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A PRELIMINARY EVALUATION OF A DITHIOSEMICARBAZONE FOR THE TREATMENT OF ANAPLASMOSIS

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A Preliminary Evaluation of a Dithiosemicarbazone for the Treatment of Anaplasmosis

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SUMMARY. *Trials were conducted on 3 splenectomized calves treated with a single intravenous (I.v.) inoculation of a dithiosemicarbazone (356C61) using 5 mg./kg., at different stages of induced anaplasmosis infection. When compared to an untreated control this compound was effective in reducing the severity of the infection. Haematological response was least severe in the animal receiving treatment before signs of parasitaemia or a decrease in packed cell volume had occurred.*

Treatment with compound 356C61 (5 mg./kg. i.v.) of 5 splenectomized calves, and 6 intact adult cattle, early in the course of an artificially induced Anaplasma marginale *infection prevented death loss and reduced the severity of the subsequent reaction when compared with non-treated controls.*

TREATMENT IS NOT considered the ideal means of disease control, but it is important when adequate preventive measures do not exist or have not been used.

Treatment assumes a more important function in anaplasmosis because with this infection a complete sterile immunity has never been demonstrated. Under some circumstances it may be desirable to infect an animal in order to protect it. Such 'vaccinating' infections with virulent organisms can result in severe losses if not tempered by treatment.

The intentional establishment of carrier animals is contraindicated in areas where efforts toward eradication seem feasible, but in enzootic zones such as tropical South America and Africa premunization appears to have merit and has been used quite extensively.

Barrett *et al.* (1965) described the successful use of α-dithiosemicarbazone compounds against a number of micro-organisms including *A. marginale*, recording activity comparable to and possibly greater than the tetracyclines. Brown *et al.* (1968) and Roby *et al.* (1968) have reported the specific activity of this compound in the treatment of anaplasmosis.

In this study observations were made concerning the efficacy of compound 356C61† when administered during different stages of induced infection in splenectomized calves and when used early in the course of infection in a group of splenectomized calves and adult intact cows. Response to treatment was compared with non-treated infected splenectomized calves and intact cattle.

MATERIALS AND METHODS

Four 9-month-old, splenectomized, Holstein-Friesian calves were injected with *A. marginale* at Day 0 by identical subcutaneous inocula consisting of 1 ml. of a 30% suspension of washed erythrocytes having a 1% parasitaemia.

Treatment was administered to 3 calves, 1, 2 and 3, at varying stages of infection. Calf 4 remained as an untreated control. Each of the treated calves was given 1 i.v. injection of compound 356C61 at the rate of 5 mg./kg.

Calf 1 was treated on Day 14 when the packed cell volume (PCV) was 31%, the parasitaemia nil, and the complement fixation (CF) titre 1 : 5. Calf 2 was treated on Day 22 when the PCV was 33%, the parasitaemia 1%, and the CF titre 1 : 80. Calf 3 was treated on Day 22 when the PCV was 25%, the parasitaemia 20% and CF titre 1 : 160.

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† Gloxazone (α-ethoxyethylglyoxal dithiosemicarbazone), Burroughs Wellcome & Co., Inc. Laboratories, Tuckahoe, New York.

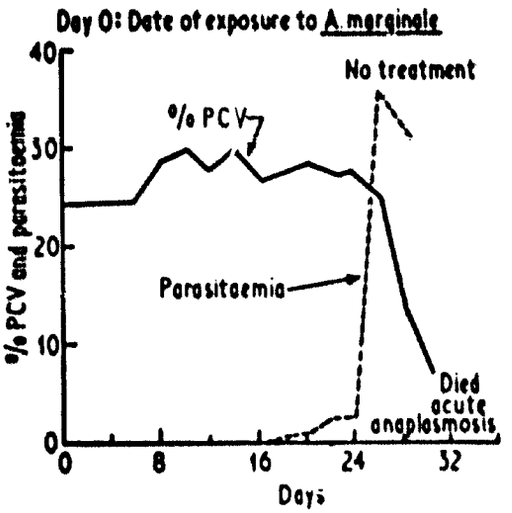

FIG. 1.—Calf 4.

Ten intact, adult Aberdeen Angus cattle were infected by the subcutaneous inoculation of 1 ml. whole blood collected from an *A. marginale* carrier cow. Of these, 6 were treated and 4 remained as untreated controls. Treatment consisted of the I.V. inoculation of compound 356C61 at the rate of 5 mg./kg., which was given on an average of 3 days after the first evidence of infection was detected, when the average PCV was 34% (±5·5) and the parasitaemia 0·9% (±2·6).

A third group of 11 splenectomized Holstein-Friesian calves was infected by the subcutaneous inoculation of *A. marginale* infected blood. Of these, 5 were treated and 6 remained as untreated controls. Treatment consisted of the I.V. inoculation of compound 356C61 at the rate of 5 mg./kg., which was given on an average of 3 days after the first evidence of infection was detected, when the average PCV was 33% (±5·3) and the parasitaemia 1·5% (±2·0).

Blood samples were collected from all animals weekly for 4 weeks before infection and at least twice a week after infection for the duration of the experiment. Complement-fixation tests were conducted using the basic procedures outlined by the U.S.D.A. (1958) but employing a micro-technique for titrations (Hidalgo & Dimopoullos, 1967). PCV determinations were made using a haematocrit centrifuge. Giemsa stained blood smears were used in the determination of parasitaemias.

An analysis of variance (Snedecor, 1956) was used for the determination of statistical differences in averages between treated and untreated controls.

RESULTS

The course of induced anaplasmosis in a splenectomized calf without treatment is shown in Fig. 1. The results of treatment when administered at varying stages of infection in 3 similar calves are shown in Figs. 2, 3 and 4.

Death due to acute anaplasmosis occurred on Day 30 in the untreated control. Treatment in the very early stages of infection (Calf 1) resulted in a prolonged course of infection, with

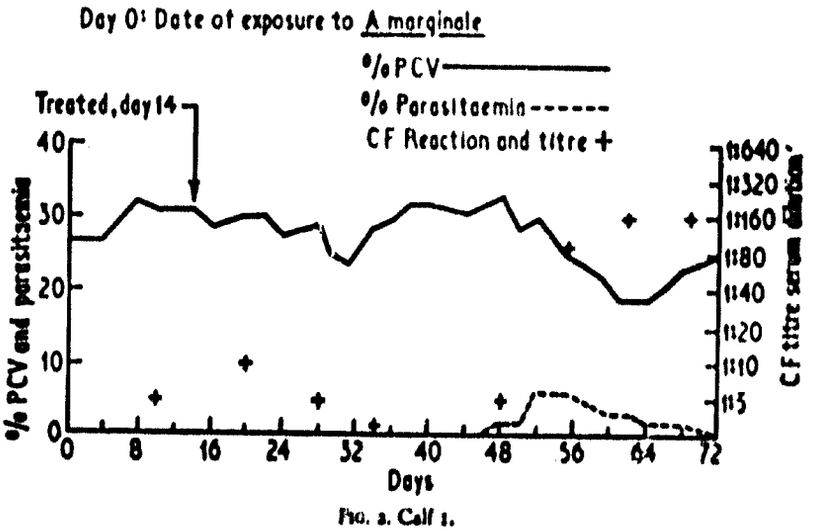

FIG. 2.—Calf 1.

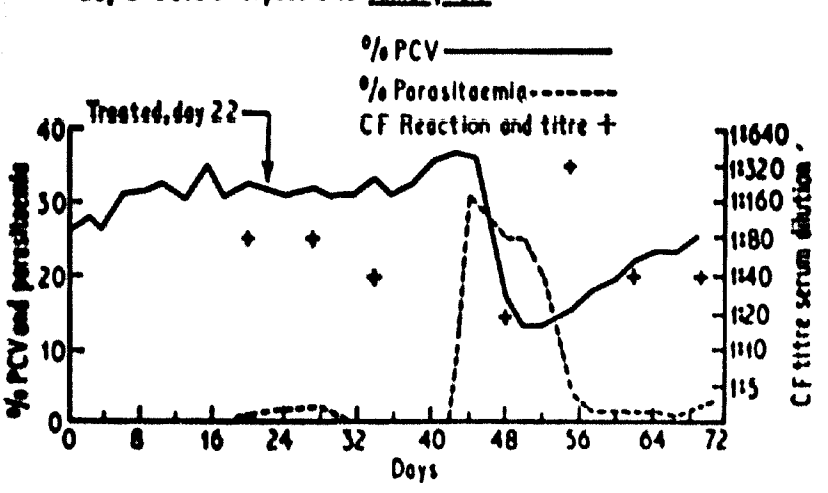

FIG. 3. Calf 2.

no demonstrable parasitaemia until Day 50. The parasitaemia in this calf remained low and was accompanied by a relatively mild drop in PCV to 19%. Treatment of Calf 2 following the observance of a low level parasitaemia on Day 22 resulted in a comparatively rapid disappearance of the parasites. A secondary response occurred at Day 44 which was characterized by a rapid increase in parasitaemia to 30% and corresponding decrease in PCV to a low of 14%. Recovery occurred following this secondary response. Treatment of Calf 3 on Day 22 following a primary 20% parasitaemia resulted in a disappearance of *Anaplasma* bodies. The primary parasitaemia was accompanied by a low PCV of 17%, which returned to a normal pre-infection range following treatment. A secondary response be-

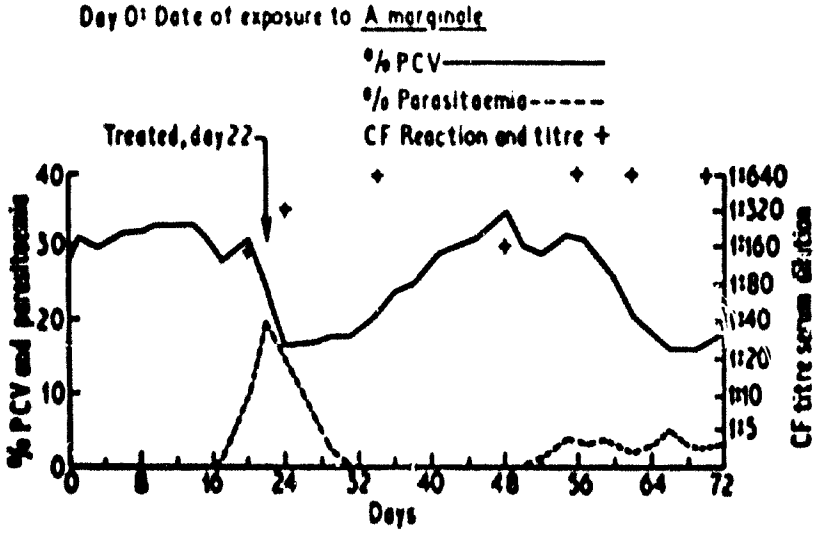

FIG. 4. Calf 3.

TABLE I
TREATMENT WITH COMPOUND 356C61 OF ADULT INTACT CATTLE AND SPLENECTOMIZED CALVES

	Treatment* data					Anaplasmosis reaction			
	No. of Animals	Av. age (mths)	Av. parasitaemia at time of treatment %	Av. PCV at time of treatment %	Av. low PCV %	Av. high CF	Av. high parasitaemia %	Time for low PCV to occur (days)	Deaths
Intact cattle treated	6	46·2 ± 28·3	0·9 ± 2·0	34 ± 5·5	22	1 : 320	8·3	28·2	0
Intact cattle not treated	4	84·0 ± 56·3	NT	33 ± 1·3	12·0	1 : 900	21·0	20·0	1
Significance				NS	P<0·01	P<0·05	P<0·01	P<0·05	
Calves† treated	5	6·9 ± 2·4	1·5 ± 2·0	33 ± 5·3	16·4	1 : 320	22·4	41·0	0
Calves† not treated	6	6·8 ± 3·5	NT	30 ± 4·7	11·7	1 : 1000	37·3	12·8	2
Significance				NS	P<0·05	P<0·05	NS	P<0·01	

* Treatment was administered on an average of 3 days after the first evidence of infection (either a positive CF response or a diagnostic parasitaemia).

NS: Not significant
NT: No test
±: Standard deviation
†: Splenectomised

ginning on Day 52 resulted in an 8% parasitaemia and a 16% PCV. Recovery occurred following the secondary response.

The effects of treatment on adult cattle and splenectomized calves are recorded in Table I. The pattern of response was similar in both groups. The anaplasmosis response was retarded, and the severity was reduced as the result of treatment. Among 10 untreated animals, 3 deaths occurred. Among 11 treated animals, no deaths occurred.

DISCUSSION

In no instance did a single treatment with compound 356C61 result in complete removal of the *A. marginale* infection. Treatment was effective in eliminating temporarily a demonstrable parasitaemia.

Treated animals characteristically developed a secondary response, which was usually milder than the primary infection seen in untreated controls.

Compound 356C61 when administered early in the course of infection as a single i.v. injection at the rate of 5 mg./kg. was effective in preventing death loss, and in reducing the severity of the anaplasmosis reaction.

In areas where premunition with virulent *A. marginale* is the method of choice for prophylaxis, treatment with compound 356C61 early in the course of infection might be a feasible approach toward the elimination of losses.

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REFERENCES

BARRETT, P. A., BEVERIDGE, E., BRADLEY, P. L., BROWN, C. G. D., BUSHBY, S. R. M., CLARKE, M. L., NEAL, R. A., SMITH, R. & WILDE, J. K. H. (1965) *Nature, Lond.* **206**, 1340

BROWN, C. G. D., WILDE, J. K. H. & BERGER, J. (1968) *Br. vet. J.* **124**, 325

HIDALGO, R. J. & DIMOPOULLOS, G. T. (1967) *Am. J. vet. Res.* **28**, 245

ROBY, T. O., AMERAULT, T. E. & SPINDLER, L. A. (1968) *Res. vet. Sci.* **9**, 494

SNEDECOR, G. W. (1957) 'Statistical Methods', 5th ed., The Iowa State College Press, Ames, Iowa.

UNITED STATES DEPARTMENT OF AGRICULTURE (1958) 'A Manual for Conducting the Complement-Fixation Test for Anaplasmosis.'