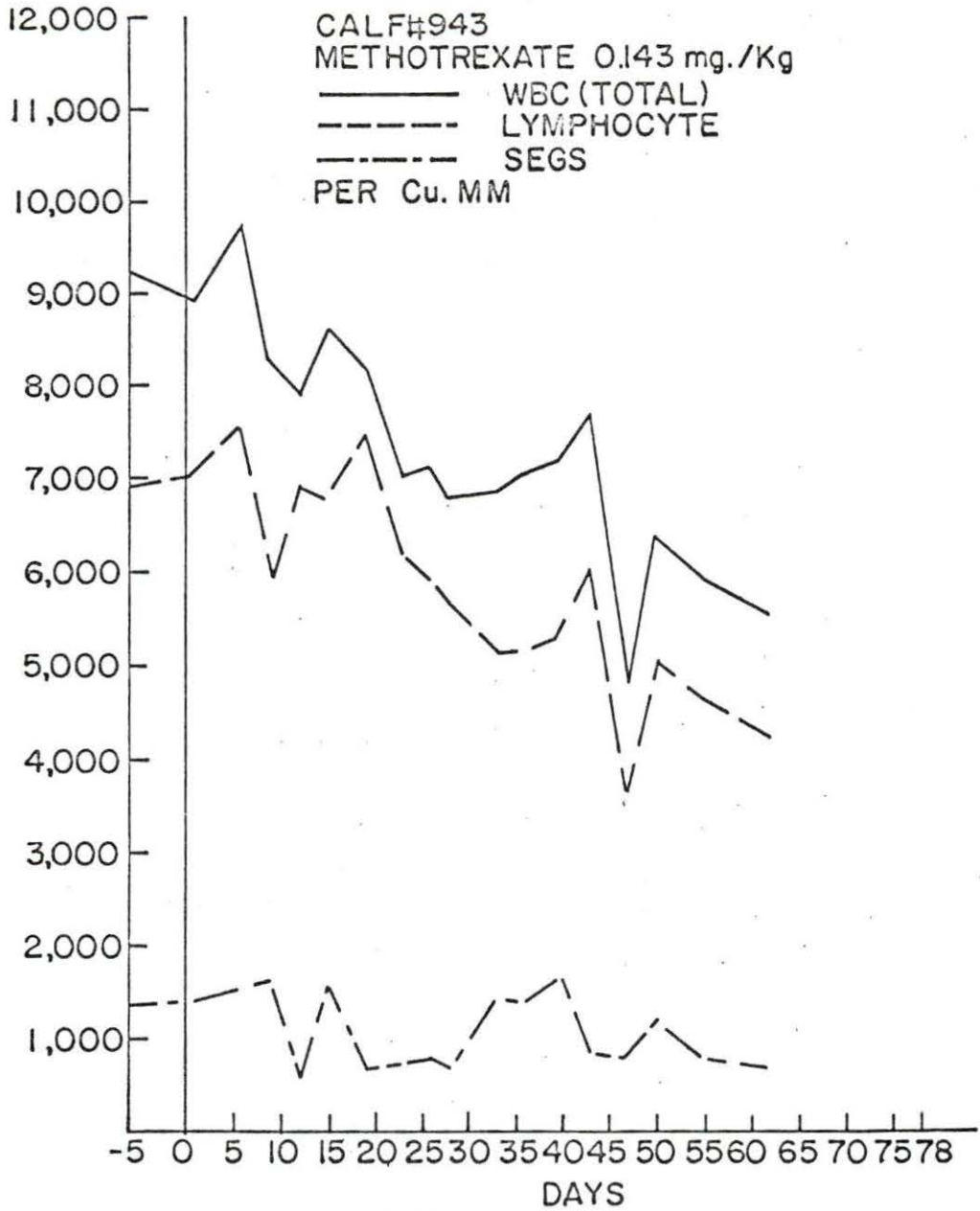


Figure 73. Calf 943. Leukocyte counts

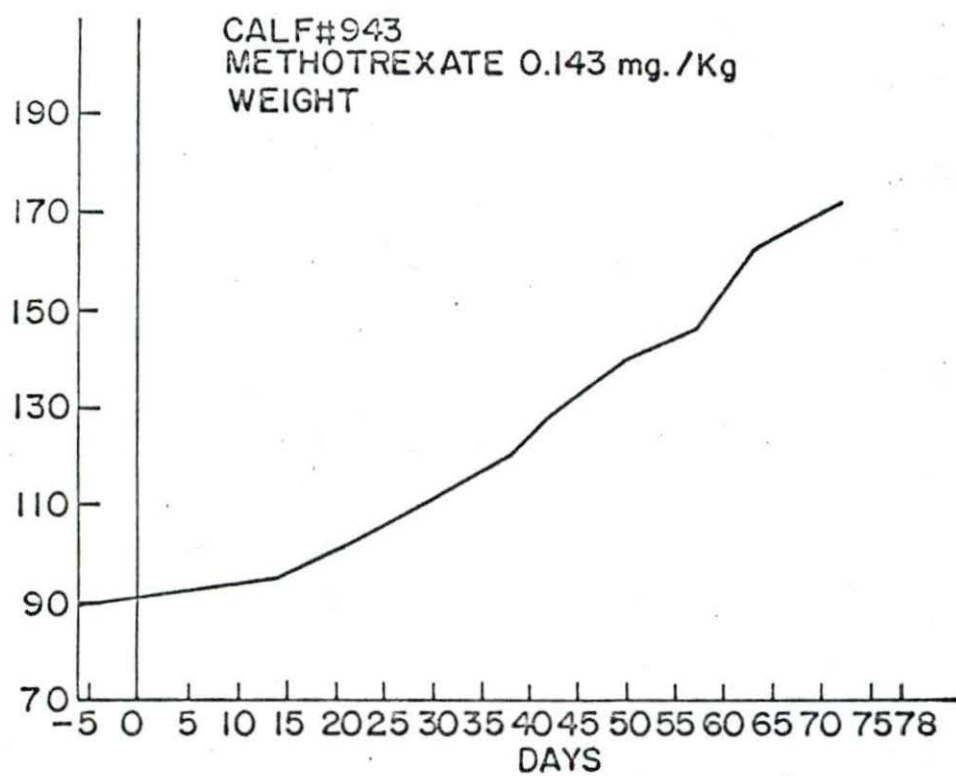


Figure 74. Calf 943. Weight and clinical data

CALF # 978
METHOTREXATE 0.277mg./Kg
THROMBOCYTES /Cu.MM

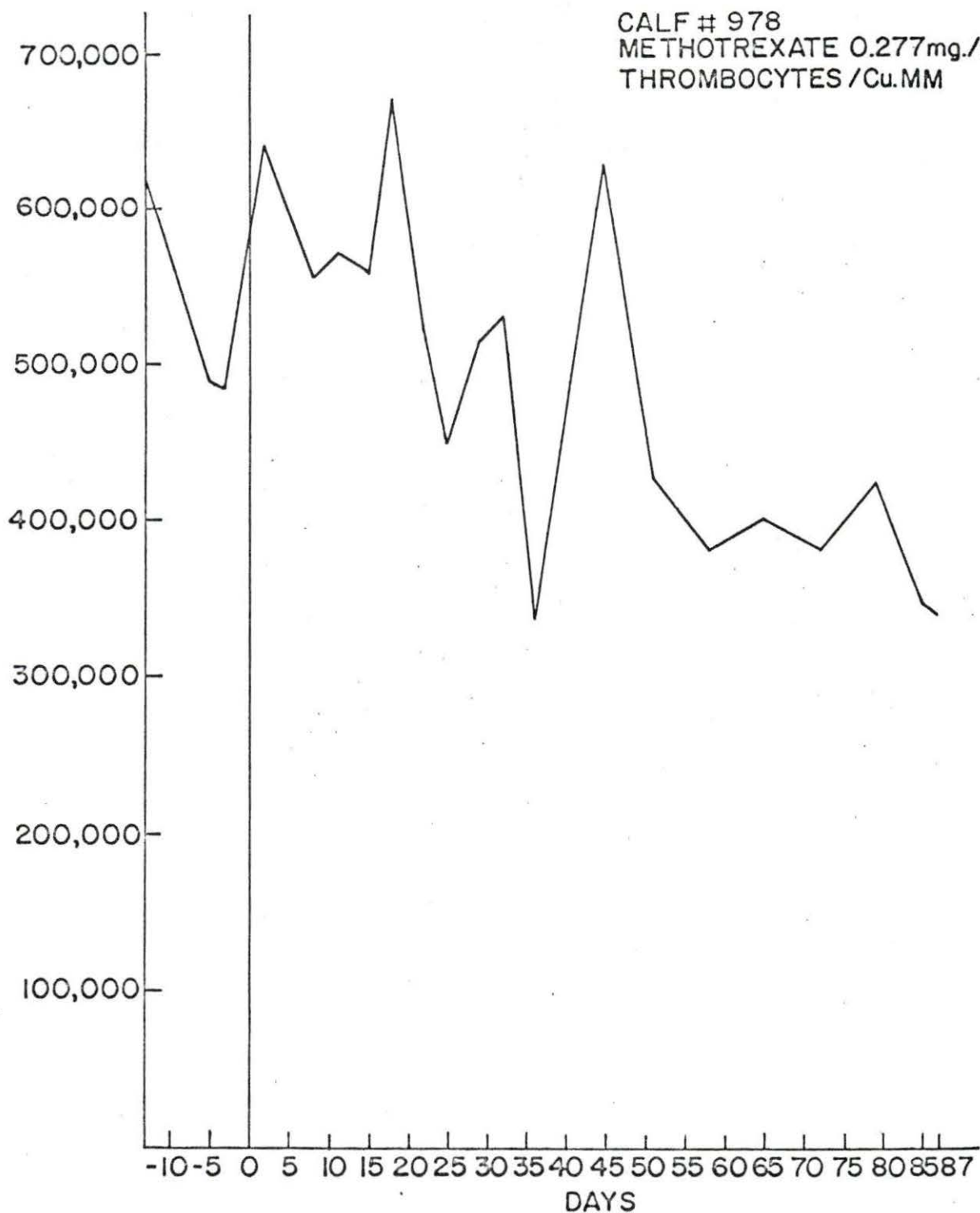


Figure 75. Calf 978. Thrombocyte counts

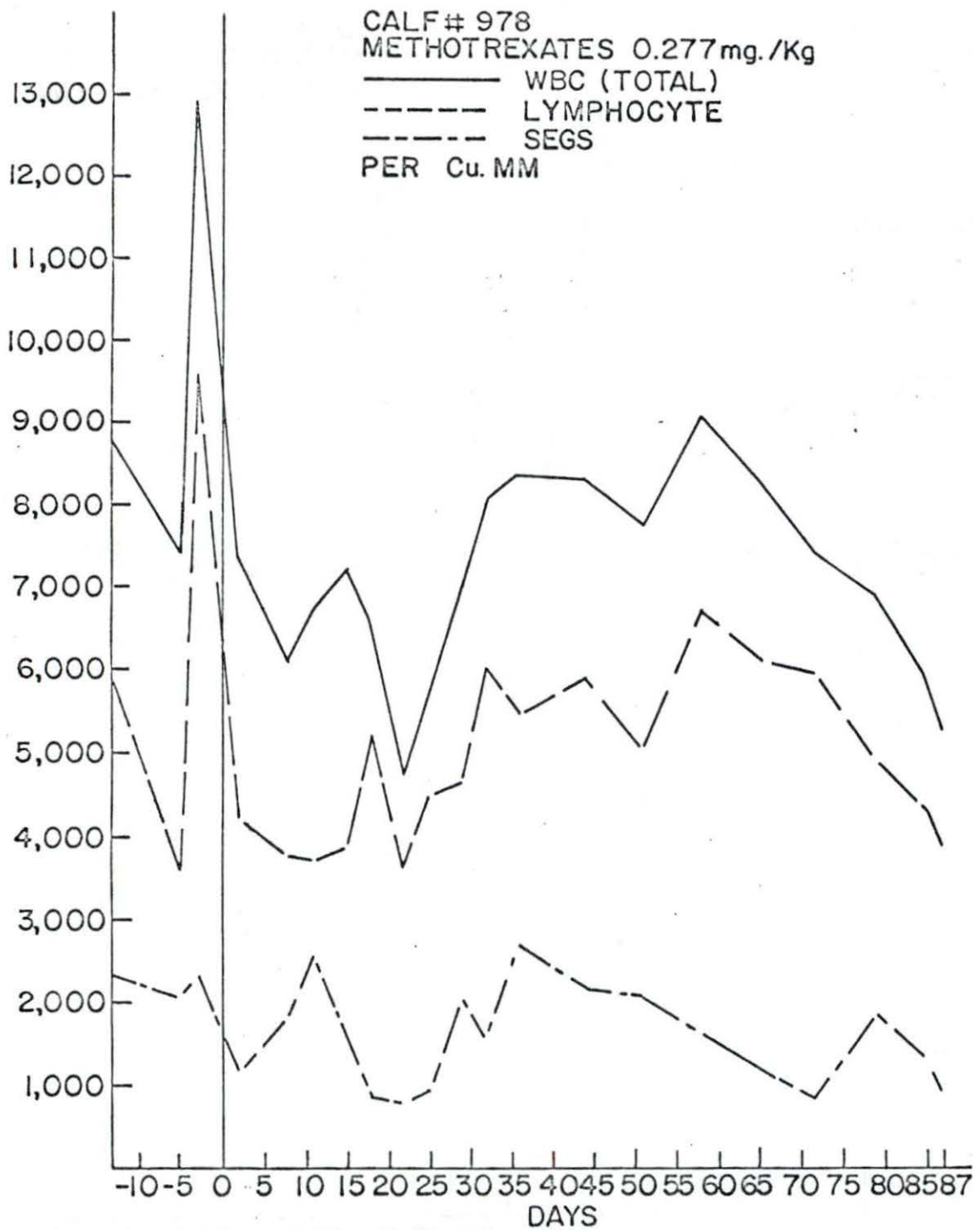


Figure 76. Calf 978. Leukocyte counts

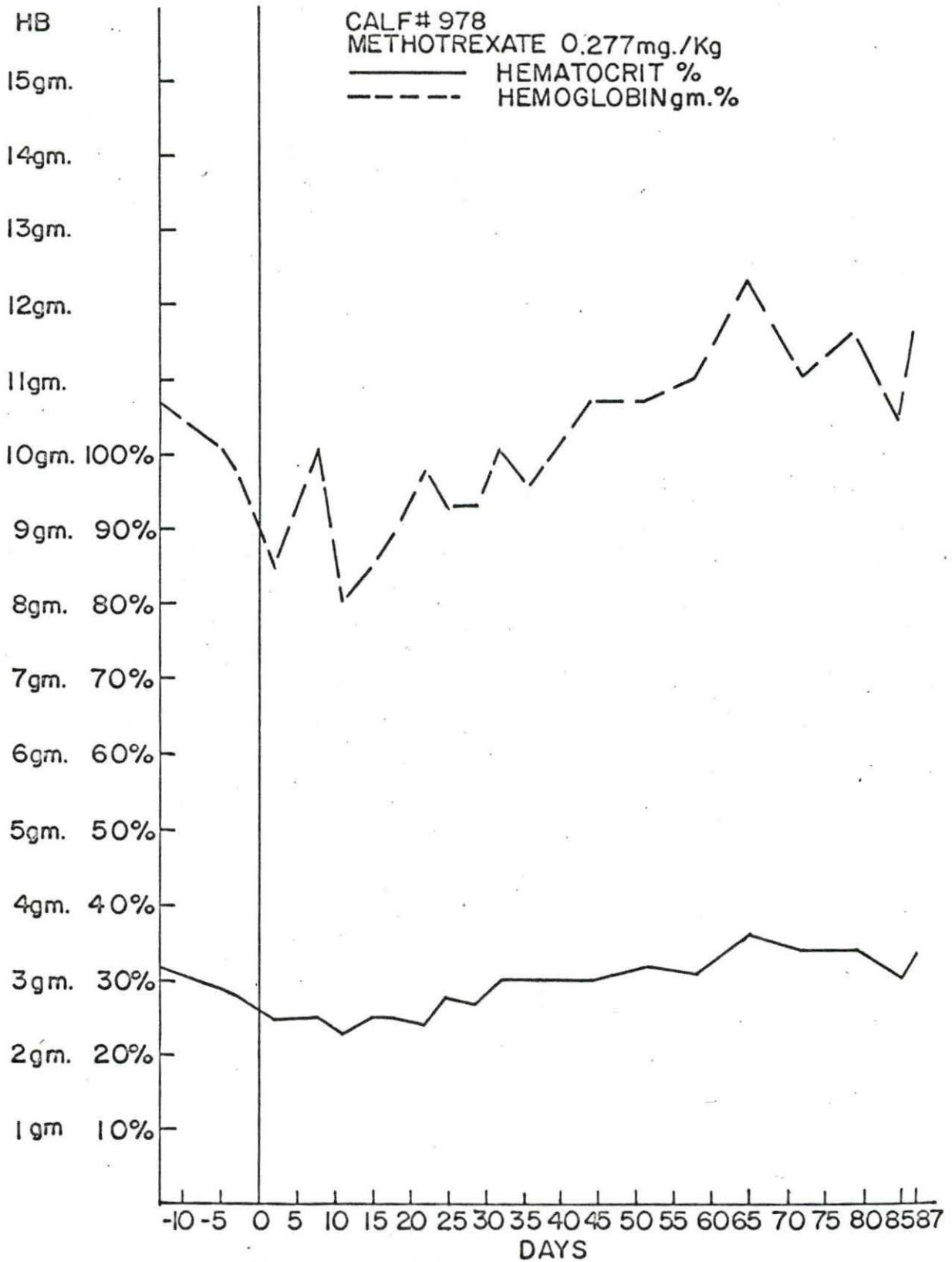


Figure 77. Calf 978. Hematocrit and hemoglobin

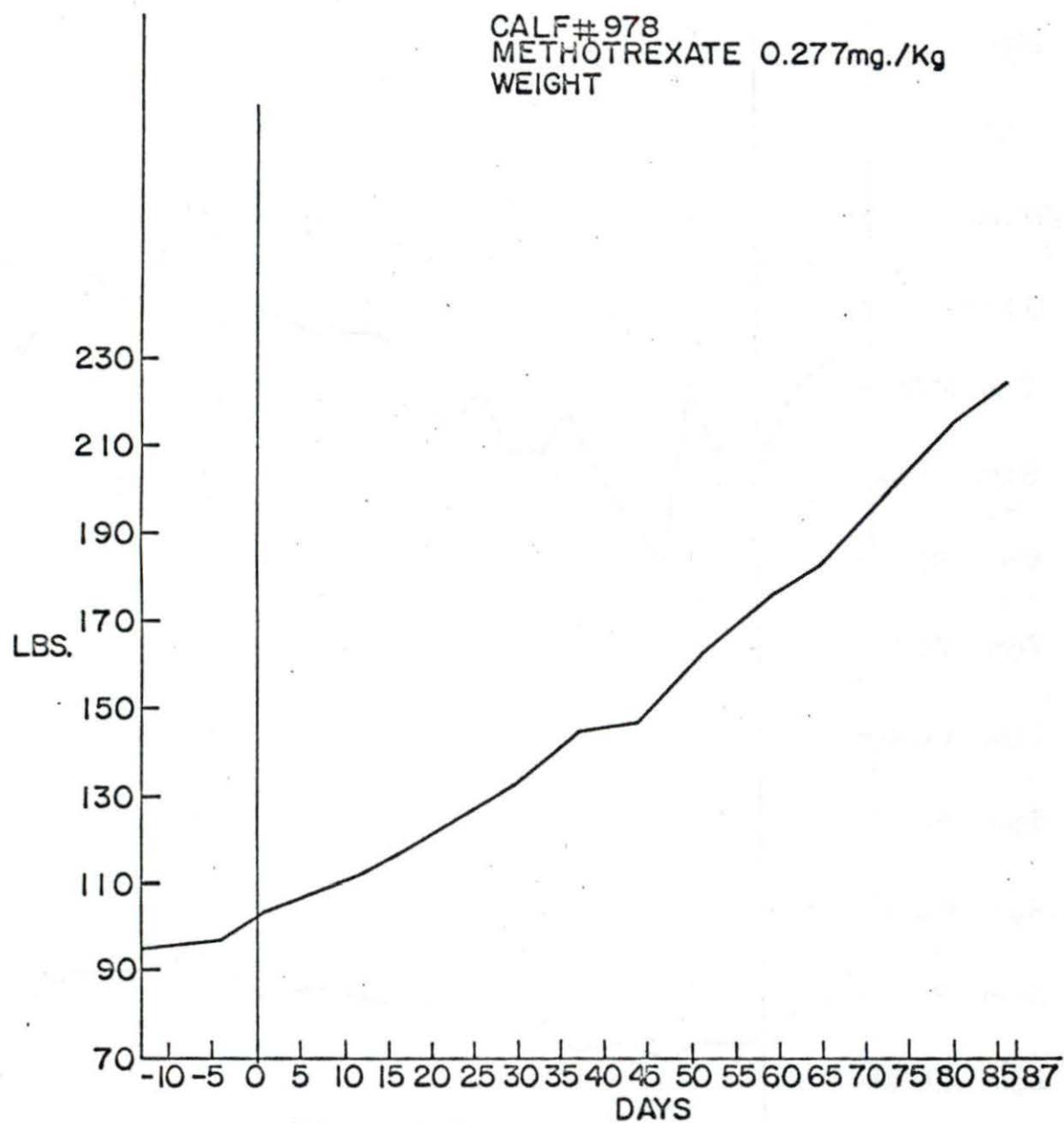


Figure 78. Calf 978. Weight and clinical data

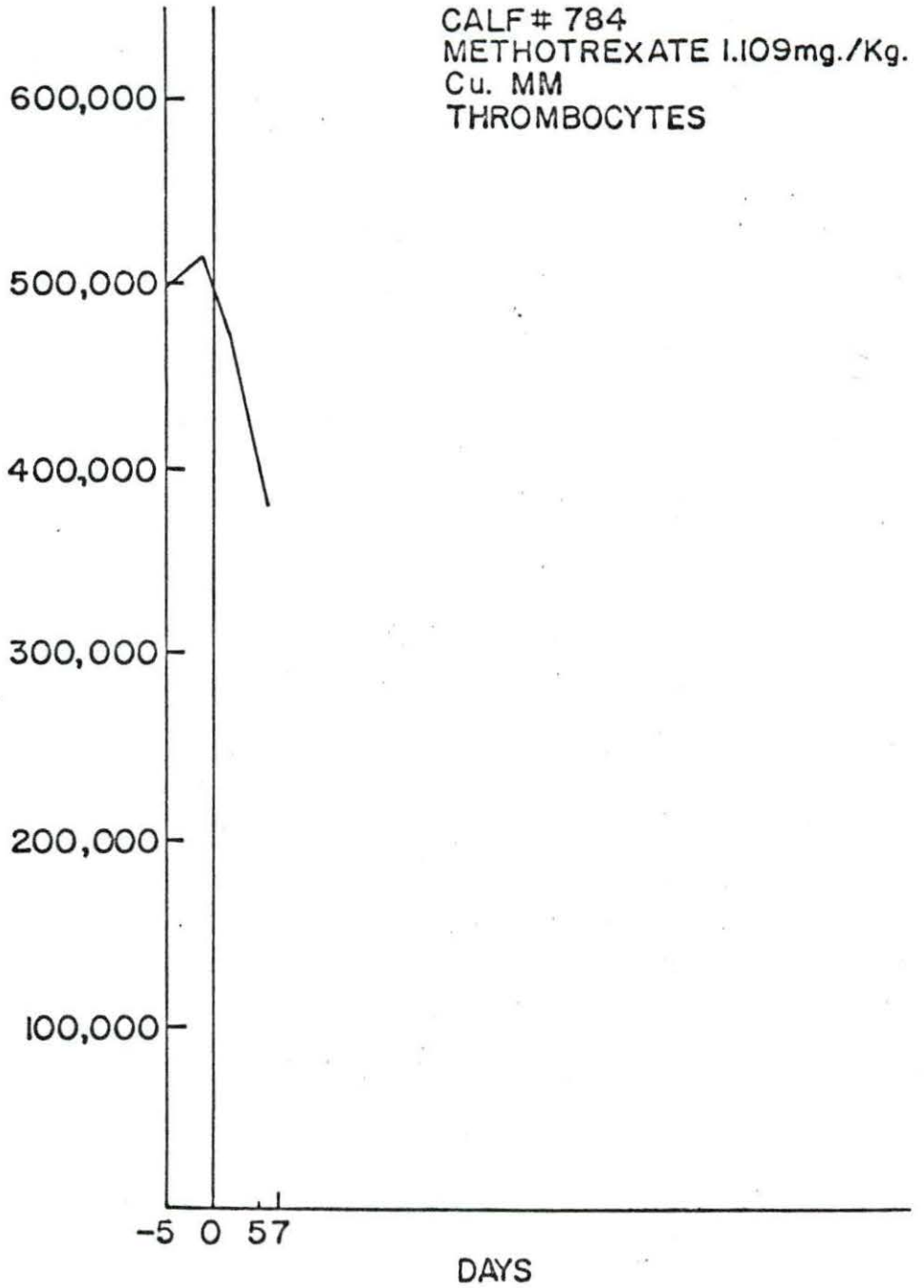


Figure 79. Calf 784. Thrombocyte counts

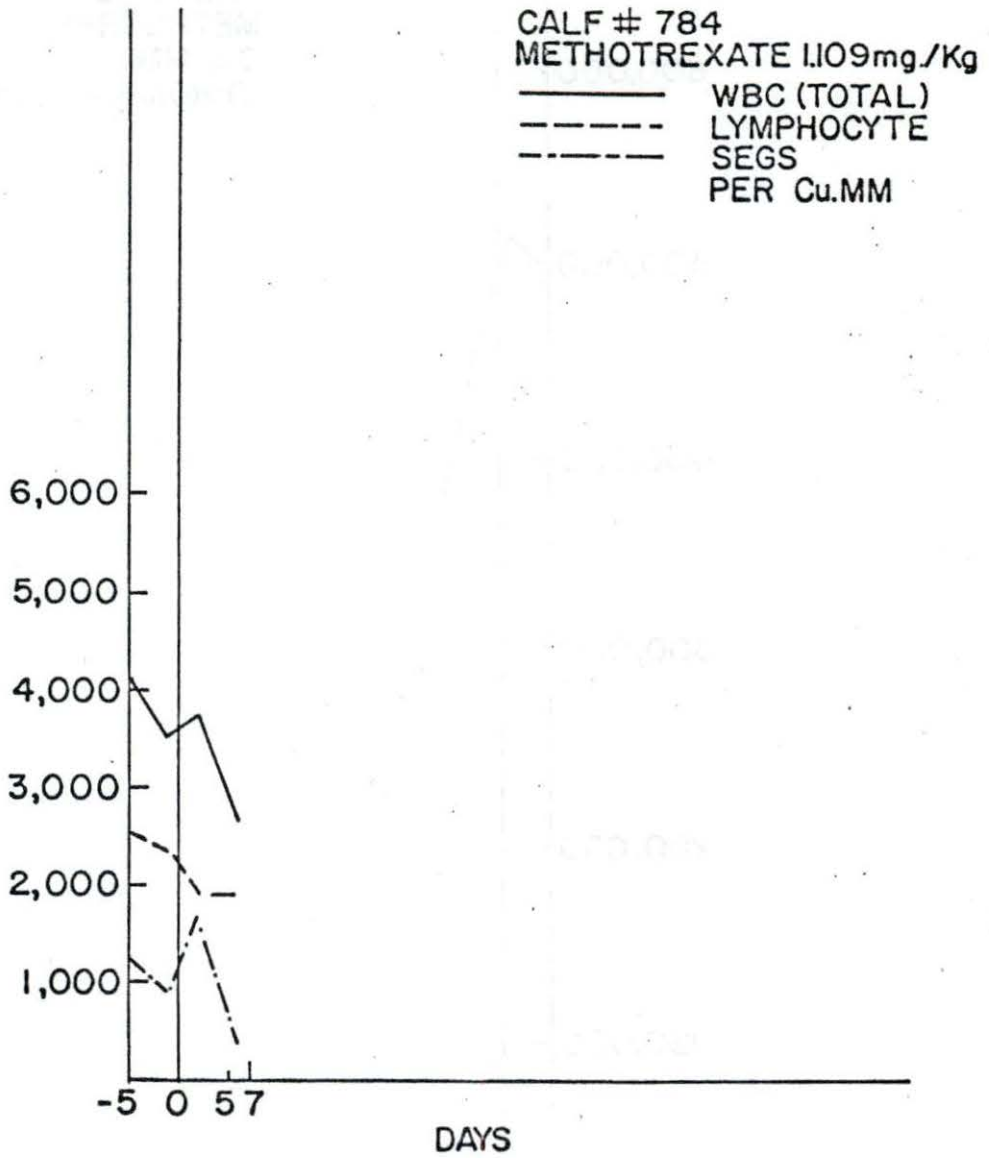


Figure 80. Calf 784. Leukocyte counts

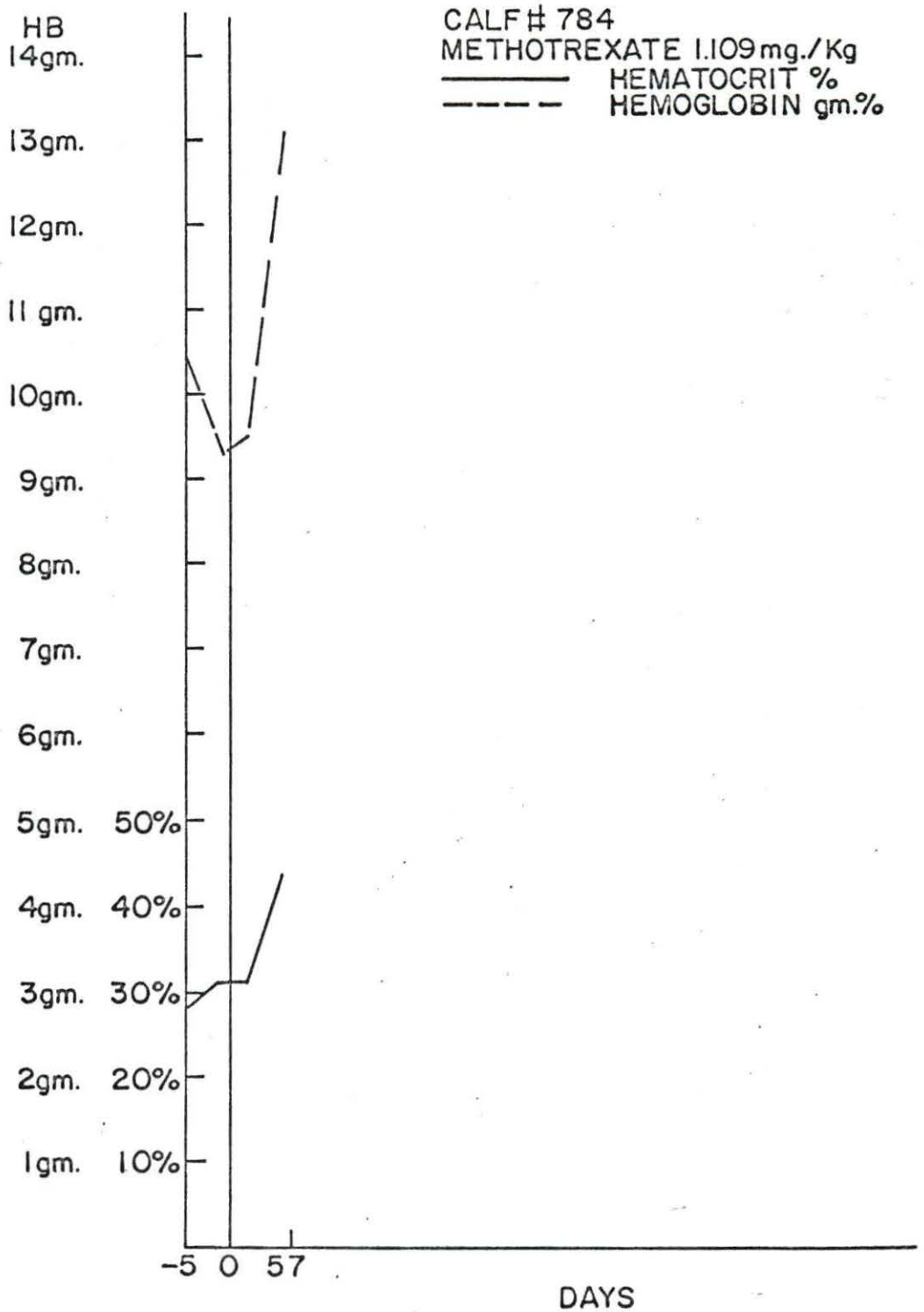


Figure 81. Calf 784. Hematocrit and hemoglobin

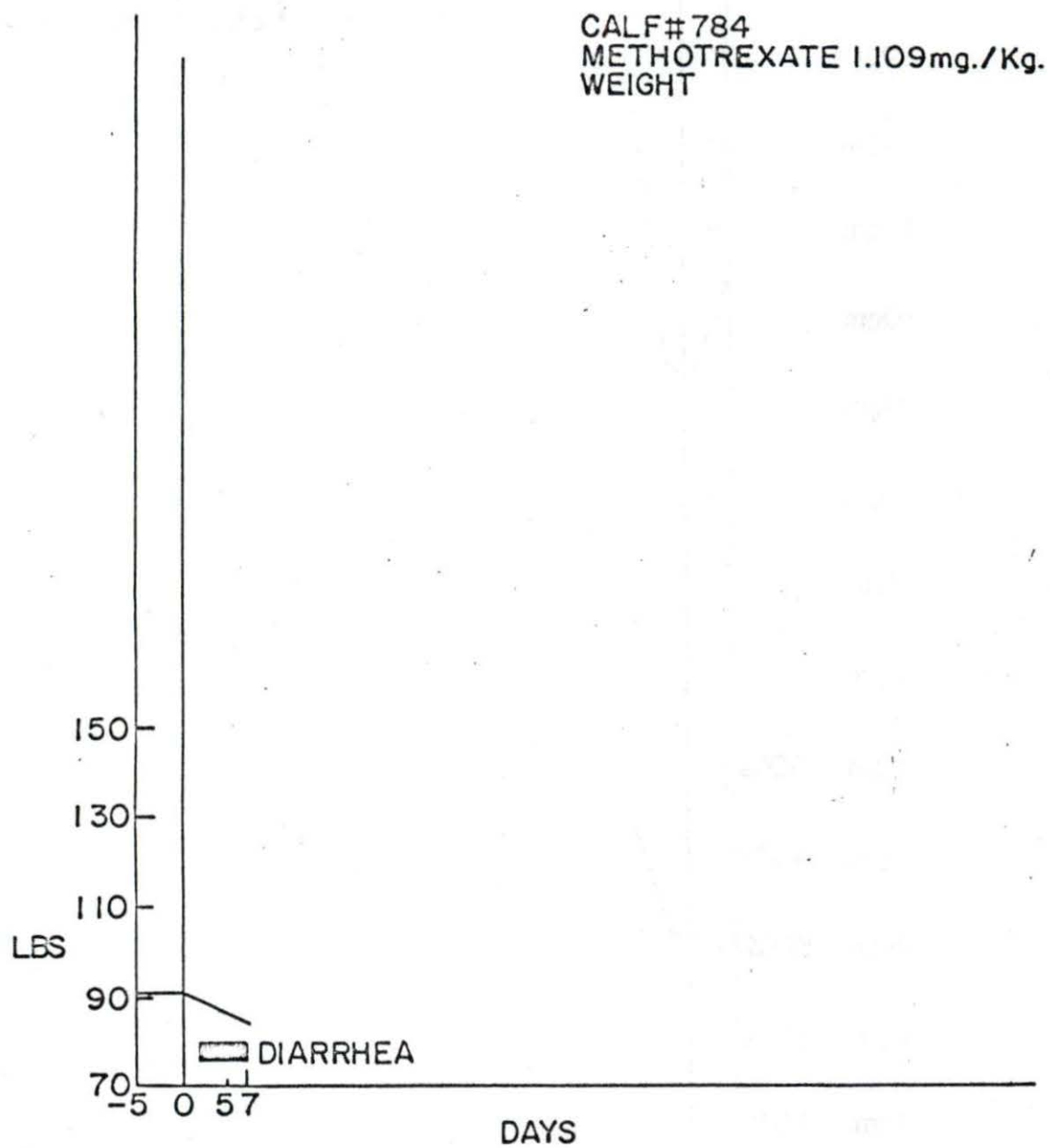


Figure 82. Calf 784. Weight and clinical data

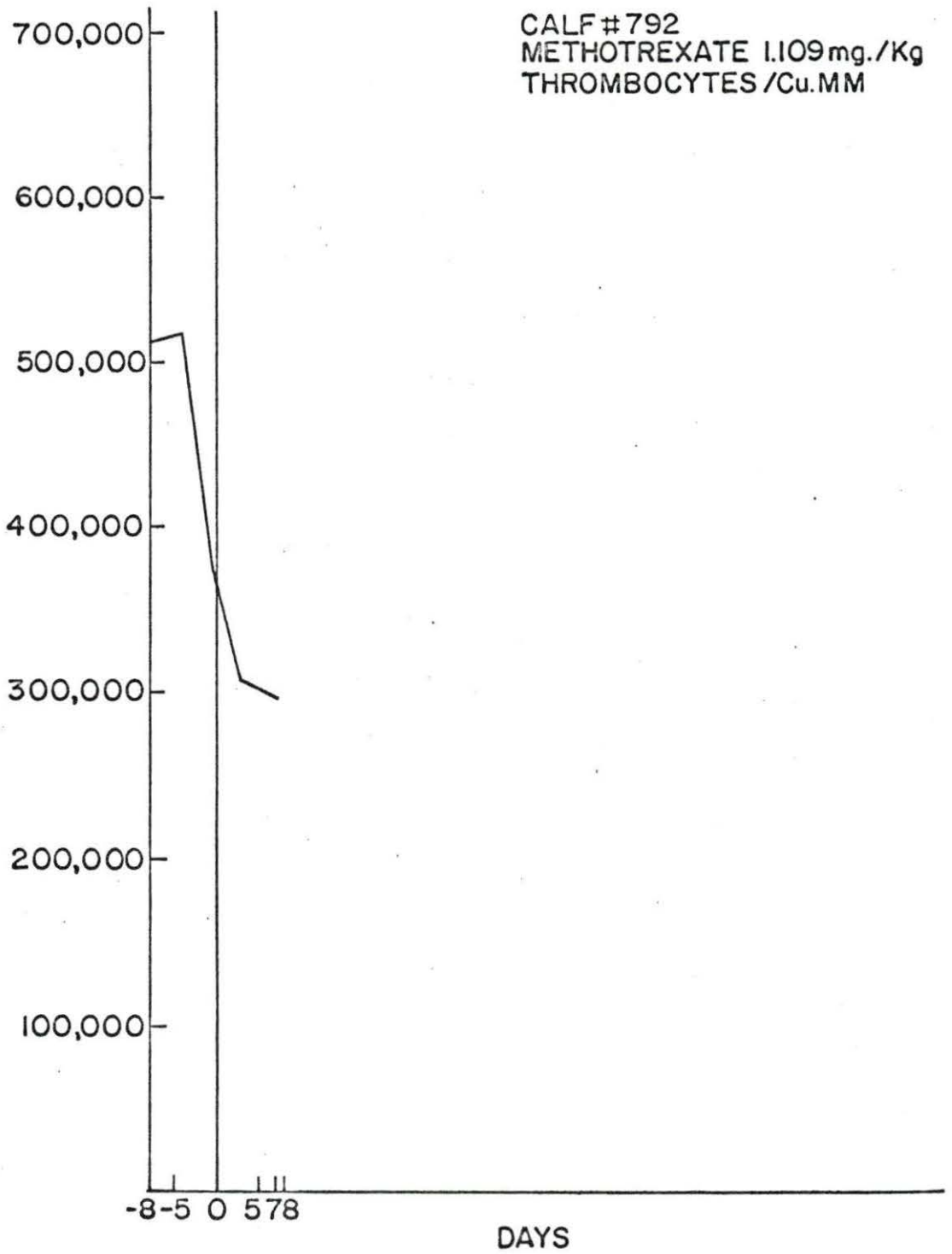


Figure 83. Calf 792. Thrombocyte counts

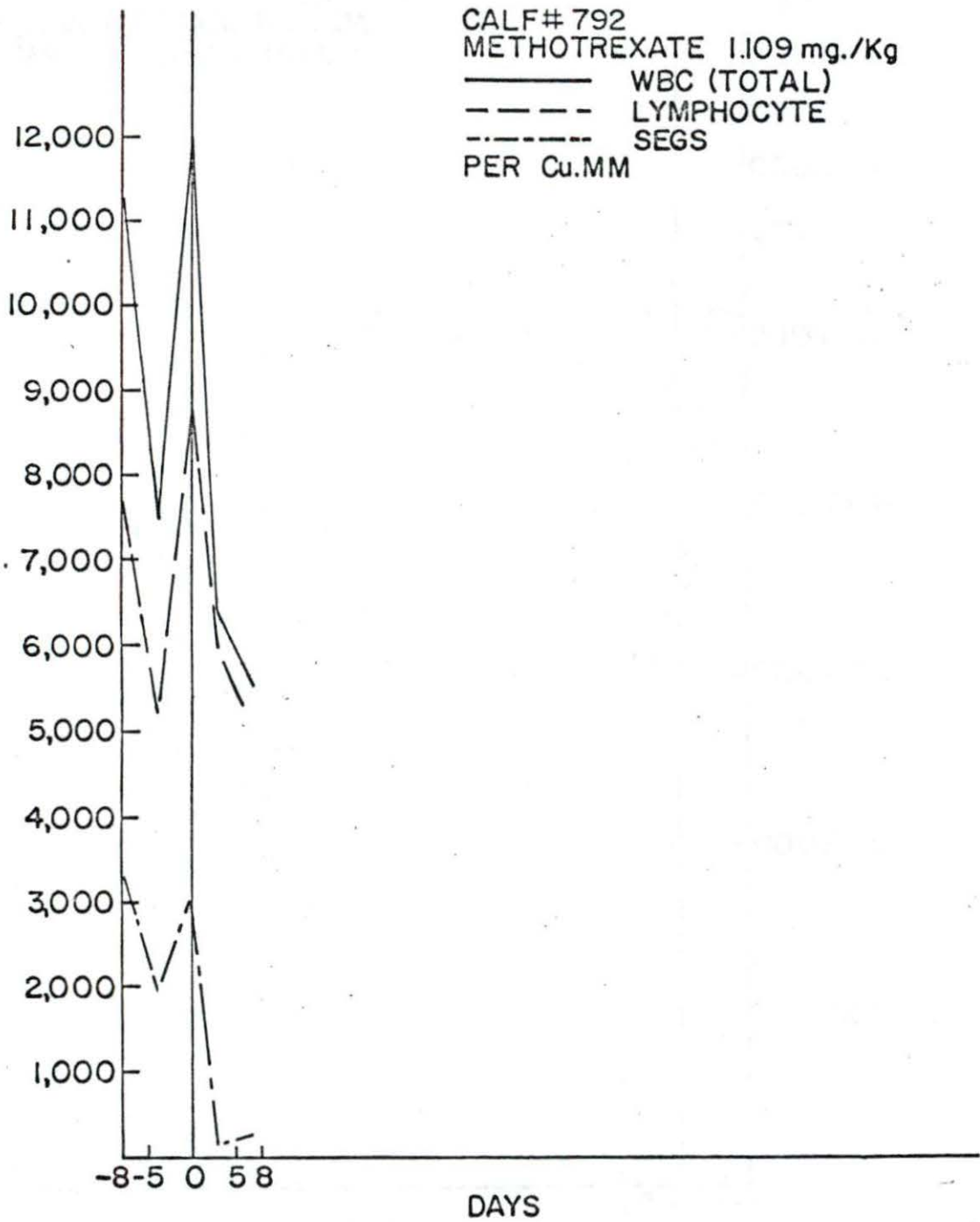


Figure 84. Calf 792. Leukocyte counts.

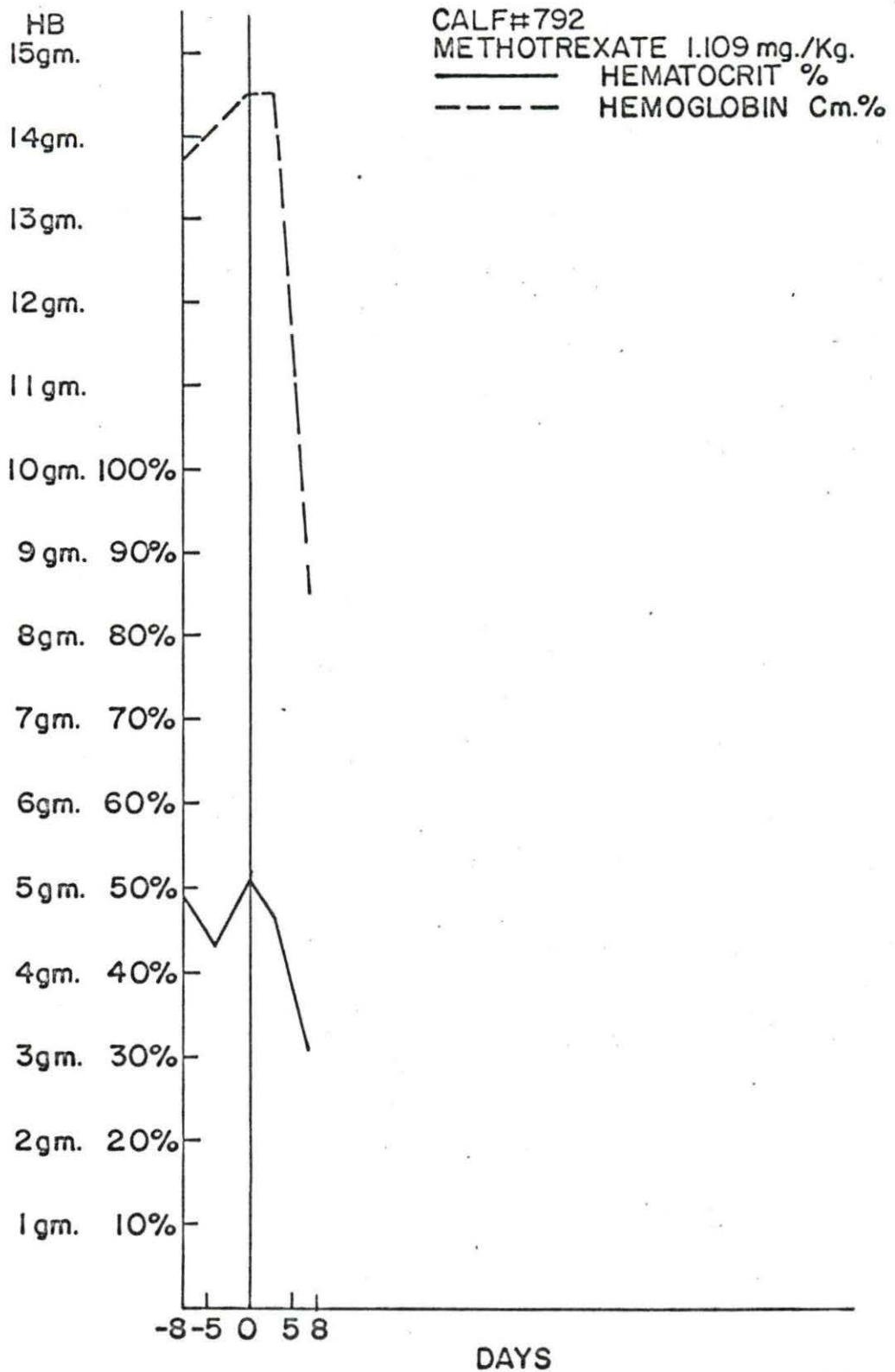


Figure 85. Calf 792. Hematocrit and hemoglobin

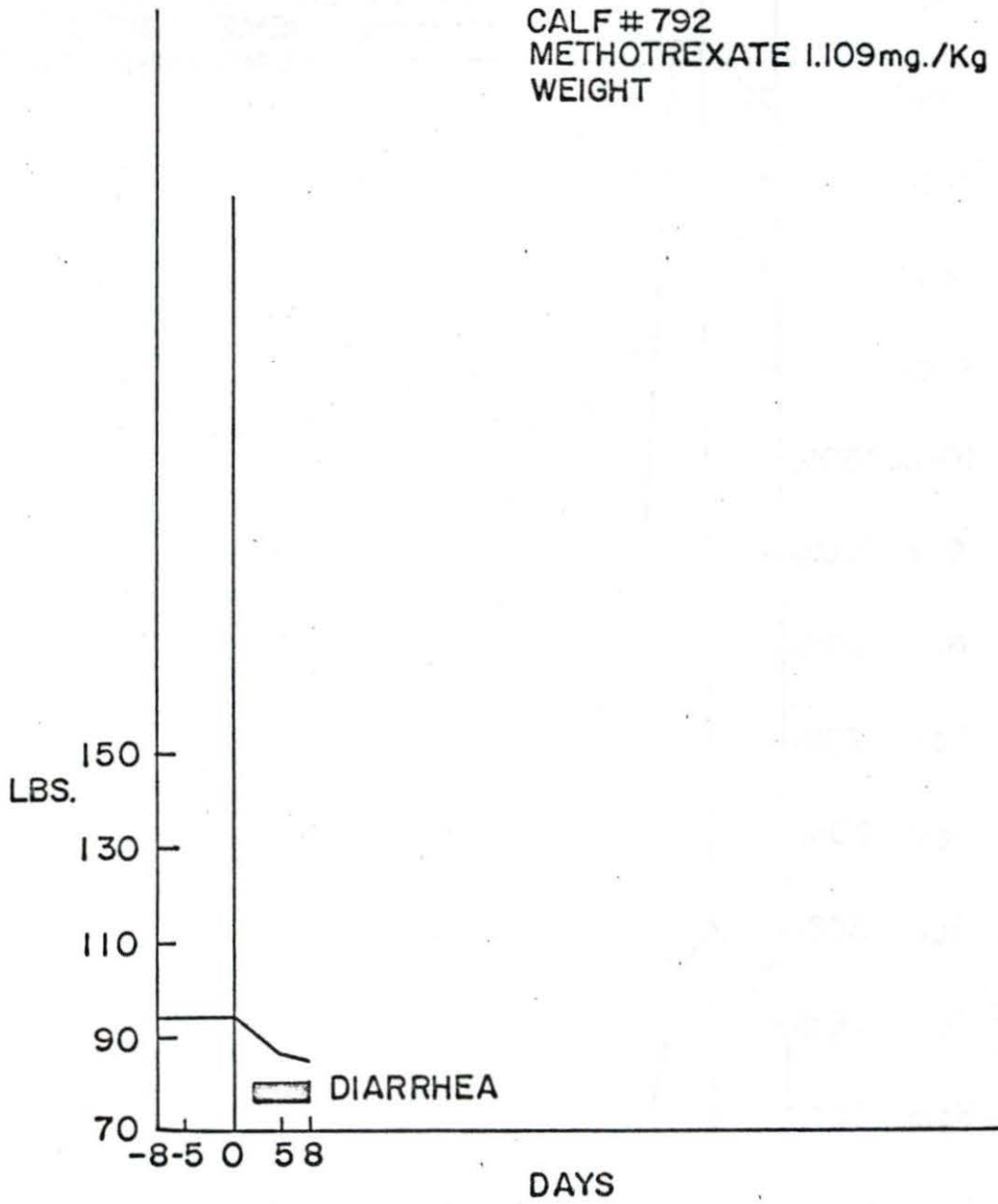


Figure 86. Calf 792. Weight and clinical data