ALTERATIONS IN HOST METABOLISM BY THE SPECIFIC AND ANORECTIC EFFECTS OF THE CATTLE TICK (*BOOPHILUS MICROPLUS*)

III.* METABOLIC IMPLICATION OF BLOOD VOLUME, BODY WATER, AND CARCASS COMPOSITION CHANGES

By P. H. SPRINGELL,† J. C. O'KELLY,† and R. M. SEEBECK†

[Manuscript received July 31, 1970]

Abstract

Plasma and red cell volumes were determined and the amounts of circulating metabolites deduced following heavy *B. microplus* infestation of Hereford steers fed a high quality diet. The experiment was designed to separate out effects of the cattle tick on host metabolism caused by reduced food intake ("anorectic effect"), and by other factors ("specific effect"). The specific effect caused a depression of the red cell volume and of the amounts of circulating haemoglobin, albumin, and total cholesterol and increases in the amount of circulating globulin. Anorectic effects were not significant.

Body water determinations were carried out at the end of the infestation period and again at slaughter. The specific effect of the tick resulted in a deficit in body weight, most of which could be accounted for by the reduced total body water. Weight increase during the post-infestation period was accomplished largely by means of water accretion.

Carcass composition during recovery revealed no significant differences between previously infested and uninfested steers. The composition at the end of infestation, as deduced by extrapolation from body water and dissection data indicated, however, that the ticks could have induced the loss of muscle relative to fat.

The observed losses in haemoglobin, plasma albumin, and plasma cholesterol in infested steers were approximately the same as the amounts consumed by the ticks. The apparent failure of the animals to make up these losses may be attributable to metabolic interference by tick toxin. By contrast, the amount of plasma globulin was not only maintained, but the net amount in the host increased, possibly as a result of an immunological response.

I. INTRODUCTION

Reduction in the host haematocrit as a result of cattle tick (*Boophilus microplus*) infestation is well known (Riek 1957). This could be caused by a drop in the red cell volume, or by an increase in the plasma volume or both. In order to understand more fully how the parasite exerts its effect on the host, it was necessary to determine whether

* Part II, *Aust. J. biol. Sci.*, 1971, **24**, 381–9.

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

apparent changes in the concentration of metabolites (O'Kelly, Seebeck, and Springell 1971) reflected genuine alterations in the amounts of each metabolite, or whether the changes were caused by water transfer in or out of the vascular system.

With information available on the amounts of total circulating metabolites, the mean tick burdens, and an estimate of the average blood uptake by ticks (Seifert, Springell, and Tatchell 1968), it was possible to draw up a balance sheet for the metabolites in question. This gives an insight into how well the animals were able to maintain metabolic steady states of particular metabolites. Carcass composition data, together with body water measurements at appropriate times, also provided an indication of how particular metabolic disfunctions resulting from tick infestation might be translated into compositional changes.

II. Materials and Methods

(a) *Experimental Animals and their Treatment*

The experimental design is fully described in a previous paper (Seebeck, Springell, and O'Kelly 1971). Twenty-one 13-months-old Hereford steers at pasture were allocated to three groups of seven animals, so that each group covered a similar weight range. In this study four animals of each group were selected so as to maintain comparable weights between groups. All animals were subjected to two artificial infestations of 0·5 and 1·0 g larvae at pasture before the start of the experiment. The subsequent diet for all animals was high quality feed in the form of cubes. One group (TI) was infested twice weekly with 2 g tick larvae and without restrictions on feed intake; a second group (PF) was kept tick-free and pair-fed to the tick-infested group. A third control group (AL) was kept tick-free and without restrictions on feed intake. Tick infestations had to be reduced on days 40–63 because of its serious effects on food intake and body weight. Female ticks maturing in the TI group of animals were counted by the procedure described by Seebeck, Springell, and O'Kelly (1971). Studies were performed immediately prior to infestation, then again at the end of infestation (11 weeks later), and finally at slaughter. The diet during the recovery phase consisted of free access to pasture together with cattle cube supplementation (Seebeck, Springell, and O'Kelly 1971).

(b) *Measurement of Blood Parameters*

The 12 animals were each injected with 5 mCi [^{51}Cr]chromic chloride (Australian Atomic Energy Commission) in 10 ml isotonic saline. A blood sample was collected 10 min after injection of the label for estimation of plasma volume, as described previously (Vercoe and Springell 1969). Counting was done in a Packard Autogamma scintillation spectrometer. At least 10,000 counts were accumulated, except for blanks which were counted for not less than 10 min.

The red cell volume and total blood volume were calculated for each steer from the observed venous haematocrit and the plasma volume. No corrections were applied to the haematocrit for possible whole body/venous differences or for trapped plasma (Springell 1968*a*).

Concentrations of most parameters were measured in blood samples collected before and at the end of the infestation period, as described elsewhere (O'Kelly, Seebeck, and Springell 1971). By multiplying the concentration by the plasma volume (or by the blood volume in case of haemoglobin), the value for the total circulating material in question was obtained (Table 1). Plasma samples were assayed for total bilirubin (Biochemica Test Combination, Boehringer & Sons) only at the end of the infestation period.

(c) *Blood Lost to Ticks*

Adult female ticks (4·5–8·0 mm long) were counted 19 and 20 days after each infestation (i.e. on 4 days each week) on one side of the animal. The mean count (Seebeck, Springell, and O'Kelly 1971) over the 51-day period (when engorging female ticks were present) was multiplied by 2 to give the mean daily tick burden (Table 8). The mean blood loss was then estimated on the assumption that each adult female imbibes 0·3 ml (Seifert, Springell, and Tatchell 1968).

(*d*) *Total Body Water Estimation*

This was carried out by the tritium dilution method at the end of the infestation period and just prior to slaughter, as described previously (Springell 1968*b*). The body weights used for water content calculations were the mean of the weights at injection and at collection. They are close to, but not identical with previously listed weights (Seebeck, Springell, and O'Kelly 1971).

(*e*) *Carcass Composition*

The animals were slaughtered at various times during recovery from tick infestation or nutritional restriction (Table 3 in Seebeck, Springell, and O'Kelly 1971). Although only four representatives of each group were involved in the experiments described here, slaughter weights were allocated over all seven animals within a group at random. Target slaughter weights were separated by 14, 16, and 18 kg, with top weights being 398, 395, and 392 kg for the previously tick-free *ad lib.*, tick-free pair-fed, and tick-infested *ad lib.* groups, respectively. Animals 1, 3, 5, and 6 in each of the treatments in that table are discussed in this paper.

After removal of the hide and viscera, the half carcass was dissected into muscle, fat, bone, and fascia plus tendon (Seebeck and Tulloh 1968). The total dissectable fat consisted of subcutaneous and intermuscular fat, as well as the kidney and channel fat portions.

(*f*) *Analysis of the Data*

Adjusted means for the various blood parameters (other than bilirubin) in the final period were arrived at by analysis of covariance (Snedecor 1956), in which account was taken of the initial differences between animals in that parameter. Composition, both in terms of body water and of dissected carcass components, was adjusted to constant body weight using logarithmic models (Seebeck 1968). Predictions of carcass composition at the end of tick infestation were made using models in the logarithmic form based on the composition at slaughter and water weights at slaughter and the end of tick infestation. In order to allow for the fact that blood component differences may be due to differences in body weight caused by the treatment, components were compared in the arithmetic form at the same final body weight and at the same initial value. These were then divided by the mean body weight and are referred to in the text as "contents" and correspond to values obtained by simply dividing components by body weight, without the assumption of linearity of regression through the origin. In some calculations the unadjusted values were also used.

The design of the experiment was such that the contribution of reduced food intake ("anorectic effect") and that of factors other than reduction in food intake ("specific effect") towards the combined effect could be assessed as follows:

Combined effect = value for tick-infested *ad lib.* group − value for control group
= specific effect + anorectic effect.

Specific effect = value for tick-infested *ad lib.* group − value for tick-free pair-fed group.

Anorectic effect = value for tick-free pair-fed group − value for control group.

An effect with a negative sign indicates a reduction in value. Statistical significance was assessed by comparison of the adjusted means using *t*-tests.

III. Results

(*a*) *Weight Changes*

Liveweight changes in the group of 21 animals have been discussed in detail elsewhere (Seebeck, Springell, and O'Kelly 1971). The subgroup of 12 animals under consideration here showed a similar trend, with the initial mean of 226 kg rising to a final adjusted mean of 289 kg for the four tick-free *ad lib.* animals. The specific and the anorectic effects (involving deficits of 19 and 28 kg respectively) were not significant,

TABLE 1

CHANGES IN BLOOD AND SOME OF ITS CIRCULATING COMPONENTS FOLLOWING TICK INFESTATION

Parameter, with Initial Mean of All Groups	Adjusted Final Mean of Controls†	Changes Due to:		
		Anorectic Effect	Specific Effect‡	Combined Effect
Red cell volume (l.), 3·574	5·482	−0·517	−2·035*	−2·552**
Plasma volume (l.), 8·555	10·927	−0·807	0·534	−0·273
Blood volume (l.), 12·129	16·260	−1·276	−1·151	−2·426
Albumin (g), 177·9	295·1	−5·9	−82·4**	−88·2**
Globulin (g), 358·0	492·0	−47·1	126·2**	79·1
Total plasma protein (g), 589·0	794·0	−63·8	52·9	−10·9
Haemoglobin (kg), 1·425	2·304	−0·184	−0·893*	−1·077**
Total cholesterol (g), 7·354	11·707	−2·229	−3·787*	−6·015**
Free cholesterol (g), 1·460	2·345	−0·454	−0·438	−0·892*

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

† Values in tick-free animals on an *ad lib.* diet following adjustment for initial differences between the animals of the three groups.

‡ In determining significance of the specific effect, use was made of the fact that pair-feeding was done on an individual basis.

TABLE 2

CHANGES IN BLOOD AND SOME OF ITS CIRCULATING COMPONENTS FOLLOWING TICK INFESTATION, ADJUSTED FOR DIFFERENCES IN BODY WEIGHT

Parameter	Adjusted Final Mean of Controls‡	Changes Due to:		
		Anorectic Effect	Specific Effect	Combined Effect
Red cell content (ml/kg)	18·89	0·38	−6·71**	−6·33*
Plasma content (ml/kg)	37·91	0·73	5·00**	5·73**
Blood content (ml/kg)	56·81	0·86	−1·22	−0·36
Albumin content (g/kg)	1·056	0·039	−0·246*	−0·207*
Globulin content (g/kg)	1·727	−0·014	0·563**	0·549**
Total plasma protein content (g/kg)	2·772	0·022	0·387*	0·409*
Haemoglobin content (g/kg)	7·948	0·356	−3·124**	−2·768*
Total cholesterol content (mg/kg)	38·55	−2·82	−8·09	−10·91†
Free cholesterol content (mg/kg)	7·546	−0·386	−0·291	−0·678

† $P < 0{\cdot}10$. * $P < 0{\cdot}05$. ** $P < 0{\cdot}01$. *** $P < 0{\cdot}001$.

‡ Amounts in tick-free animals on an *ad lib.* diet, following adjustment for initial amounts of the parameter and for body weight at the end of tick infestation, and then divided by the mean body weight at the end of tick infestation (263·7 kg).

because of the large variance and small numbers of animals, but the combined effect of −47 kg was significant ($P < 0{\cdot}05$).

(b) Blood Changes

(i) *Blood Volume Changes*

The specific effect of tick reduced both the red cell volume and content and increased the plasma content, while the plasma volume was relatively unaffected (Tables 1 and 2). In the case of the red cell volume the anorectic effect was reinforcing, the combined effects producing a greater reduction than the specific effect alone. There was no significant correlation between tick numbers on the individual animals and the observed variables.

To check whether differences in excitation between infested and non-infested cattle could be a factor contributing towards the observed differences in red cell volume, a separate experiment was performed using two uninfested and two heavily tick infested steers. Each animal was given intravenously a single dose of adrenalin (30 μg/kg), which caused the plasma glucose concentration to double. The adrenalin-induced increases in the venous haematocrit of the tick-infested steers (0·1 and 3·2%) were no larger than those of the tick-free animals (2·6 and 6·3%).

(ii) *Blood Protein Changes*

Significant reductions in total circulating albumin and haemoglobin were due to the specific effects of tick (Table 1). There were also significant decreases in the corresponding contents (Table 2). On the other hand, the specific effect increased the amount of globulin, while both the globulin and total plasma protein contents rose significantly (Tables 1 and 2). No blood protein parameters were correlated with tick numbers.

(iii) *Plasma Lipid Changes*

The amount and content of circulating total and free cholesterol were significantly reduced by the combined anorectic and specific effects (Table 1). None of the effects due to reduced food intake were significant (Tables 1 and 2), while the specific effect was significant only for the circulating total cholesterol ($P < 0 \cdot 05$). None of the observed lipid components were related to tick numbers.

(iv) *Plasma Bilirubin Levels*

At the end of tick infestation the plasma bilirubin of the tick-infested *ad lib.* group (0·24 mg/100 ml) lay between that of the tick-free *ad lib.* group (0·28 mg/100 ml) and the tick-free pair-fed group (0·15 mg/100 ml). The anorectic effect (—0·13 mg/100 ml) was significant ($P < 0 \cdot 05$), but the specific effect (+0·09 mg/100 ml) was not.

(c) Body Water Changes

At the end of the tick-infestation period there was a significant specific effect on the amount of body water when adjusted for differences in body weight (Table 3). At slaughter, however, the slopes of the regressions of log body water on log body weight were significantly different for the three groups. Adjusted means, calculated ignoring this difference in slopes, were not significantly different. The body water was also dependent on the number of days to slaughter (from the end of the tick-infestation period) and when adjusted for both body weight and the number of days to slaughter, the differences in body water were also not significant.

TABLE 3

WATER COMPOSITION OF STEERS BEFORE AND AFTER RECOVERY FROM TICK INFESTATION AND NUTRITIONAL RESTRICTION

Group	Adjusted Means at End of Tick Infestation (kg)†	Adjusted Means at Slaughter (kg)‡
Tick-free *ad lib.* (AL)	191·2	261·1
Tick-free pair fed (PF)	188·4	264·8
Tick-infested *ad lib.* (TI)	183·0	262·8
Significant differences between groups	PF > TI*	—

* Effect significant, $P < 0\cdot05$. A comparison was made using variances from PF and TI groups only.

† Adjusted for final body weight (X) in the form $\log Y = \mu + b_1 \log X$.

‡ Adjusted for body weight at slaughter (X_1) in the form $\log Y = \mu + b_1 \log X_1$. Significant difference between slopes ($-0\cdot695 \pm 0\cdot531$, $1\cdot087 \pm 0\cdot269$, and $0\cdot774 \pm 0\cdot278$ for the AL, PF, and TI groups respectively) of the individual group regressions ignored in adjusting along the common regression line.

The previously listed rates of growth (Table 3 in Seebeck, Springell, and O'Kelly 1971), were now examined in more detail (Table 4) using the body water measurements.

TABLE 4

GROWTH RATE OF STEERS DURING RECOVERY AFTER TICK INFESTATION AND NUTRITIONAL RESTRICTION

Group	Days to Slaughter	Rate of Gain (kg/day)		
		Total	Water	Body Solids
Tick-free *ad lib.* (AL)	179±58	0·53±0·05***	0·50±0·14*	0·02±0·09
Tick-free pair fed (PF)	102±33	0·89±0·16**	1·09±0·43*	−0·20±0·33
Tick-infested *ad lib.* (TI)	162±25	0·63±0·07***	0·55±0·06***	0·08±0·04†
Significant differences between groups	‡	PF > AL*	—	—

† $P < 0\cdot10$. * $P < 0\cdot05$. ** $P < 0\cdot01$. *** $P < 0\cdot001$. ‡ Not applicable.

It can be seen that most of the differences in growth rate between groups can be accounted for by water accretion.

(d) *Carcass Composition*

Amounts of fat, muscle, and bone in the dressed carcass at slaughter, adjusted for differences in body weight, are given in Table 5. Although the tick-infested animals tended to be the fattest and the tick-free pair-fed contained the most amount of muscle, the differences between groups were not significant.

TABLE 5

CARCASS COMPOSITION OF STEERS BEFORE AND AFTER RECOVERY FROM TICK INFESTATION AND NUTRITIONAL RESTRICTION, ADJUSTED TO CONSTANT BODY WEIGHT AT THAT TIME

Group	Adjusted Means at Slaughter (kg)			Adjusted Means at End of Tick Infestation (kg)‡	
	Muscle	Fat	Bone	Muscle	Fat
Tick-free *ad lib.* (AL)	118·9	32·9	28·8	85·5	18·9
Tick-free pair fed (PF)	121·2	36·0	28·3	86·7	21·0
Tick-infested *ad lib.* (TI)	114·8	46·0	27·3	81·7	25·3
Significant differences between groups	—	—	—	PF > TI* AL > TI†	TI > AL*

† $P < 0 \cdot 10$. * $P < 0 \cdot 05$.

‡ Muscle and fat weights were calculated using the relationships given in Table 6 as prediction formulae, in which were inserted values for body weight and body water at the end of the tick infestation, and for the tick-infested animals the number of days deviation from the mean date of their slaughter.

Table 6 shows the constants and standard errors in the relationship of each of muscle and fat, and body weight, body water, and deviations from the mean date of slaughter (for tick-infested animals only). The treatment effects (T_i) estimated in these relationships indicated that there was more fat and less muscle in the tick-infested animals when adjusted for the covariates, than in the other two groups. These relationships, allowing for the differences in T_i values, were used to estimate the amounts of muscle and fat at the end of the tick infestation. Means for these components, adjusted to constant body weight, are included in Table 5, and show that the specific effect of tick reduced muscle (5·0 kg) and increased fat (4·3 kg), at constant body weight. There was no significant relationship between tick numbers and estimated body composition at the end of the tick-infestation period. Estimated bone weights are not included in the table because they depended essentially on body weight rather than body water or slaughter date, and thus the estimated composition was only a reflection of body weight.

Table 7 shows the fat and muscle weight differences attributable to the specific and anorectic effects at the end of the infestation period. There was a significant reduction in muscle weight due to the combined effect. Table 7 also shows the specific

Table 6

CONSTANTS, COEFFICIENTS, AND STANDARD ERRORS IN THE ANALYSIS OF THE RELATIONSHIP BETWEEN MUSCLE AND FAT WEIGHT AT SLAUGHTER AND BODY WEIGHT, BODY WATER, AND DAY OF SLAUGHTER‡

Relationship expressed in the form

$$Y_{ij} = \mu + T_i + b_1 W_{ij} + b_2 H_{ij} + b_3 D_{ij} + e_{ij},$$

where Y is log muscle and fat weight (kg) at slaughter, W is log body weight (kg), H is log body water (kg), D is day of slaughter, T_i are treatment effects, μ is an effect common to all animals, and e is the error term

Effect	Muscle			Fat		
	Constants and Coefficients	Standard Errors§		Constants and Coefficients	Standard Errors§	
μ	−0·3090			−4·9342		
T_i		AL	PF		AL	PF
Ad lib. (AL)	0·0052			−0·0719		
Pair-fed (PF)	0·0075	0·0109		−0·0103	0·0547	
Tick-infested (TI)	−0·0127	0·0108	0·0098†	0·0822	0·0540*	0·0489
b_1	−0·0018	± 0·1895		6·7947**	± 0·9478	
b_2	0·9862**	± 0·1743		−4·4548**	± 0·8717	
b_3	−0·000029	± 0·000164		0·000420	± 0·000821	
R^2	0·963			0·880		

† Contrast significant ($P < 0{\cdot}10$) between TI and PF (specific effect).

* Contrast significant ($P < 0{\cdot}05$) between TI and AL (combined effect).

** Coefficient significantly greater than zero ($P < 0{\cdot}01$).

‡ Day of slaughter was included in the model by using the deviations in days from the mean date of slaughter of the tick infested animals and using the value of zero for animals of the other two groups. In this way the coefficient could be calculated for the tick infested animals without causing bias in the other constants estimated.

§ Standard errors given are for contrasts between the appropriate T_i constants, and the standard error of regression coefficient for b_1, b_2, and b_3.

Table 7

BODY COMPOSITION OF STEERS AT THE END OF THE TICK-INFESTATION PERIOD

Parameter	Mean of Tick-free *ad lib.* Group at End of Infestation	Changes Due to:		
		Anorectic Effect	Specific Effect	Combined Effect
Fasting body weight (kg)‡	284	−24	−19	−43†
Total body water (l.)	203	−14	−18	−32†
Body dry matter (kg)	81·3	−10·0	−1·5	−11·5
Fat weight (kg)§	24·0	−3·3	−0·5	−3·8
Muscle weight (kg)§	92·6	−6·3	−10·9	−17·2*

† $P < 0{\cdot}10$. * $P < 0{\cdot}05$.

‡ Animals were weighed before injection of tritiated water and at sampling time (16 hr later). The weights recorded represent the mean of these two weighings.

§ Muscle and fat weights were calculated using the relationships given in Table 6 as prediction formulae, in which were inserted values for body weight and body water at the end of the tick infestation and for the tick-infested animals the number of days deviation from the mean date of their slaughter.

and anorectic effects on the weight of body solids. In terms of body solids, the anorectic effect appears to be 90% of the combined effect, but none of the effects are significant.

(e) *Metabolites Ingested by Ticks*

A mean burden of 417 ticks/day was estimated in the four tick-infested steers. This can be calculated (Seifert, Springell, and Tatchell 1968) to result in a daily blood loss of about 125 ml, or approximately 1% of the host blood volume. From present results and from earlier data (O'Kelly, Seebeck, and Springell 1971) it can be deduced that during the 51-day experimental period, when engorged adult females were present, an average total of 960 g protein were imbibed per beast. The likely uptake of component proteins is shown in Table 8. It is interesting that the amounts of albumin, haemoglobin, and cholesterol lost by the steers roughly correspond to the amounts withdrawn by ticks. In the case of globulin, however, there was an increased amount found in the host, despite the parasite-induced losses.

Table 8

COMPARISON OF METABOLITE LOSSES DUE TO SPECIFIC TICK EFFECTS AND AMOUNTS IMBIBED BY TICKS

Metabolite	Change in Host Due to Specific Effect† (g)	Amount Imbibed by Parasites‡ (g)	Amount Needed to Maintain Host Level§ (g)
Albumin	-83 ± 27*	98 ± 4***	$+15 \pm 24$
Globulin	$+141 \pm 10$***	217 ± 16***	$+358 \pm 8$***
Haemoglobin	-809 ± 294*	642 ± 96***	-168 ± 336
Total cholesterol	$-3 \cdot 78 \pm 1 \cdot 33$*	$3 \cdot 64 \pm 0 \cdot 45$***	$-0 \cdot 14 \pm 0 \cdot 95$

* $P < 0 \cdot 05$. *** $P < 0 \cdot 001$.

† Mean values from four pairs of animals before adjustment for initial differences between animals of the three groups. These values are close to, but not identical with, those in Table 1. The + indicates an increase, the − a decrease in the metabolite.

‡ Values are based on the following assumptions:

(1) A daily blood uptake of 0·3 ml/tick (Seifert, Springell, and Tatchell 1968) by 417 ticks over a 51-day period when adult females were present.

(2) Means of initial and day 70 values for the haematocrit, albumin, globulin, haemoglobin and total cholesterol (O'Kelly, Seebeck, and Springell 1971).

§ The + indicates retention, the − a loss of metabolite over and above that removed by the ticks.

IV. Discussion

The results of tick infestation in cattle can be partitioned into anorectic and specific effects. Early workers did not attempt this, but only reported the combined effects. It was shown in Part I of this series (Seebeck, Springell, and O'Kelly 1971), that the anorectic component was a significant factor in the retarded growth of infested animals, but no significant effects associated with anorexia were found in the parameters studied in this paper, except for a reduction in bilirubin concentration. Further discussion of our results is confined to consideration of the specific effect.

The mechanism of the anaemia in tick-infested cattle has not been elucidated and has been defined only in terms of a reduced haematocrit. Reduction in haematocrit could be caused by a decrease in red cell volume or by an increase in plasma volume or both. The results (Table 1) show that only the red cell volume was significantly changed, and that this effect was independent of body weight (Table 2).

Red cell volumes could be lowered by infestation-induced storage of erythrocytes in organs such as the spleen. However, adrenaline injection failed to reveal the presence of such stores of red cells. It is therefore concluded that the reduced haematocrit in infested cattle can be taken as a valid indicator of a reduced number of red cells.

The problem remains as to the cause of the anaemia. Examination of blood for changes in the number of reticulocytes (O'Kelly, Seebeck, and Springell 1971) gave no indication of a change in the rate of synthesis of erythrocytes as a result of tick infestation. On the other hand, measurement of bilirubin in this study also provided no clear evidence for an increase in red cell destruction on infestation. The possibility remains that the blood taken up by the tick is a contributing factor. If a balance sheet is drawn up similar to that outlined in Table 8, it can be calculated that the mean loss of red cells by the host (1·85 litres, not adjusted for initial differences) very nearly balances the mean amount consumed by ticks (1·63 litres). By contrast the plasma volume actually increased slightly (by 0·87 litre unadjusted), but insignificantly, despite a withdrawal of 4·75 litres of plasma by the ticks. Since ticks do not preferentially remove red cells (Seifert, Springell, and Tatchell 1968), the tick-induced imbalance between plasma and cells in the host must have been due to an inability on the part of the host to replace the imbibed cells. A similar conclusion is reached with regard to haemoglobin, as will be discussed in more detail later. Indeed, the parallel behaviour of haemoglobin and the red cell volume (Tables 1 and 2) is consistent with an earlier finding (O'Kelly and Seifert 1969, 1970) that the tick-induced anaemia is not accompanied by changes in red cell haemoglobin content.

Tick infestation had only a slight effect on the blood content of steers (Table 2), because the decrease in red cell content was almost balanced by an increase in plasma content. The plasma volume rose only very slightly (Table 1) as a direct result of infestation, so that the observed increase in plasma content is a reflection of changes in the body weight.

In Part II of this series (O'Kelly, Seebeck, and Springell 1971), correlations were found to exist between concentrations of metabolites and tick numbers. While the specific effect on circulating blood proteins were pronounced (Table 1), no such relationship could be demonstrated here, possibly because of the smaller number of animals involved. However, another feature of the previous work was the uncertainty of whether the tick-induced concentration changes were attributable to alterations in the degree of hydration of the vascular fluid, or to alterations in the absolute amounts of circulating protein, or both. Thus, the previously observed reduction in plasma albumin concentration and the increased globulin concentration by ticks could have come about in several ways. The present work (Table 1) enables one to state categorically that the total amount of plasma albumin had declined and that of globulin raised, because there was no haemodilution or haemoconcentration. Similarly, the previously reported tick-induced drop in the haemoglobin concentration must have been associated with a decrease in the total amount of circulating haemoglobin, rather than with haemodilution. It is further evident from Tables 1 and 2 that changes in amounts of blood proteins were independent of body weight, because of the correspondence with changes in contents. The total circulating protein was maintained at virtually the same level, possibly due to a compensatory mechanism dictated by osmotic requirements and involving albumin and globulin. Additional factors could be an immunologically induced increase in globulin, and transfer of albumin from the intravascular to the extravascular pool.

The body water data made it possible to partition the components of the specific effect on body weight (Seebeck, Springell, and O'Kelly 1971). If it is assumed that the animals had equal water contents at the start of the infestation, then the specific effect in terms of body solids amounted to only 1·5 kg (Table 7). Differences in growth subsequent to tick infestation were also largely explained by differences in body water (Table 4). The higher growth rate of the previously tick-free pair-fed group is explicable in terms of a high rate of water accretion. The only evidence of body solid accumulation occurred during recovery of the previously infested group.

The body composition (relative to the same body weight) showed no statistical difference between groups at slaughter (Table 5), although as one might expect on recovery from severe restriction, the previously tick-infested animals tended to be fatter. After adjustment for differences in body weight, weight of body water, and date at slaughter, there was more fat and less muscle in the tick-infested animals at slaughter than in the other two groups (Table 6). These differences imply that, even subsequent to tick infestation, the normal relationships between body water and composition parameters were disturbed. However, there were no treatment differences in the coefficients for body water and body weight in this relationship, because the observed differences in slope for the relationship between body water and body weight (Table 3) was compatible with differences (non-significant) in the relationships of dissected muscle and fat to body weight at slaughter.

The relationships given in Table 6 were used for extrapolation of body composition back to the end of the tick-infestation period. Although the regressions were based on between-animal within-group differences, they are assumed to represent within-animal changes. This is assisted by the random allocation of animals to a range of slaughter weights, but extrapolation back to the end of the infestation period is speculative, since there may have been a different rate of change in physiological status in the tick-infested animals than allowed for by adjustment of those animals by the covariate for deviations from their mean date of slaughter.

Loss of blood also contributes to the specific effect of ticks on body weight. Calculations based on an average uptake of 0·3 ml blood per tick per day (Seifert, Springell, and Tatchell 1968) lead to the conclusion that 960 g protein are withdrawn from the host. This would be equivalent to a loss of only about 4 kg protein of similar hydration to muscle, whereas the loss of muscle as extrapolated from body water and carcass competition (Table 7) was estimated to be 11 kg. This would suggest that the loss of blood *per se* is not the major factor in the protein deficit of infested animals.

It is also unlikely that the removal of blood by the parasites had an appreciable effect on the host's water balance. Since ticks return up to 70% of the imbibed water back into the host (Tatchell 1967), the overall water deficit on tick infestation would have amounted to about 1·5 litres over the 51-day period during which engorged females were present.

The amount of albumin, haemoglobin, and cholesterol imbibed by the ticks was not significantly different from that apparently lost by the steers (Table 8). A number of interpretations are possible, the simplest of which would be that there had been no alteration in the rate of breakdown or synthesis of these metabolites by the host as a result of infestation. Alternatively, if there had been a change in breakdown and synthesis, then both alterations must have balanced one another out, so as to maintain the *status quo*. In the case of haemoglobin at least, there is no evidence of an overall change in the amount of catabolism, as gauged by the constancy of the bilirubin concentration. Unfortunately, there is a lack of data on phlebotomy of the magnitude found here. It would have been expected that the small losses of albumin, haemoglobin, and cholesterol encountered here would have been replaced in a non-infested animal. This raises the possibility that the failure to replenish these blood constituents could be due to the intervention of a toxin secreted by the ticks. Fluctuations in the level of toxin, or the varying host susceptibility to it, could explain the high standard error in the apparent losses sustained by the host (Table 8), as compared to the smaller variation in the uptake of components by the parasites.

The reason for the relative increase in fat and decrease in muscle postulated for the end of the infestation period (Table 5) could not be resolved, because of a lack of information concerning fat metabolism. At present all evidence points to a disturbance of protein metabolism, at least part of which may be due to the uptake of blood protein by the tick, and part of which could be toxin-induced.

V. Acknowledgments

It is a pleasure to thank Miss J. J. Creed, Mr. A. Dougan, and Mrs. S. A. Frisch for skilful technical assistance, the Australian Meat Board for provision of facilities, and the Australian Meat Research Committee for part support.

VI. References

O'Kelly, J. C., Seebeck, R. M., and Springell, P. H. (1971).—Alterations in host metabolism by the specific and anorectic effects of the cattle tick (*Boophilus microplus*). II. Changes in blood composition. *Aust. J. biol. Sci.* **24**, 381–9.

O'Kelly, J. C., and Seifert, G. W. (1969).—Relationships between resistance to *Boophilus microplus*, nutritional status, and blood composition in Shorthorn × Hereford cattle. *Aust. J. biol. Sci.* **22**, 1497–506.

O'Kelly, J. C., and Seifert, G. W. (1970).—The effects of tick (*Boophilus microplus*) infestations on the blood composition of Shorthorn × Hereford cattle on high and low planes of nutrition. *Aust. J. biol. Sci.* **23**, 681–90.

Riek, R. F. (1957).—Studies on the reactions of animals to infestation with ticks. I. Tick anaemia. *Aust. J. agric. Res.* **8**, 209–14.

Seebeck, R. M. (1968).—Developmental studies of body composition. *Anim. Breed. Abstr.* **36**, 167–81.

SEEBECK, R. M., SPRINGELL, P. H., and O'KELLY, J. C. (1971).—Alterations in host metabolism by the specific and anorectic effects of the cattle tick (*Boophilus microplus*). I. Food intake and body weight growth. *Aust. J. biol. Sci.* **24**, 373–80.

SEEBECK, R. M., and TULLOH, N. (1968).—Developmental growth and body weight loss of cattle. III. Dissected components of the commercially dressed carcass, following anatomical boundaries. *Aust. J. agric. Res.* **19**, 673–88.

SEIFERT, G. W., SPRINGELL, P. H., and TATCHELL, R. J. (1968).—Radioactive studies on the feeding of larvae, nymphs, and adults of the cattle tick, *Boophilus microplus* (Canestrini). *Parasitology* **58**, 415–30.

SNEDECOR, G. W. (1956).—"Statistical Methods" 5th Edn. p. 394. (Iowa State College Press: Ames, Iowa.)

SPRINGELL, P. H. (1968*a*).—Red cell volume and blood volume in beef cattle. *Aust. J. agric. Res.* **19**, 145–60.

SPRINGELL, P. H. (1968*b*).—Water content and water turnover in beef cattle. *Aust. J. agric. Res.* **19**, 129–44.

TATCHELL, R. J. (1967).—Salivary secretion in the cattle tick as a means of water elimination. *Nature, Lond.* **213**, 940–1.

VERCOE, J. E., and SPRINGELL, P. H. (1969).—Effect of subclinical helminthosis on nitrogen metabolism in beef cattle. *J. agric. Sci., Camb.* **73**, 203–9.