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Chemoprophylaxis (Imidocarb) Against *Babesia bigemina* and *Babesia argentina* Infections

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SUMMARY

The chemoprophylactic effects of imidocarb (3,3'-bis-(2-imidazolin-2-yl)-carbanilide dihydrochloride) against bovine babesiasis were evaluated in 29 calves. The compound had prophylactic and therapeutic properties in calves artificially and naturally infected with *Babesia bigemina* and *Babesia argentina* of Colombian (South American) origin. Administered intramuscularly at the dose level of 2 mg./kg., imidocarb suppressed the development of acute babesiasis in calves treated 46 days previously and later exposed to a lethal dose of *Babesia* spp.-infected blood. Imidocarb failed to protect against *Anaplasma marginale* infection. Calves treated intravenously with imidocarb at dose level of 2 mg./kg. and challenge inoculated 20 days later with a lethal dose of *Babesia* spp.-infected blood were protected. For 90 days after challenge, none of the calves had *Babesia* spp. parasitemia, as determined by examination of stained blood films and by subinoculation of blood into susceptible splenectomized calves. Calves intravenously treated 21 days previously with 3 mg. of imidocarb per kilogram resisted tick-borne challenge of *Boophilus microplus*. This resistance was evidenced for 15 weeks of field exposure by negative results of examinations of stained blood films and death of nontreated calves from acute babesiasis. All calves treated with imidocarb and subsequently exposed to blood or tick-borne *Babesia* spp. responded with an increase of complement-fixing antibodies.

Imidocarb readily controlled very severe acute infections with *B. bigemina* and *B. argentina* when the compound was given at dose rates of 1 mg./kg. by both intramuscular or subcutaneous routes. Signs of acute toxicosis were observed in calves given intravenous injections of 3 mg./kg. Three calves died, having signs of embarrassed respiration, oral respiration, excessive salivation, muscular fasciculations, urination, defecation, incoordination, and prostration. Signs of toxicosis were milder with intramuscular or subcutaneous injections of imidocarb.

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The possibilities of chemoprophylaxis against bovine babesiasis caused by *Babesia divergens* were investigated by Ryley,[10] *B. argentina*, by Newton and O'Sullivan,[11] and *B. bigemina* and *B. argentina*, by Callow and McGregor.[5] These investigators found that quinuronium derivatives had some prophylactic effect against *Babesia* spp., but not enough to prevent infection with these organisms in prophylactically treated cattle. Pipano[12] reported that diamidine derivatives given at the dose of 5 mg./kg. during the incubation period of infection with *Babesia berbera* had the effect of decreasing the severity of disease; however, the administration of the drug 4 days before infection did not confer any protection.

Further search for the development of a new drug with longer prophylactic effect was obviously needed. Beveridge[2] reported that the compound now known as imidocarb (3,3'-bis-(2-imidazolin-2-yl)-carbanilide dihydrochloride)* had a greater babesiacidal effect and a better chemoprophylactic index than did quinuronium, diamidine, and amicarbalide derivatives against *Babesia rodhaini* in mice and rats. Imidocarb was selected from a series of carbanilides on the basis of screening tests against *B. rodhaini* by Schmidt et al.[17]

Following these laboratory observations, imidocarb was found to have a high level of activity against *B. bigemina* and *B. argentina* in Australian cattle. Callow and McGregor[4] found that imidocarb cured (sterilized) *B. argentina* at the dose level of 2 mg./kg., but not at 1 mg./kg. It was found that *B. bigemina* was more susceptible to this compound. Imidocarb at the dose level of 0.6 or more mg./kg. sterilized *B. bigemina* infections in splenectomized steers. The observations made by these authors that imidocarb had a prophylactic effect has an important practical significance. Imidocarb injected at the dose level of 2 mg./kg. reduced susceptibility of cattle to *B. argentina* infection for 33 days, and to *B. bigemina* infection for 10 weeks after treatment. Roy-Smith[15] reported that injection of 2 mg. of imidocarb per kilogram at the time of tick-borne chal-

* No. 4A65, Burroughs, Wellcome Company, Tuckahoe, N.Y.

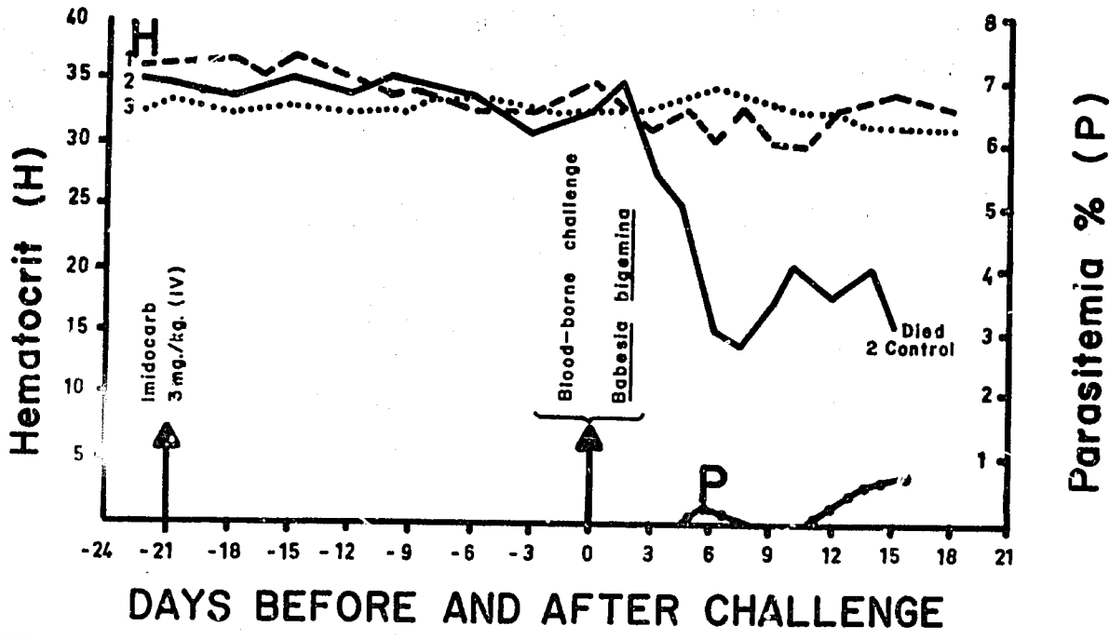

Fig. 1—Hematologic responses of calves given (intravenous administration) imidocarb 21 days before blood-borne challenge with *Babesia bigemina* (experiment 1). Graphic representation of anemia (H), parasitemia (P), and their relationships to the prophylactic effect of drug and blood-borne challenge. Calves 1 and 3 were chemoprophylactically treated and calf 2 not treated died 16 days after blood-borne challenge inoculation (experiment 1).

lenge exposure prevented development of clinical signs of *B. bigemina* and *B. argentina* infections; however, treatment 36 days before infection was less effective. Brown and Berger[3] found imidocarb at dose level(s) of 1 mg. or more mg./kg. given intramuscularly eliminated *B. bigemina* infection from cattle in Kenya. In Ireland, Wood[19] found that imidocarb was very effective against *B. divergens* infections at dose levels of 1 mg./kg. for therapy and of 2 mg./kg. for prophylactic treatment. Hart et al.[7] reported that imidocarb protected cattle from naturally occurring *B. argentina* infections for 44 days; however, protection against *B. bigemina* was not clearly demonstrated.

The purpose in the present experiments done in Colombia was to evaluate prophylactic effect, dosage, route of injection, and toxicity of imidocarb given to cattle before they were infected with *B. bigemina* and *B. argentina*.

Materials and Methods

Calves.—Twenty-nine Holstein-Friesian calves 3 to 5 months of age were used as experimental animals. The calves were kept in a tick-free environment during the experimentation in Bogotá (alt. 2,600 m.), but in Palmira, Cauca Valley (alt. 1,000 m.), calves were exposed to infected ticks (*Boophilus microplus*) 3 weeks after drug administration. Prior to the experimental or natural infection, all calves were examined for the presence of current or previous infection with *B. bigemina*, *B. argentina*, *A. marginale*, *Trypanosoma vivax*, and *Trypanosoma theileri*, or other hemotropic infections. Samples of blood were collected from all calves for complement-fixation tests, preparation of stained blood films, and for inoculation into splenectomized calves.

Medication.—Imidocarb,[b] received as a water soluble salt, was dissolved in sterilized distilled water as a 10% solution and used within 24 hours after preparation. This solution was administered intravenously, intramuscularly, or subcutaneously at dose levels of 1 to 3 mg./kg., according to the experimental approach of treatment.

Chemoprophylaxis

Experiment 1.—Three 85- ± 8-kg., male, 4-month-old Holstein-Friesian calves at the Veterinary Medical Research Laboratory of the Colombian Agricultural Institute, Bogotá, were used in the preliminary experiment for the prophylactic evaluation of imidocarb. Two of the calves were given (intravenously) 3 mg. of imidocarb per kilogram; the other calf was not treated and was used as a control. The 2 treated calves and the nontreated calf control were challenge inoculated 21 days later (day 0) with 100 ml. of blood containing 0.1% *B. bigemina* parasitemia from a splenectomized calf acutely affected. At the time of blood collection, the calf had a packed cell volume (PCV) of 26%. Blood was collected in ethylenediaminetetraacetic acid (EDTA) disodium salt (2 mg./ml. of blood) as anticoagulant and was intravenously injected into the 3 calves immediately after collection.

Experiment 2.—Five 90-kg., male, 4-month-old Holstein-Friesian calves at the Veterinary Medical Research Laboratory, Colombian Agricultural Institute, Bogotá, were used in this experiment. Four calves were given (intramuscularly) 2 mg. of imidocarb/kg.; 1 calf was not treated and was used as a control. The 5 calves were each challenge inoculated 46 days after drug treatment (day 0) with 100 ml. of pooled blood containing 0.5% *B. bigemina*, 0.01% *B. argentina*, and 1% *A. marginale* parasitemias. Blood was collected from 2 splenectomized calves, 1 infected with *Babesia* spp. and the other with *A. marginale*. The calf infected with *Babesia* spp. had 23% PCV, and the calf infected with *A. marginale* had 18% PCV at the time of blood collection for challenge inoculums. Blood was collected in EDTA and was immediately injected intravenously into the calves.

[b] Burroughs, Wellcome Company, Tuckahoe, N.Y.

Experiment 3.—Four 90- ± 7-kg., male, 3-month-old Holstein-Friesian calves at the Veterinary Medical Research Laboratory, Colombian Agricultural Institute, Bogotá, were used. Three calves were intravenously treated with imidocarb at the dose level of 2 mg./kg., and the remaining calf control was not treated. The calves were challenge inoculated 20 days later with 100 ml. of blood containing 0.5% *B. bigemina* and 0.1% of *B. argentina* parasitemia. Blood was collected from a splenectomized calf which had 20% PCV at the time of blood collection. The calf was acutely affected; blood was collected with EDTA as anticoagulant and was intravenously injected immediately.

Experiment 4.—Eight 80- ± 6-kg., male, 4-month-old Holstein-Friesian calves at the Colombian Agricultural Institute Palmira Experiment Station, Palmira, Cauca Valley, were used for a field evaluation of imidocarb. Eight calves were given imidocarb intravenously at dose level of 3 mg./kg., and the calf control was not treated. All calves were kept in tick-free units for 21 days after drug treatment, and thereafter were exposed to field challenge with *B. microplus* ticks which were naturally infected with *B. bigemina, B. argentina,* and *A. marginale.*

Chemotherapy

Experiment 5.—This experiment was done to determine the therapeutic effect of imidocarb against bovine babesiasis caused by *B. bigemina* and *B. argentina.* The 2 organisms were isolated from natural field cases located in enzootic areas for bovine babesiasis in Fusagasuga, Cauca Valley, and Montería, Colombia. The blood was collected in EDTA solution from infected cattle and inoculated into splenectomized calves at the Veterinary Medical Research Laboratory, Colombian Agricultural Institute, Bogotá. When signs of acute babesiasis infection were evidenced by anemia (decreased PCV), parasitemia, hemoglobinuria, and temperature elevation, the calves were treated with imidocarb. Three calves (No. 3, 5, 6) were injected intramuscularly with 1 mg./kg.; 2 calves (No. 2, 4), intramuscularly with 2 mg./kg.; and 1 calf (No. 1), subcutaneously with 1 mg./kg. Three infected calves (No. 7-9) were not treated and were used as controls.

Determination of Drug Efficacy.—The prophylactic effect of imidocarb experiments 1 through 4 was determined by resistance to artificial and natural infection with *B. bigemina* and *B. argentina.* The therapeutic effect of imidocarb experiment 5 was determined by recovery of calves from acute infection after drug administration. The recovery from *Babesia* spp. infection was determined by a return of certain measured values to normal. All calves were examined daily and then once each week before and after treatment with imidocarb for hematocrit values (H), percentage of parasitemia (P), rectal temperature (T), complement-fixing antibody titers (AT) when applicable, and death. The complement-fixation antibody test was performed according to techniques described by Todorovic et al.[5] Observations were made on drug toxicity at the time of treatment and during the experiment. Inoculation of 500 ml. of blood from the experimental calves into susceptible splenectomized calves was carried out to determine the presence or absence of *Babesia* spp. infection before or after treatment.

Results

Chemoprophylactic Experiments

The results of experiments 1 through 4 are recorded (Fig. 1, 2, 3, and 4).

Experiment 1.—After intravenous injection of imidocarb at the dose level of 3 mg./kg., the 2 treated calves had excessive salivation, lacrimation, micturation, dyspnea, defecation, muscular tremor, coughing, and prostration. The first signs were observed 1 to 2 minutes after drug inoculation. Calves were continuously watched for several hours after drug injection and then only observed at intervals for drug toxicity. Acute toxic effects abated at 12 hours, but the calves remained with

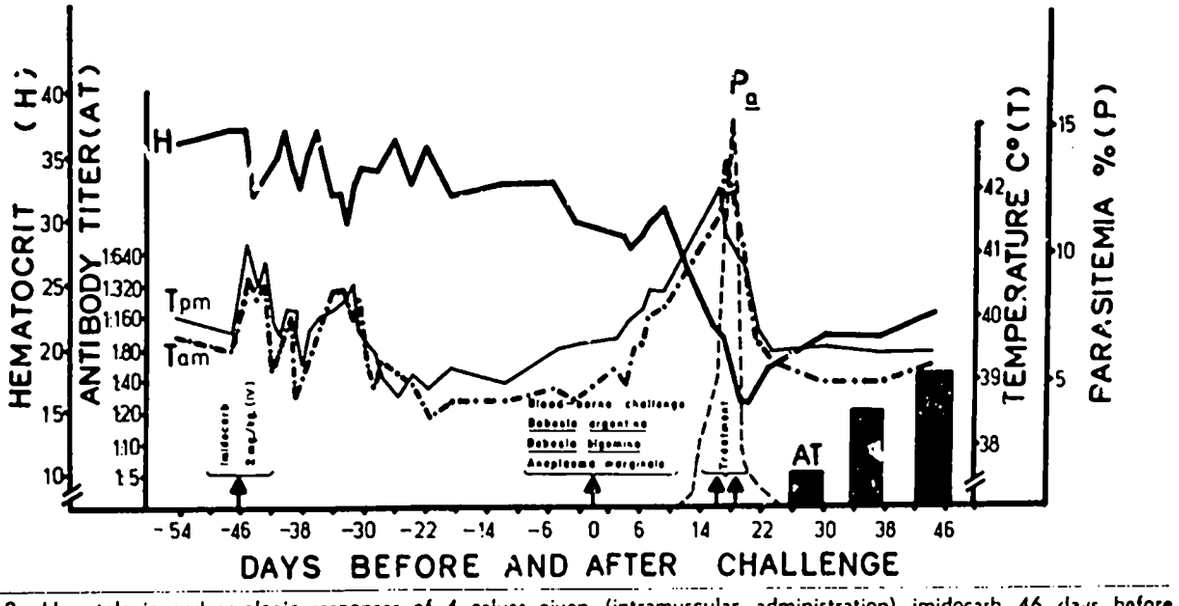

Fig. 2—Hematologic and serologic responses of 4 calves given (intramuscular administration) imidocarb 46 days before blood-borne challenge with *B. bigemina, Babesia argentina, Anaplasma marginale* (experiment 2). Graphic representation of mean values for anemia (H), parasitemia (*A. marginale* Pa), rectal temperature (Tam, morning; Tpm, afternoon), and *Babesia* spp. complement-fixing (CF) antibody titer (AT), and their relationships to the prophylactic effect of imidocarb and blood-borne challenge inoculation (experiment 2).

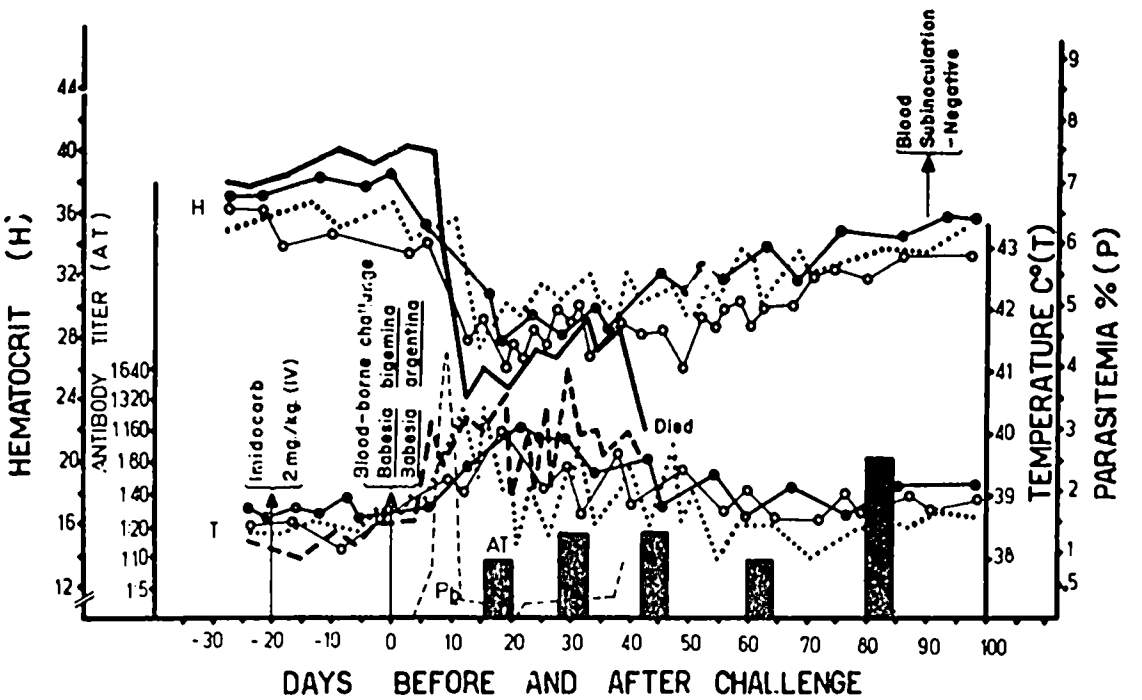

Fig. 3—Hematologic and serologic responses of 4 calves given (intravenous administration) imidocarb 20 days before blood-borne challenge with *B. bigemina* and *B. argentina* (experiment 3). Graphic representation of mean values for anemia (H), parasitemia (P), temperature (T), and *Babesia* spp. CF antibody titer (AT) and their relationships to the prophylactic effect of drug and blood-borne challenge. Control calf not chemoprophylactically treated died of babesiasis 42 days after challenge exposure (experiment 3).

roughened coats and refused food for another 10 to 12 hours. Death losses due to drug toxicity did not occur, and PCV values remained within normal limits.

At day 0 (or 21 days after drug treatment), the 2 treated calves and the 1 control were injected intravenously with 100 ml. of blood infected with *B. bigemina*. At the time of challenge, all calves had PCV of 35 to 36%. Four days after blood-borne challenge inoculation was done, the calf not treated with imidocarb had parasitemia of 0.5% with *B. bigemina* which persisted until day 16 when this calf died. Anemia was evidenced by low PCV of 15% which coincided with the *B. bigemina* parasitemia. The necropsy findings were typical for an acute babesiasis. The 2 calves treated with imidocarb for the 21 days before blood-borne challenge exposure with *B. bigemina* did not have any demonstrable parasitemia, and their PCV remained at normal values during the experiment (Fig. 1).

Experiment 2.—The 4 calves given (intramuscular administration) 2 mg./kg. doses appeared to tolerate imidocarb better than did the calves injected intravenously (experiment 1). These calves had signs of toxicity, however; these were less pronounced than those in experiment 1 calves treated with 3 mg./kg. It was evidenced only with slight salivation and lacrimation. Apart from some roughness of the hair coat, calves also showed a 1 degree (Celsius) increase of rectal temperature on the day of drug treatment and during the first week after treatment (Fig. 2). A slight decrease of PCV at the time of temperature elevation was also observed.

On day 0, or 46 days after drug treatment, all calves (4 treated; 1 control) were exposed to blood-borne challenge inoculation of *B. bigemina*, *B. argentina*, and *A. marginale*. At the time of challenge inoculations, the calves had average PCV of 30%, morning rectal temperatures of 38 C., and afternoon rectal temperatures of 39 C. Thirteen days after challenge exposure, all calves had *A. marginale* parasitemia which reached 15% on day 18 when all calves were treated with compound 356C61* twice. Rectal temperature increased to 42 C. and PCV decreased to 16% at the time of highest parasitemia. Demonstrable parasitemia was not observed in stained films of peripheral blood of the calves treated with imidocarb. The first increase of *Babesia* and *Anaplasma* complement-fixing antibody titers occurred 26 days after blood challenge exposure. Titers of 1:10 were detected on 26 and then 36 and 46 days later (Fig. 2). The control calf, not treated but given the same inoculum of blood as the treated ones, died 17 days after challenge exposure, having had signs of acute babesiasis and anaplasmosis and had 4% parasitemia with *B. bigemina*, 0.3% with *B. argentina*, and 0.3% with *A. marginale*.

Experiment 3.—The 3 calves treated intravenously with 2 mg./kg. doses of imidocarb had acute signs of toxicity. Those calves had dyspnea, oral respiration, excessive salivation, lacrimation, muscular fasciculations, acute incoordination, urination, defecation, and coughing with mucous or bloody discharge from both nostrils immediately after drug treatment; these signs were transient and disappeared within 24 hours. Calves refused food at the time of treatment.

On day 0, or 20 days after drug treatment, all calves

* Gloxazone (γ-ethoxyethylgloxal dithiosemicarbazone), Burroughs, Wellcome Company, Tuckahoe, N.Y.

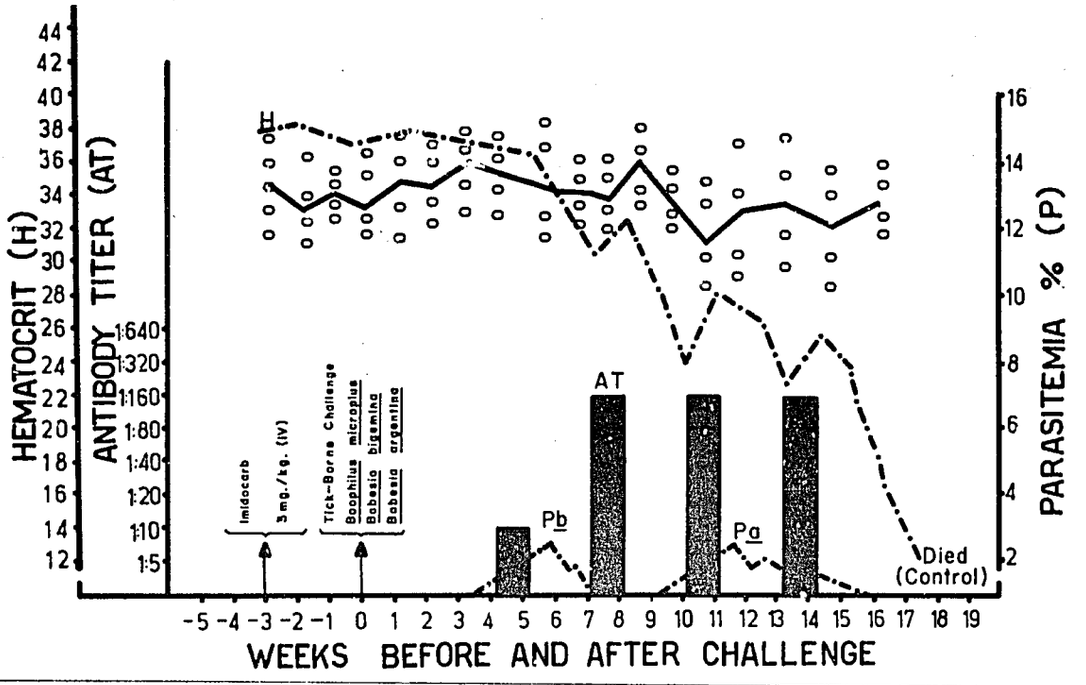

Fig. 4—Hematologic and serologic responses of 6 calves given (intravenous administration) imidocarb 21 days before tick-borne (*Boophilus microplus*) challenge. Ticks were naturally infected with *Babesia* spp. and *A. marginale* (experiment 4). Graphic representation of mean values for anemia (H-continuous line; circles represent individual variation), parasitemia (*Babesia* spp. Pb, and *A. marginale* Pa), and *Babesia* spp. CF antibody titer (AT) and their relationships to the prophylactic effect of drug and tick-borne challenge. Control calf not chemoprophylactically treated died of babesiasis 17 weeks after tick-borne challenge exposure (experiment 4).

were exposed to blood-borne challenge with *B. bigemina* and *B. argentina*. Four days later, *B. bigemina* was found in the blood films of the nontreated calf (control); however, parasitemias were not detected in the treated calves. All calves had lessened PCV on day 10 after challenge exposure and gradually recovered from this transient anemia. Rectal temperature was increased and coincided with the appearance of signs of anemia. Increase of temperature was also transient and coincided with the changes in rectal temperature of the control calf which was infected with *Babesia* spp.

The first complement-fixing antibodies were detected

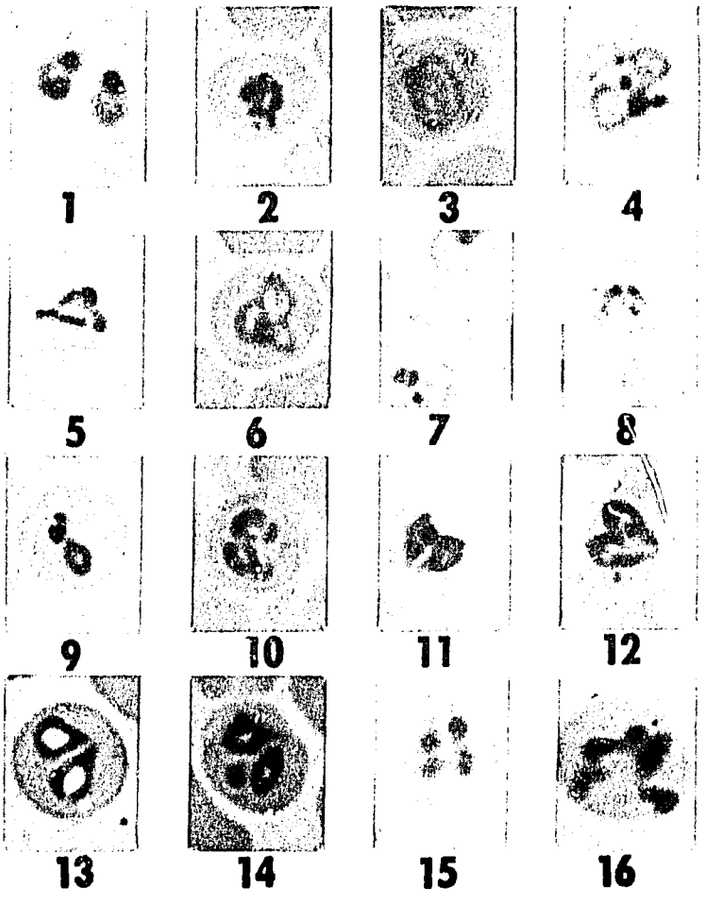

Fig. 5—Stained films of peripheral blood of Colombian cattle acutely infected with *B. bigemina* and *B. argentina*. The typical paired piriform body of *B. bigemina* lies outside the erythrocyte (1). Various forms, amoeboid, rounded or irregularly shaped of *B. bigemina* (2, 3, and 4). Multiplication of *B. bigemina* in the erythrocytes occurs more frequently by budding than by binary fission (5 and 6). *Babesia argentina* is a smaller form which lies in the center of erythrocytes; paired parasites form an obtuse angle (7, 8, and 9). *Babesia bigemina* is a larger parasite; may assume various shapes, but most characteristically it is pear shaped, forming an acute angle in the erythrocyte (10, 11, 12, 13 and 14). Multiple infection of a single erythrocyte with *B. argentina* (15 and 16). Giemsa stain; × 1,000.

TABLE 1—Treatment of Splenectomized Calves Infected with *Babesia bigemina* and *Babesia argentina*

Identification of infected calves with *Babesia* spp. (% of parasitemia at the time of treatment)	Location where *Babesia* spp. were isolated from naturally infected cattle	PCV at time of treatment (%)	Dose (mg./kg.) and route of administration of imidocarb	Observation
Calf 1 (85 kg.) *B. bigemina* (6) and *B. argentina* (0.1)	Fusagasuga	13	1 (S.C.)	Tremor, salivation, and urination at 8 minutes after injection of drug. Died the day after treatment.
Calf 2 (76 kg.) *B. bigemina* (1) and *B. argentina* (0.5)	Fusagasuga	19	2 (I.M.)	Lacrimation, nasal and oral salivation, urination, defecation, dyspnea, and cough at 10 minutes after injection of drug. Recovered with PCV of 34% after 15 days after treatment. Parasitemia was not observed the day after treatment.
Calf 3 (54 kg.) *B. bigemina* (8) and *B. argentina* (0.5)	Cauca Valley	11	1 (I.M.) repeated the day after	Lacrimation, nasal secretion, salivation, urination, increased dyspnea, and cough at 10 minutes after injection of drug. Recovered, with PCV of 29% at 25 days after treatment. Parasitemia was not observed the day after treatment.
Calf 4 (83 kg.) *B. bigemina* (0.5) and *B. argentina* (0.01)	Cauca Valley	17	2 (I.M.)	Slight lacrimation and urination at 5 minutes after injection of drug. Recovered 3 weeks after drug treatment, with PCV of 30%. Parasitemia was not observed day after the treatment.
Calf 5 (90 kg.) *B. bigemina* (12) and *B. argentina* (0.01)	Montería	11	1 (I.M.)	Tremor, salivation, dyspnea, convulsion, and urination at 5 minutes after treatment. Died 15 minutes after injection of drug.
Calf 6 (90 kg.) *B. bigemina* (5) and *B. argentina* (0.1)	Montería	14	1 (I.M.)	Slight lacrimation, salivation, and urination. Recovered with PCV of 35% at 4 weeks after drug treatment. Parasitemia was not observed the day after treatment.
Calf 7 (87 kg.) *B. bigemina* (3) and *B. argentina* (0.1)	Fusagasuga	15	Not treated	Died of acute babesiasis
Calf 8 (94 kg.) *B. bigemina* (7) and *B. argentina* (0.2)	Cauca Valley	12	Not treated	Died of acute babesiasis
Calf 9 (91 kg.) *B. bigemina* (1) and *B. argentina* (0.01)	Montería	18	Not treated	Died of acute babesiasis

S.C. = subcutaneously; I.M. = intramuscularly; PCV = packed cell volume.

at 15 days after challenge exposure at a titer of 1:10, and this titer persisted during the time of observations. At 90 days after exposure, blood taken from each of the 3 treated calves was inoculated into a splenectomized calf and was not found to be infected with *Babesia* spp. The control calf, not treated with imidocarb but challenge exposed to the same dose of infective blood given to the 3 treated calves in day 0, died 40 days after challenge with signs of acute babesiasis (Fig. 3).

Experiment 4.—The 8 calves treated (intravenous administration) with 3 mg./kg. doses of imidocarb had acute signs of drug toxicity. Three calves died immediately after drug treatment with signs of excessive salivation, lacrimation, dyspnea, oral respiration, muscle tremor, acute incoordination, prostration, urination, defecation, and coughing, with bloody discharge from the nostrils. Five calves which recovered from drug toxicosis refused feed for 24 hours. On day 0, or 21 days after drug treatment, all calves were exposed to tick-borne challenge with *B. microplus*. At the time of exposure, all calves had PCV in the range of 32 to 36%. After 25 days of tick exposure, *B. bigemina* and *B. argentina* were found in the blood films taken from the nontreated calf (control). The characteristic morphology of *Babesia* spp. is shown (Fig. 5). However, neither *B. bigemina* nor *B. argentina* was found in the blood films of calves treated with imidocarb. All calves had a low level of *A. marginale* infection.

The first complement-fixing antibody titers (1:10) were detected at 5 weeks after challenge exposure, and thereafter the titer was 1:160 which persisted throughout the experiment. The nontreated calf (control) had a *Babesia* spp. parasitemia followed by an *A. marginale* parasitemia, and finally died 17 weeks after tick exposure with acute babesiasis and anaplasmosis (Fig. 4).

Chemotherapy

Experiment 5.—Six calves with acute babesiasis caused by *B. bigemina* and *B. argentina* were treated with imidocarb (Table 1). Calves 3 and 6 treated with an intramuscular dose of 1 mg./kg. and calves 2 and 4 treated with 2 mg./kg. doses of imidocarb injected intramuscularly recovered from acute infection. These calves had signs of excessive salivation, lacrimation, dyspnea, muscular fasciculations, and prostration after treatment. Two calves (No. 1 and 5) treated subcutaneously or intramuscularly with imidocarb at doses of 1 mg./kg. died at the time of treatment due to the drug or the effect of hemotropic disease, or both. Calves which recovered from babesiasis were not tested after treatment for the presence (or absence) of *Babesia* spp. parasites. Calves did not have a relapse of babesiasis during the 3-month period of observations. The 3 calves (No. 7–9) acutely infected with the same isolates of *B. bigemina* and *B. argentina* and not treated with imidocarb died from acute babesiasis.

Discussion

Bovine babesiasis continues as a major threat to the livestock industry throughout the world. Since the natural transmission of *Babesia* spp. parasites is dependent on ticks, the disease can be eradicated by an

adequate tick control program.[13] In many countries, however, vector control program cannot be implemented for various reasons. At present, the alternative measure to control the disease and prevent losses is an effective vaccination or chemotherapeutic program.[10]

The search for a new drug which can be administered into susceptible cattle as a prophylactic measure at some time before they are exposed is obviously needed. The introduction of such a drug as a prophylaxis of babesiasis will have a great practical application in the countries where babesiasis is prevalent.[16]

The mechanism of action of the majority of chemotherapeutic drugs available against babesiasis has not been studied sufficiently and their action is not known.[8] The same statement could be made for the new experimental compound imidocarb which was found to have the dual properties: (1) therapeutic, and (2) chemoprophylactic. These properties undoubtedly exert multiple actions on the *Babesia* parasites, as well as on the infected host, through various tissue mechanisms which are not presently understood.[2]

To obtain chemoprophylactic effects, a compound with long residual activity should be administered to cattle before they are exposed to *Babesia* spp. This procedure prevents the development of the *Babesia* infection, stops the establishment of parasites in tissues, and protects the bovine host from invading *Babesia* parasites. In cattle given imidocarb, residues were found which persisted for a long time, and this probably resulted in the prophylactic effect observed. This could be an explanation of the prophylactic mechanism of imidocarb. Wood[19] reported that imidocarb has long residual properties. He reported that the drug persisted in concentrations of 0.01 μg./Gm. in bovine adipose tissue up to 8 weeks, and 0.03 μg./Gm. in muscle, up to 12 weeks, 0.51 μg./Gm. in kidney, and 0.34 μg./Gm. in liver. In bovine fetuses, imidocarb persisted for 4 weeks at concentrations of 2.4 μg./Gm. in liver and 1.1 μg./Gm. in kidney.

Although several compounds are used to treat clinical babesiasis, there is not a commercially available drug showing prophylactic properties. In Australia, Callow and McGregor[4] reported (1970) that cattle treated with imidocarb at dose level of 2 mg./kg. were refractory to infection with *B. bigemina* or *B. argentina* up to several weeks after treatment. This work confirmed their observation on the prophylactic effect of imidocarb against *B. bigemina* and *B. argentina* of South American origin.

Pipano[12] reported that berenil (4,4′-(diazoamino) dibenzamidine diaceturate) given to calves in doses of 5 mg./kg. during the incubation period of infection with *B. berbera* had the effect of lengthening the periods of incubation and infection and decreasing the severity of the disease. One or 2 doses did not interfere with the co-infectious immunity of recovered calves. The administration of the drug to calves 4 days before infection did not produce any protection.

Ryley[16] subcutaneously injected rats with sparingly soluble salts of quinuronium derivatives of known therapeutic activity to test the formation of drug depots. The drugs which formed a depot were tested for their capability to prevent clinical babesiasis in splenectomized calves when the calves were later challenge exposed to *B. divergens*. Encouraging, but not satisfactory, results were obtained with quinuronium suraminate, quinuronium 5,5′-methylene bis-salicylate, and quinuronium embonate.

Barnett[1] reported on the chemotherapy effects of treatment, subsequent relapses, and immunity of *B. bigemina* in a series of experiments in splenectomized cattle. Amicarbalide in doses of 10 mg./kg. was effective, but the treated calves subsequently proved susceptible to reinfection. Co-infectious immunity (latent) followed the administration of 7.5 mg. or less/kg., but some fatal relapses occurred. Homidium bromide was active at dose level of 1 or more mg./kg. but reduction of the number of parasites after treatment was slow and one severe relapse occurred after treatment. Phenamidine isothionate at dose level of 13.3 mg./kg. acted rapidly; co-infectious immunity followed treatment and clinical relapse never occurred with doses at 8 to 10 mg./kg. Acaprin at dose level of 1 mg./kg. acted rapidly, producing co-infectious immunity with clinical relapses; but with a dosage of 0.75 mg./kg., a fatal relapse occurred after treatment. Berenil (Ganaseg) in doses of 3.5 mg. or less/kg. caused rapid elimination of the parasites which were not demonstrable thereafter. The calves were immune for up to 109 days after treatment. Berenil at dose level of 5 mg./kg. produced a sterile cure and the calf was completely susceptible to reinfection 68 days later.

Newton and O'Sullivan[11] reported that a 20% suspension of the quinuronium compound, 5,5′-methylene bis-salicylate, in arachis oil administered subcutaneously in 1-Gm. doses had a satisfactory prophylactic effect against *B. argentina* in young calves. A 2-Gm. dose produced acute toxicosis with fatal nephritis in some calves; however, protective effects were demonstrated in calves that recovered. *Babesia argentina* was established in calves infected by inoculation with infective blood or by ticks, but none of the 31 calves injected with the drug 7 to 35 days previously developed clinical babesiasis; the control calves did. The control calves had significantly higher febrile reactions and the differences in the hemoglobin levels and PCV were statistically significant.

Callow and McGregor[5] reported that cattle treated with the babesiacidal depot compound, quinuronium 5,5′-methylene bis-salicylate, within the period 4 weeks before to 1 week after inoculation with live *B. argentina* vaccine had lower parasitemias than did the nontreated controls. Treated and vaccinated steers had adequate immunity 15 weeks after vaccination. Steers and splenectomized calves treated at the times of inoculation and onset of primary parasitemias, respectively, were not sterilized of babesial infection. Drug resistance did not develop in *B. argentina* passaged through 7 calves treated with the depot compound. Renal damage was observed in 4 calves that died 2 to 10 weeks after treatment.

Callow and McGregor[4] reported that imidocarb readily controlled very severe infections with *B. argentina* at dose rates of 1 to 20 mg./kg. *Babesia bigemina*

infections were treated with dose rates of 0.4 to 20 mg./kg. Signs of toxicosis were acute following intravenous dosages of 10 and 20 mg./kg., although the calves survived. Signs of toxicosis were mild or absent in calves given subcutaneous dosages of 10 mg. or less/kg. The imidocarb completely cured (sterilized) infections with *B. argentina* in clinically recovered steers treated subcutaneously at dose rates from 2 to 10 mg./kg., but not at the dosage of 1 mg./kg. The steers treated at dose rates of 10 mg./kg. carried *B. bigemina* as well, and these infections were also sterilized. Splenectomized calves with acute *B. bigemina* infections were cured (sterilized) when treated subcutaneously with dose levels of 0.6 and 0.4 mg./kg. The compound also reduced the susceptibility of cattle treated with it before infection. Protection was evident in a group of steers treated subcutaneously with imidocarb at dose level of 2 mg./kg. 33 days before inoculation with *B. argentina* and was pronounced in groups treated 24, 23, and 13 days before inoculation. In tests of prophylaxis against *B. bigemina*, 2 calves given the 2 mg./kg. dose were strongly protected 12 weeks after treatment and 3 others given larger doses were refractory to infection up to 10 weeks after treatment.

The chemoprophylactic activity of imidocarb against *B. rodhaini* in the mouse and rat is not exactly comparable with that against bovine *Babesia* spp. infections. The differences in prophylactic activity between imidocarb and amicarbalide were negligible, except perhaps when they were administered within 1 week of challenge exposure. When given within this period, the effect of imidocarb was better than of amicarbalide.[2] According to Hart et al.[7] and Roy-Smith,[15] imidocarb protected cattle against *B. argentina* up to 44 days; however, protection against *B. bigemina* was not clearly demonstrated. In addition, there is an implication that the *Babesia* spp. in blood-borne-infected cattle lose their virulence and resistance to chemotherapeutic agents when maintained by serial passage in cattle.[10] This decrease may occur only after the 2nd or 3rd passage in intact cattle and after the 6th passage in splenectomized cattle. Different authors obtained different results in regard to the action of imidocarb, because *Babesia* spp. of different drug resistances may have been used.[10]

Results of the experiments in which antibody responses of calves treated with imidocarb and exposed to *B. microplus* infected ticks were similar to those obtained by Roy-Smith.[15] Complement-fixing antibody titers were detected in treated calves without detectable parasitemias with *Babesia* spp. The increase of complement-fixing antibody took place at the same time as infection of nontreated control calves. This method of antibody stimulation is a matter of speculation and worthy of detailed investigation.[15] One explanation possible is that invading parasites from the tick were killed at the site of entry and because of their large numbers, they produced antigenic stimulation, whereby the host responded with the production of antibodies which were detected by the complement-fixation test.

The results of the experiment under field conditions in Palmira confirm the laboratory findings in Bogotá of the prophylactic effect of imidocarb against *B. bigemina* and *B. argentina*. These results are not in agreement with findings of Roy-Smith[15] that *B. bigemina* is somehow imidocarb resistant, in view of the fact that the parasitemia was detectable after drug administration. The authors observed that some cattle had a detectable *B. bigemina* parasitemia, but none of them had clinical signs of disease. In contrast, calves in the control group did have clinical signs following parasitemia.

Roy-Smith[15] reported the results of a series of 4 field trials. The chemoprophylactic effect of imidocarb given subcutaneously at a dose rate of 2.0 mg./kg. against *B. argentina* and *B. bigemina* infections was evaluated in the field trials by exposing treated and nontreated cattle to natural infection. In these circumstances, nontreated cattle developed clinical babesiasis with *B. argentina*. Treatment with imidocarb at the time of exposure prevented development of clinical signs of the disease, but permitted the development of *Babesia* spp. antibodies. Treatment of a small group of cattle 36 days before exposure was less effective.

Imidocarb was highly effective against acute infections of *B. bigemina* and *B. argentina*, according to several workers (Callow and McGregor,[4] Brown and Berger,[3] Wood[19]), and this was supported by the results of the experiment reported in the present paper. The mechanism of imidocarb action is not clear. Imidocarb apparently acts directly on the *Babesia* spp. parasites, causing an alteration in number, size, vacuolation, and morphology of the nucleus and cytoplasm. These changes in *Babesia* spp. parasites apparently occur due to the malfunction of the metabolic and enzymatic processes that ordinarily ends in the death of the organism. Simultaneously, the drug causes changes in the tissue reactivity, stimulates the active mesenchyme and reticuloendothelial system, increases the phagocytosis or the number of macrophages, helps to produce defensive substances, and increases the immunologic properties of the host. Imidocarb effectively controls parasitemia; the day following injection, *Babesia* spp. parasites disappear.

Severe toxic reactions of calves treated with imidocarb in the present experiments are not substantially supported by the results of other investigators in respect to dosage of drug. In the present experiments, the dose of 3 mg./kg. injected intravenously killed 3 of 8 calves and that of 2 mg./kg. injected subcutaneously or intramuscularly caused severe toxic reactions, but did not kill calves. Callow and McGregor[4] reported that only the dose of 50 mg./kg. injected intravenously might be severely toxic; however, they found that only 1 of 6 calves dying within several hours of treatment appeared to die from drug toxicity. This calf was given 50 mg./kg. intravenously for treatment of a *B. argentina* infection. Other calves were considered to have died either of peracute babesiasis or from the effects of procedures involved in the collection of the blood vaccine. Two calves given imidocarb intravenously at dose level of 20 mg./kg. and 1, at dose level of 10 mg./kg., had incoordination, dyspnea, and salivation, but they recovered within 24 hours. The 8 steers treated subcutaneously at dose level of 10 mg./kg. were not affected.

A local reaction taking the form of a flat swelling up to 15 cm. in diameter was seen in some of the calves treated subcutaneously at dosages of 5 or 10 mg./kg. The swelling gradually subsided without any external evidence of necrosis.

Brown and Berger[3] reported that imidocarb at dose levels of 10 mg./kg. injected intramuscularly can be toxic. They reported that 1 of 12 calves injected with this dose died after treatment. Calves develop signs of acute toxicosis, with salivation, hyperventilation, and lacrimation, the severity of which correlated with the size of dosage. Eight other calves given total subcutaneous or intramuscular doses of 25 to 35 mg./kg., as repeated daily doses of 5 to 10 mg./kg., died of cumulative toxicosis 8 to 14 days later. Critical observations of 24 calves, 6 to 15 months old, and of 22 steers after slow intravenous administration (2 to 6 minutes) and fast intravenous administration (10 seconds) of doses of 0.125 to 4 mg. of base or dihydrochloride/kg. indicated that reactions were similar with both preparations. There were no cases of fatal toxicosis and there were no obvious differences in the susceptibility of calves and steers to either base or dihydrochloride. In 56 calves observed, transient signs of salivation, lacrimation, hyperventilation, coughing, and frequent micturition were severe at dose levels of 2 and 4 mg./kg. and were moderate or slight correlated with the size of the imidocarb dose and the route of injection. At dose level of 3 mg./kg., signs were moderate in both calves and adult cattle—salivation and micturition being more evident in the adult cattle and mild distress in the calves. At dose level of 5 mg./kg., signs, although transient, were more severe in calves, showing a greater tendency to distress and hyperventilation, and repeated micturition was more marked in adult cattle.

Kuttler[9] also reported that imidocarb injected intravenously into cattle produced excessive salivation, lacrimation, and labored breathing. These side effects appear less severe when the drug is administered intramuscularly or subcutaneously, and the efficacy was not altered. Death was not observed in cattle given imidocarb by intramuscular injection 3 times at 24-hour intervals at the dose level of 6 mg./kg. or when given the compound by subcutaneous injection once at the dose level of 15 mg./kg.

Carbrey et al.[6] suggested that the side effects of imidocarb could be diminished by dividing the daily dose and giving the 2 parts within 3 to 6 hours. Roby and Mazzola[14] also found that imidocarb dihydrochloride caused marked swelling and inflammation at the injection site in cattle. They suggested that this is due to the high acidity of the aqueous solution of imidocarb; however, the diproprionate salt form of imidocarb did not cause local irritation.

The potential use of imidocarb as a prophylactic drug is indicated in a number of circumstances. To protect susceptible cattle which are being moved from a tick- and *Babesia*-free country through a tick-infested area, imidocarb can be used to replace hazardous vaccination procedures with virulent *Babesia* spp. Furthermore, application of imidocarb and exposure of cattle to infected ticks with *Babesia* spp. might help in the development of natural co-infectious immunity without hazardous losses.

Finally, on the basis of results of the present experiments, imidocarb has proven to be highly efficacious in the treatment of acute babesiasis caused by *B. bigemina* and *B. argentina* of Colombian origin. In addition, the prophylactic effect of imidocarb lasted longer than any babesiacidal drug tested. However, toxic effects were found which might limit its application.

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