RELATIONSHIPS BETWEEN RESISTANCE TO *BOOPHILUS MICROPLUS*, NUTRITIONAL STATUS, AND BLOOD COMPOSITION IN SHORTHORN × HEREFORD CATTLE

By J. C. O'KELLY* and G. W. SEIFERT†

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Summary

Relationships between nutrition, blood composition, and tick resistance were studied in three groups of six yearling Shorthorn × Hereford steers. Feeding was *ad libitum* and group I was offered lucerne hay and safflower seeds, group II lucerne hay only, and group III wheat straw supplemented with molasses. All animals were infested five times with 0·5 g tick larvae during the treatment period of 62 days.

The mean number of mature female ticks counted on one side of each animal and the mean percentage change in body weight during treatment were respectively: group I, 1797 and +20·1; group II, 1921 and +14·0; and group III, 3555 and −13·3.

At the end of the experimental period there were significant increases ($P < 0·05$) in haematocrit, haemoglobin, and plasma protein-bound iodine in groups I and II. Significant decreases ($P < 0·01$) were found in haematocrit, haemoglobin, plasma protein-bound iodine, and serum globulin, cholesterol, phospholipid, and triglyceride in group III. Serum albumin, total protein levels, and red cell counts were significantly lower ($P < 0·01$) in group III than in the other two groups. The free : total cholesterol ratio in plasma was significantly elevated ($P < 0·01$) in all groups.

The influence of both nutrition and tick infestation on blood composition is discussed. It was concluded that at the tick dosage used the level of tick resistance is stable on an adequate diet and that dietary deficiency influences the breaking down of resistance.

I. INTRODUCTION

It is now well established that there are marked differences between breeds of cattle in their resistance to the cattle tick, *Boophilus microplus*, and also that within breeds there are wide animal variations in susceptibility to tick infestation (Wilkinson 1955; Riek 1962; Francis and Little 1964; Roberts 1968). However, the mechanism of tick resistance in cattle remains largely undetermined. Many workers have mentioned a loss of resistance in animals to parasites in general as a result of malnutrition (Dogiel 1964) but there is a lack of published information on the nutritional component of tick resistance in cattle.

Most papers dealing with the relationships between cattle and tick report the changes of haematocrit and haemoglobin in the host following infestation with the parasite but there are few reports of changes in other blood components. Francis and Ashton (1967) found an association between the distribution of amylase genes and tick burden which reached significance in Droughtmaster but not in *Bos taurus*

* Division of Animal Genetics, CSIRO, Cattle Research Laboratory, Rockhampton, Qld. 4700.

† Division of Animal Genetics, CSIRO, National Cattle Breeding Station, Belmont, Rockhampton, Qld. 4700.

cattle. In a study of grazing Zebu and Shorthorn × Hereford cattle in a tropical environment O'Kelly (1968*a*) found that within breeds, animals with high plasma cholesterol carried significantly fewer mature cattle tick.

In studying possible blood factors related to resistance it is important to know the effects of levels of tick burden on blood composition. Poor nutrition is also reflected by changes in blood components such as haemoglobin and serum protein (Meacham *et al.* 1964) and plasma lipids (O'Kelly 1968*b*). A study of blood composition alterations during tick infestation of cattle on different planes of nutrition may thus contribute to a more complete knowledge of host–parasite relationship and of factors that might augment host resistance.

As part of a series of studies on the interactions between ticks and nutrition this paper reports the response to tick infestation of Shorthorn × Hereford steers on planes of nutrition which simulate natural tropical summer and dry season conditions, i.e. diets designed to increase and reduce body weight respectively, and also changes in blood composition on these treatments.

II. Materials and Methods

(*a*) *Animals, Diets, and Tick Infestations*

Eighteen yearling British-breed steers (Hereford × Shorthorn) × (Shorthorn × Hereford) were selected at random from a group of 26.

The initial tick resistance of the 18 animals was assessed while they were on pasture from November 2 to December 15, 1966 (Fig. 1). Each animal was infested with 0·5 g tick larvae on two occasions and the female ticks maturing 19–23 days after each dose were counted. The number of adult female ticks between 0·45 and 0·8 cm on the complete left side of the animal resulting from each infestation was used as the index of susceptibility. The observed tick counts on this occasion are referred to as the selection counts.

Animals were ranked on tick resistance and allocated by restricted randomization to three groups; each group was then allocated at random to its respective nutritional treatment from January 4 to March 16, 1967. Each group was yarded separately and all animals were fed *ad libitum* and had access to shade and water. Group I received medium quality lucerne hay and untreated safflower seed; group II received only medium quality lucerne hay and group III wheat straw. Body weights of the animals were recorded before and after a 16-hr overnight fast. To curb excessive weight loss of group III animals, when they had been on straw for 5 weeks they were given 8 oz of molasses per animal per day at a 1 : 20 dilution sprayed over the straw. This increased the intake of straw as judged by the weight loss during the 16-hr fast and was responsible for the weight gain from February 9–16. The molasses was subsequently reduced to 4 oz per animal per day. The mean empty body weights (lb) at the beginning of treatment were: group I, 398; group II, 425; group III, 387. Each animal was infested with 0·5 g tick larvae on five occasions during the treatment period and tick resistance was assessed as described above for the selection period.

(*b*) *Analytical Procedures*

The techniques for the analyses of plasma lipids have been fully described previously (O'Kelly 1968*a*) and all values are given as milligrams per 100 ml of plasma except the non-esterified fatty acids which are in microequivalents per litre.

Haematocrit percentages of the packed cells were determined with an International Equipment Co. microcapillary centrifuge (15,000 *g* for 4 min). Haemoglobin was determined as the oxyhaemoglobin and red cell counts were made with an EEL electronic cell counter.

The method employed for the estimation of plasma protein-bound iodine (P.B.I.) was that of Brown, Reingold, and Samson (1953) as modified by Lennon and Mixner (1957) and with an adaptation of the colorimetric procedure to the Technicon auto-analyser. Other plasma con-

stituents were determined by the following methods: Albumin and globulin (Fernandez, Sobel, and Goldenberg 1966), total protein by the biuret method as described by King and Wootton (1956), and sodium and potassium with an EEL flame-photometer. Electrophoretic separation of serum proteins was obtained using cellulose acetate strips and veronal (barbital) buffer, pH 8·6, ionic strength 0·05. The cellulose acetate strips were stained with Ponceau S, the bands were eluted with alkali, and the optical density of the solution was measured at 525 nm.

III. Results

(*a*) *Tick Infestation and Dietary Treatments*

The weight gains and mean weekly tick counts for the groups are presented in Figure 1. The counts before the commencement of treatment represent the mean of two selection counts. Differences between the selection counts of the groups were non-significant.

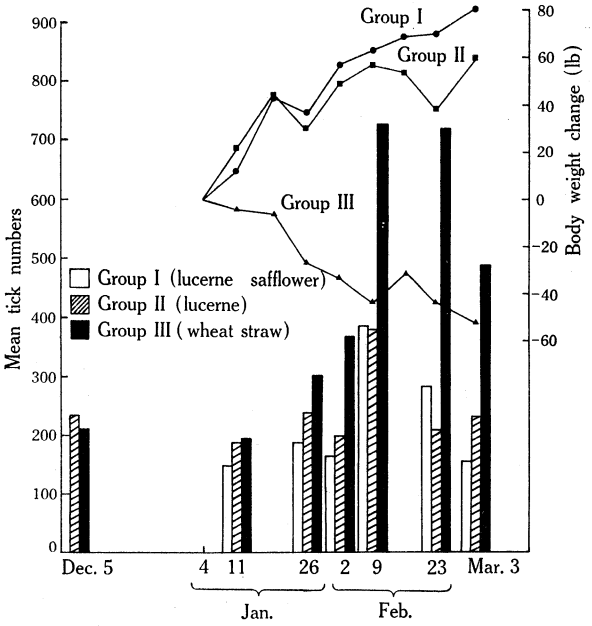

Fig. 1.—Change in body weight and mean tick numbers per animal in the treatment period (January 4–March 3, 1967). The tick counts in the selection period (December 5, 1966) are also shown.

Weight gains of animals on the two high planes of nutrition (groups I and II) were similar while weight losses of animals on the low plane of nutrition (group III) increased rapidly after the second week of treatment. The loss of weight from January 19–26, 1967, of groups I and II could possibly have been due to extremely hot and humid conditions experienced during that period. These conditions similarly affected group III animals which experienced their greatest mean weekly loss (21 lb). Weight losses for group II from February 9–23, 1967, were possibly the result of eating mouldy hay, which apparently did not affect group I because of the safflower seed supplement.

The relationship between weight change and tick numbers is apparent from Figure 1; increased tick numbers were associated with decreasing weight in group III. The mean number of ticks carried per animal and the mean percentage change in body weight during treatment were respectively; group I, 1797 and +20·1; group II, 1921 and +14·0; and group III, 3555 and −13·3. The group III values were

significantly different ($P < 0{\cdot}01$) from the values for the other two groups. It may be significant that tick scores (February 9) obtained from infestations of January 19–26, when groups I and II were losing weight, were the highest recorded for these two groups.

TABLE 1

CORRELATIONS OF THE SELECTION COUNTS TO THE EXPERIMENTAL COUNTS

Date	Correlation Coefficient		
	Group I	Group II	Group III
11.i.67	0·85*	0·99***	0·58
26.i.67	0·76	0·95**	0·03
2.ii.67	0·80	0·85*	0·90*
9.ii.67	0·74	0·84*	0·79
23.ii.67	0·64	0·31	0·01
3.iii.67	0·44	0·30	−0·22

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$. *** $P < 0{\cdot}001$.

TABLE 2

SERUM LIPID CONCENTRATIONS IN THE SELECTION PERIOD (S) AND 34 AND 62 DAYS AFTER THE BEGINNING OF THE TICK-INFESTATION PERIOD

Values given are means ± standard errors

Lipid	Period (days)	Dietary Treatment*		
		Group I: Lucerne + Safflower Seeds	Group II: Lucerne	Group III: Wheat Straw
Total cholesterol (mg/100 ml)	S	63·6± 3·6	61·2± 2·5	61·8± 2·3
	34	101·7±13·0	64·2± 2·8	43·0± 2·6
	62	75·2± 6·8	60·8± 3·5	37·4± 4·2
Free cholesterol (mg/100 ml)	S	12·1± 0·6	11·7± 0·4	11·9± 0·4
	34	18·7± 2·3	12·2± 0·5	9·7± 0·9
	62	18·3± 1·0	13·9± 1·0	10·2± 1·1
Ratio of free to total cholesterol (%)	S	19·2± 0·2	19·1± 0·2	19·1± 0·1
	34	18·3± 0·4	19·2± 0·3	22·3± 1·0
	62	24·9± 1·4	23·0± 1·7	27·9± 2·8
Phospholipid (mg/100 ml)	S	83·0± 4·0	85·8± 2·7	91·1± 5·4
	34	135·0±12·9	95·5± 6·8	70·8± 4·4
	62	110·1± 9·1	83·2± 7·7	50·7± 3·8
Triglyceride (mg/100 ml)	S	25·1± 0·9	24·1± 2·3	22·8± 1·5
	34	37·4± 4·5	33·9± 1·6	14·4± 0·8
	62	32·0± 4·8	24·7± 3·0	11·6± 1·8
Non-esterified fatty acids (μ-equiv/l)	S	859 ±60	978 ±92	921 ±61
	34	465 ±24	432 ±18	542 ±74
	62	404 ±33	320 ±18	483 ±79
Total lipids (mg/100ml)	S	234·3±10·7	236·3± 8·2	242·1± 9·8
	34	336·1±32·6	240·7± 7·8	166·2± 5·5
	62	282·0±21·2	214·1±14·2	131·8±12·2

* In selection period S all animals were at pasture.

The correlations between the selection counts and all the other counts on individual animals are given in Table 1. The correlations declined with time but those of group III were erratic, the final correlation being negative.

Weight gain on pasture (November 17–December 14, 966) was negatively correlated ($r = -0{\cdot}49$, $P < 0{\cdot}05$, 16 d.f.) to the selection tikc counts. By contrast the correlations between the total number of tick carried during treatment and the gain over the whole period were very low for all groups.

TABLE 3

SERUM PROTEINS, PLASMA ELECTROLYTES, AND PLASMA PROTEIN-BOUND IODINE IN THE SELECTION PERIOD (S) AND 34 AND 62 DAYS AFTER THE BEGINNING OF THE TICK-INFESTATION PERIOD

Values given are means ± standard errors

Determination	Period (days)	Dietary Treatment*		
		Group I: Lucerne + Safflower Seeds	Group II: Lucerne	Group III: Wheat Straw
Serum albumin (g/100 ml)	S	2·3 ±0·1	2·3 ±0·1	2·2 ±0·2
	34	2·2 ±0·2	2·4 ±0·1	2·1 ±0·2
	62	2·7 ±0·1	2·6 ±0·1	1·9 ±0·1
Serum globulin (g/100 ml)	S	4·9 ±0·2	4·7 ±0·2	4·7 ±0·1
	34	5·1 ±0·2	5·1 ±0·2	4·1 ±0·1
	62	4·3 ±0·3	4·3 ±0·2	3·4 ±0·1
Albumin/globulin ratio (%)	S	46·8 ±4·5	48·1 ±0·9	50·6 ±4·4
	34	44·5 ±4·6	47·5 ±2·5	51·8 ±5·3
	62	64·9 ±4·2	60·7 ±2·4	56·4 ±2·8
Serum total proteins (g/100 ml)	S	7·2 ±0·1	6·9 ±0·3	6·9 ±0·3
	34	7·3 ±0·2	7·4 ±0·2	6·2 ±0·2
	62	7·0 ±0·3	6·8 ±0·1	5·3 ±0·2
Relative percentages of serum proteins:				
Albumin	62	46·7 ±1·1	46·0 ±0·9	41·9 ±0·6
α-Globulin	62	12·1 ±0·4	11·4 ±0·4	13·9 ±0·2
β-Globulin	62	10·4 ±0·1	10·6 ±0·1	5·6 ±0·3
γ-Globulin	62	30·8 ±3·9	32·0 ±0·5	38·6 ±0·1
Plasma sodium (m-equiv/l)	62	141·4 ±0·7	140·7 ±0·7	140·8 ±0·8
Plasma potassium (m-equiv/l)	62	4·6 ±0·2	4·6 ±0·1	4·9 ±0·1
Plasma protein-bound iodine (μg/100 ml)	S	3·37±0·22	2·92±0·29	3·75±0·27
	34	3·41±0·18	3·12±0·26	1·69±0·15
	62	4·44±0·31	4·10±0·24	1·73±0·19

* In selection period S all animals were at pasture.

(*b*) *Blood Composition*

The results of the blood composition analyses are given in Tables 2–4. There were no significant differences between the groups in any of the parameters determined in the selection period. After 34 days in the treatment period significant changes in blood composition were an increase in group I total and free cholesterol ($P < 0{\cdot}05$),

triglyceride ($P < 0{\cdot}05$), and total lipid ($P < 0{\cdot}05$), and group II haematocrit ($P < 0{\cdot}001$), haemoglobin ($P < 0{\cdot}001$), and triglyceride ($P < 0{\cdot}01$), and a decrease in group III total cholesterol ($P < 0{\cdot}001$), phospholipid ($P < 0{\cdot}05$), triglyceride ($P < 0{\cdot}001$), total lipid ($P < 0{\cdot}001$), and P.B.I. ($P < 0{\cdot}001$).

At the end of the experimental period (62 days) there were significant increases from the initial values in haematocrit ($P < 0{\cdot}05$), haemoglobin ($P < 0{\cdot}001$), and P.B.I. ($P < 0{\cdot}05$) in groups I and II and in free cholesterol ($P < 0{\cdot}001$) and phospholipid ($P < 0{\cdot}05$) in group I. Significant decreases ($P < 0{\cdot}01$) were found in group III in haematocrit, haemoglobin, P.B.I., and serum globulin, cholesterol, phospholipid, triglyceride, and total lipid levels. The free : total cholesterol ratio

TABLE 4

HAEMATOLOGICAL VALUES IN THE SELECTION PERIOD (S) AND 34 AND 62 DAYS AFTER THE BEGINNING OF THE TICK-INFESTATION PERIOD

Values given are means ± standard errors

Determination	Period (days)	Dietary Treatment*		
		Group I: Lucerne + Safflower Seeds	Group II: Lucerne	Group III: Wheat Straw
Haematocrit (%)	S	28·9±1·8	27·5±0·7	27·8±0·7
	34	32·8±1·0	33·1±1·0	28·0±0·3
	62	33·6±0·6	33·0±0·4	21·5±1·8
Haemoglobin (g/100 ml blood)	S	12·2±0·8	11·2±0·3	11·9±0·3
	34	14·8±0·6	14·8±0·4	12·6±0·3
	62	14·6±0·4	14·0±0·2	9·0±0·7
Red cell count (millions/mm^3)	62	7·8±0·4	7·1±0·3	5·3±0·5
Mean corpuscular haemoglobin concn. (%)	S	42·2±0·3	40·8±0·8	42·3±0·6
	34	44·9±0·6	44·8±0·6	45·0±0·8
	62	43·2±0·4	42·5±0·2	41·8±1·4
Mean corpuscular volume (μm^3)	62	43·9±2·4	46·5±2·1	41·2±2·1
Mean corpuscular haemoglobin content (pg)	62	19·1±1·2	19·8±1·0	17·2±0·8

* In selection period S all animals were at pasture.

in plasma was significantly elevated ($P < 0{\cdot}01$) in all groups but the albumin : globulin ratio was significantly increased ($P < 0{\cdot}01$) in only groups I and II. Serum albumin, total protein concentrations, and red cell counts were significantly lower ($P < 0{\cdot}01$) in group III than in the other two groups. The albumin and β-globulin serum fractions were significantly lower ($P < 0{\cdot}01$) while the α-globulin was significantly higher ($P < 0{\cdot}05$) in group III than in the other groups. γ-Globulin was significantly higher ($P < 0{\cdot}001$) in group III than in group II. There were no significant differences between groups in plasma sodium and potassium, mean corpuscular haemoglobin concentration, mean corpuscular volume, or mean corpuscular haemoglobin content.

(c) *Relationships between Tick Resistance and Blood Composition*

The 18 animals were bled at the beginning and end of the selection period and showed significant animal differences in both plasma cholesterol ($r = 0{\cdot}922$, $P < 0{\cdot}001$) and total lipid ($r = 0{\cdot}878$, $P < 0{\cdot}001$). Tick score was negatively correlated with some blood components in this period as indicated in the following tabulation:

Blood Character	Correlation Coefficient	
	Initial Day	Final Day
Plasma total cholesterol	−0·469*	−0·590**
Plasma total lipid	−0·829***	−0·765***
Haematocrit		−0·194
Haemoglobin		−0·216
Plasma protein		−0·105

d.f. 16, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Correlations between total tick score in the treatment period and the mean plasma lipid concentration of that period are shown in the following tabulation:

Plasma Lipid	Correlation Coefficient		
	Group I	Group II	Group III
Total cholesterol	−0·650	−0·093	−0·661
Total lipid	−0·763	−0·160	−0·686

These are not statistically significant but if treatment differences were ignored there were significant negative relationships between tick counts and plasma cholesterol and total lipid; this arose because group III had lower plasma cholesterol and total lipid and much higher tick numbers than the other two groups.

IV. Discussion

The breakdown of tick resistance in cattle due to malnutrition and consequent loss of body weight found in this study is analogous to results with other hosts and parasites (Lapage 1951, 1956; Dogiel 1964). The effect of licking may be an important aspect of resistance (Snowball 1956). It is possible that the increased tick numbers found in the low energy–low protein intake group bear some relationship to the available energy of the animals and therefore to their ability to groom effectively.

Young animals during the rapid phase of growth are very responsive to environmental stress and as such are affected to a larger degree than mature animals by malnutrition. The magnitude of the breakdown of tick resistance, as judged by the increased tick burdens in the relatively short treatment period, may therefore have been less striking if mature animals had been used.

The animals were fed in groups so individual feed intakes were not recorded. Maintenance of the lipaemia which develops in cattle on an oil seed diet depends on the continued ingestion of lipid (O'Kelly and Robinson 1968). In the group supplemented with oil seed the plasma lipid levels fluctuated greatly within individual animals during the experimental period suggesting an irregular intake of dietary oil.

There may also have been relatively large daily variations in the ratio of safflower seed to lucerne hay ingested by individual animals. This would be another factor contributing to fluctuating plasma lipid levels since the effect of dietary fat in raising the plasma lipid concentrations is modified by other factors such as the level of protein intake (Bohman, Wade, and Torell 1962). This study shows then that supplementing high protein diets with oil seeds in *ad libitum* feeding regimes is not a successful way of artificially raising and maintaining high plasma cholesterol levels. The mean number of ticks per animal and the mean percentage change in body weight during treatment were respectively 1797 and +20·1 in the oil-supplemented group and 1921 and +14·0 in the group receiving lucerne hay alone. However, because of the fluctuations in plasma lipids in animals fed safflower seeds the results of testing the association noted previously (O'Kelly 1968*a*) between the elevated plasma lipid levels and tick resistance must be considered inconclusive.

The reduction in plasma lipids in the low quality group is consistent with a nutritional deficiency alone (O'Kelly 1968*b*) and it is therefore not possible to separate the effect of nutrition from any effects which the tick might exert on plasma lipids. Cholesterol is esterified by the liver so that liver dysfunction (which may be nutritional or caused by a toxin) might be expected to interfere with its formation and cause a significant increase in the free : total cholesterol ratio. While the increased free : total cholesterol ratio in the low quality group may be largely nutritional the significant increases in this ratio in animals on the high planes of nutrition is strongly suggestive of a toxin which interferes with the synthesis of cholesterol esters.

The animals on the low quality diet showed a marked hypoproteinaemia. Dietary protein intake affects the metabolism of serum albumin (Viteri *et al.* 1964) and feeding the protein-deficient diet presumably resulted in the reduction in plasma albumin levels. However, not only was the concentration of serum globulin reduced but the percentage distribution of the various fractions was also altered. The reduction in the β-globulin component agrees with the finding of Chandler, McCarthy, and Kesler (1968) who reported that serum β-globulin was the most responsive to dietary changes in dairy calves. An increase in γ-globulin may be relatively non-specific (see e.g. Leland 1961; Herlich and Merkal 1963) and the significant increases reported here could be the result of antibody formation against the parasite or microorganisms introduced by the tick, or a compensatory reaction to restore serum osmotic pressure that would be reduced as a result of the low albumin concentration. Reduced protein intake may also result in inability to synthesize adequate amounts of lipoproteins and so impair the transport of lipids.

In ruminants anaemias result from the activities of blood sucking parasites and are well recognized in tick infestation in cattle (see e.g. Francis 1960; Riek 1957). The results reported here for light tick burdens on growing animals suggest that adequate nutrition stimulates the erythropoietic system enough to produce significant increases in the haematocrit values. Poor nutrition produces a fall in haematocrit and haemoglobin levels (Meacham *et al.* 1964). The depressed haematocrit and haemoglobin levels of tick infested animals on poor quality feed therefore reflect the combined influence of poor nutrition and tick infestation and in this experiment it was not possible to evaluate the influence each exerted independently. Using the data of Seifert, Springell, and Tatchell (1968) it was calculated that the greatest

daily blood loss to ticks was not more than 100 ml per animal and it is not surprising then that changes in mean corpuscular volume and mean corpuscular haemoglobin concentration which are characteristic of bone marrow response to severe blood loss (Schalm 1961) were not detected.

Blood loss is often accompanied by withdrawal of fluid from the tissues to make up the blood volume and potassium and sodium are important intra- and extracellular ions which help maintain osmotic pressure equilibrium. No changes in the plasma concentration of these two ions were observed in any animals at the end of the experimental period.

P.B.I., which may be used as an indicator of thyroid activity in cattle (Post 1965*b*), is altered by nutrition (Post 1965*a*) and is positively correlated with plasma cholesterol (O'Kelly 1968*a*). Decreased P.B.I. was then expected to accompany the depressed plasma cholesterol concentrations in animals on the poor quality diet. Many of the metabolic changes in the tick infested animals on poor quality diet may then be an effect of nutrition alone exerting its influence through the hormonal axis.

The results clearly show that within 34 days of dietary treatment tick resistance had broken down in the animals fed a low quality diet. The significant changes observed at this time in the blood compostion of these animals were a decrease in P.B.I., serum globulin, and plasma lipids. By contrast the significant elevation of the ration of free : total cholesterol, indicating liver dysfunction, was found in all animals, but was not apparent until the end of the treatment period.

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