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

II.* CHANGES IN BLOOD COMPOSITION

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Abstract

Changes in the blood composition of Hereford steers kept on a high-quality diet and infested with *B. microplus* were studied. The experiment was designed so that the effects on blood composition due to reduced feed intake ("anorectic effect") and those due to the remaining factors of tick infestation ("specific effect") could be independently estimated.

The specific effect of tick depressed serum albumin and elevated globulin concentrations. Total cholesterol concentration was lowered by the specific effect but free cholesterol concentration was approximately equally reduced by anorectic and specific effects. The free : total cholesterol ratio was elevated by the specific effect of tick. The specific effect depressed haematocrit, haemoglobin concentration, serum alkaline phosphatase, and lactate dehydrogenase. Both the anorectic and specific effects reduced serum amylase activity. Glucose and non-esterified fatty acid concentrations were unaffected by treatment. Lymphocytes and eosinophils were increased and neutrophils decreased by the specific effect of tick.

Total tick numbers carried during treatment were negatively correlated with serum albumin and free cholesterol concentrations, lactate dehydrogenase, glutamate–oxaloacetate transaminase, and monocytes and positively correlated with the serum globulin concentration.

The results support the view that the tick secretes a toxin which interferes with its host's metabolism.

I. Introduction

Depression in growth rate and haematological changes have been reported in cattle following infestation with the cattle tick (*Boophilus microplus*) (e.g. see Francis 1960; Little 1963). The mechanisms producing these metabolic changes in the host had not been elucidated. As part of a series of studies on the interactions between ticks and nutrition, changes in blood composition were investigated in tick-infested Shorthorn × Hereford steers on both adequate and inadequate diets (O'Kelly and Seifert 1969, 1970). The results of these studies were compatible with the view that the tick exerts a direct influence on its host's metabolism by secretion of a toxin, although part of the observed changes in blood composition may have been associated with depressed feed intake.

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In Part I of this series (Seebeck, Springell, and O'Kelly 1971) it was demonstrated that heavy tick infestations produce anorexia in Hereford steers on a high-quality diet, and that the reduction in feed intake accounts for about 65% of the depressed growth rate. It is important, then, when studying metabolic alterations accompanying tick infestation in cattle, to dissociate quantitatively the specific effect of tick from the effect of non-specific factors such as reduced feed intake.

This paper reports an investigation of factors affecting the blood composition of Hereford steers infested regularly with large doses of tick larvae. The experiment was designed so that the effects on blood composition due to reduced feed intake ("anorectic effect") and those due to the remaining factors of tick infestation ("specific effect") could be independently estimated.

II. Materials and Methods

(a) *Experimental Animals and their Treatment*

The experimental design is fully described in Part I (Seebeck, Springell, and O'Kelly 1971). Briefly, the tick resistance of 21 13-month-old Hereford steers at pasture was assessed after two artificial tick infestations (0·5 g and 1·0 g larvae). The means of the two tick counts for each animal are referred to as the selection counts. The steers were allocated to three groups of seven animals. The diet for all animals was high-quality feed in the form of pellets. One group was infested twice weekly with 2 g tick larvae and was without restrictions on feed intake; a second group was kept tick-free and pair-fed to the tick-infested group. A third group (referred to as the control group) 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 group 1 animals were counted and the procedure is described in Part I. The total of counts following all infestations was used as a measure of the tick load on each animal. Blood samples were taken by jugular venipuncture on days 0, 33, and 70 of the 10-week treatment period.

(b) *Analytical Procedures*

Albumin and globulin were assayed by the method of Fernandez, Sobel, and Goldenberg (1966). Total protein was determined by the biuret method (King and Wootton 1956). For non-esterified fatty acids the method of Dole (1956) was modified to produce a one-phase titration system (Tarrant, Thompson, and Wright 1962). The iron reagent of MacIntyre and Ralston (1954) was used for total cholesterol, while free cholesterol was precipitated as the digitonide and measured as above for total cholesterol. Glucose was estimated using an *o*-toluidine reagent and iron by the Ness and Dickerson (1965) procedure. Haematocrit was determined with an International Equipment Co. microcapillary centrifuge (15,000 *g*, 4 min). Haemoglobin was determined as the oxyhaemoglobin. Reticulocytes were stained with brilliant cresyl blue for counting. For the total leucocyte counts, blood was diluted with acetic acid coloured with methyl violet and the cells counted in a double-squared Neubauer chamber. Differential leucocyte counts were performed on fresh blood using Leishman's stain. Eosinophils were counted in a Fuchs–Rosenthal chamber using a modification of Dunger's original solution (Speirs 1952).

Alkaline phosphatase was assayed using disodium phenyl phosphate as substrate and determining the liberated phenol with Folin and Ciocalteu's phenol reagent according to the method of King and Wootton (1956). Other enzymes were assayed as follows: amylase according to Henry and Chiamori (1960), glutamate–oxaloacetate transaminase by the Reitman and Frankel (1957) procedure, and lactate dehydrogenase using the 2,4-dinitrophenylhydrazine colour reagent (King 1965).

(c) *Analysis of the Data*

Adjusted means for the various parameters (other than leucocytes and iron) on days 33 and 70 were arrived at by analysis of covariance (Snedecor 1956), in which account was taken of the initial differences between animals of the three groups. 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 overall 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*) *Blood Composition*

The results of the blood analyses in the treatment period are given in Tables 1–4.

Table 1

Haematological values on days 33 and 70 of the treatment period

Determination, with Initial Mean of All Groups	Day	Control Group Means†	Changes Due to:		
			Anorectic Effect	Specific Effect	Combined Effect
Haematocrit (%), 29·8	33	36·8	+1·1	−10·0***	−8·9***
	70	33·8	+0·5	−10·2***	−9·7***
Haemoglobin (g/100 ml blood), 12·0	33	13·9	+0·2	−3·8***	−3·6***
	70	14·3	+0·3	−4·8***	−4·5***
Leucocyte components: (thousands/mm³)					
Total leucocytes	33	11·8	−1·0	−4·3***	−5·3***
	70	12·5	−2·4	−2·4	−4·8
Lymphocytes	33	7·21	−0·16	+0·68*	+0·52*
	70	6·72	+0·40*	+0·64**	+1·04***
Monocytes	33	0·26	+0·06	−0·13	−0·07
	70	0·41	−0·04	−0·09	−0·13
Neutrophils	33	1·68	+0·01	−0·73	−0·72
	70	2·10	−0·18	−1·31***	−1·49***
Eosinophils	33	0·40	−0·05	+0·23	+0·18
	70	0·44	−0·01	+0·45***	+0·44***

* $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$.

† Haematocrit and haemoglobin adjusted to constant initial values. Total leucocytes unadjusted. Leucocyte components adjusted to constant total leucocytes.

Plasma glucose and non-esterified fatty acid concentrations were unaffected by treatment. The lack of change in total protein concentrations was the result of the albumin and globulin fractions being altered in different directions. Alterations in the other blood components studied were due to the specific effect of tick (Tables 1–4) with only the following exceptions. The free cholesterol concentration on day 33

was significantly lowered by the combined anorectic and specific effects whereas on day 70 the specific effect alone attained significance. The anorectic effect became a

TABLE 2

PLASMA LIPID CONCENTRATIONS ON DAYS 33 AND 70 OF THE TREATMENT PERIOD

Determination, with Initial Mean of All Groups	Day	Control Group Adjusted Means	Changes Due to:		
			Anorectic Effect	Specific Effect	Combined Effect
Total cholesterol (mg/100 ml), 83·6	33	99·8	−7·5	−35·9***	−43·4***
	70	105·8	−13·1	−38·6***	−51·7***
Free cholesterol (mg/100 ml), 16·6	33	19·9	−1·7	−2·3	−4·0*
	70	21·4	−2·9	−4·5*	−7·4***
Ratio of free to total cholesterol (%), 19·9	33	20·0	+0·1	+5·2***	+5·3***
	70	20·1	−0·1	+5·8***	+5·7***
Non-esterified fatty acids (μ-equiv/l), 278	33	393	+16	−69	−53
	70	426	+152	−237	−85

* $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$.

significant factor in the depression of amylase activity on day 70. While serum transaminase was lowered by the specific effect on day 70 the depressed values failed to reach significance.

TABLE 3

SERUM GLUCOSE AND PROTEINS ON DAYS 33 AND 70 OF THE TREATMENT PERIOD

Determination, with Initial Mean of All Groups	Day	Control Group Adjusted Means	Changes Due to:		
			Anorectic Effect	Specific Effect	Combined Effect
Glucose (mg/100 ml), 69·6	33	77·8	−3·5	+0·4	−3·1
	70	73·8	−7·5	+0·5	−7·0
Albumin (g/100 ml), 2·05	33	2·43	+0·14	−0·65***	−0·51***
	70	2·73	0·00	−0·78***	−0·78***
Globulin (g/100 ml), 4·23	33	4·45	−0·04	+0·59**	+0·55**
	70	4·64	−0·27	+0·88***	+0·61***
Total protein (g/100 ml), 6·88	33	7·13	−0·02	−0·04	−0·06
	70	7·42	−0·31	+0·23	−0·08
Albumin–globulin ratio (×100), 48·8	33	55·3	+2·5	−18·1***	−15·6***
	70	58·6	+3·5	−24·1***	−20·6***

* $P < 0·05$. ** $P < 0·01$. *** $P < 0·001$.

Except for two parameters (free cholesterol concentration and lactate dehydrogenase), the changes in blood composition caused by the specific effect of tick

were apparent by day 33 when an average of 4240 mature female ticks had been counted on each animal. These changes were only slightly altered in magnitude by a further average burden of 15,360 ticks in the next 37 days. However, the specific effects on free cholesterol and lactate dehydrogenase attained significance only at the final sampling.

Plasma iron concentration was sampled only on the last day of treatment. Tick infestation reduced it significantly ($P < 0{\cdot}05$) from the control level of 93 μg/100 ml to 61 μg/100 ml. The specific effect (−24 μg/100 ml) was greater than the anorectic effect (−8 μg/100 ml), but neither was significant.

TABLE 4

SERUM ENZYME ACTIVITIES ON DAYS 33 AND 70 OF THE TREATMENT PERIOD

Determination, with Initial Mean of All Groups	Day	Control Group Adjusted Means	Changes Due to:		
			Anorectic Effect	Specific Effect	Combined Effect
Amylase	33	188·6	−14·4	−60·2*	−74·6*
(units/100 ml), 138·8	70	197·0	−49·6*	−55·4*	−105·0***
Alkaline phosphatase (King–Armstrong units/100 ml), 8·84	33	10·90	+0·18	−3·14*	−2·96*
	70	12·85	+0·58	−6·90**	−6·32*
Lactate dehydrogenase	33	968·9	−12·8	+53·0	+40·2
(m-i.u./ml), 881·9	70	1125·3	−61·1	−172·8*	−233·9*
Serum glutamate–oxaloacetate transaminase	33	29·0	+0·2	+1·0	+1·2
(i.u./l), 29·8	70	30·0	+0·6	−2·6	−2·0

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

Total initial leucocyte counts were not recorded and the results for days 33 and 70 were subjected to analysis of variance. On day 33 the total leucocyte count was significantly lower ($P < 0{\cdot}001$) in the tick-infested group than in the other two groups, but on day 70 these differences were not significant. The leucocyte components on each day were subjected to analysis of covariance in the logarithmic form at a constant total leucocyte count on that day. The adjustment to constant total leucocyte count using the logarithmic transformation was carried out to remove that part of the variation in percentages of the individual leucocyte types resulting from the curvilinear realtionships between the counts for total leucocytes and the leucocyte types. Lymphocytes were significantly elevated ($P < 0{\cdot}05$) by the specific effect on day 33. On day 70 not only were the lymphocytes more significantly elevated ($P < 0{\cdot}01$) by the specific effect, but also the eosinophils were raised ($P < 0{\cdot}001$) and neutrophils were lowered ($P < 0{\cdot}001$). Reticulocyte counts never rose above 1% of the total red cell count and there were no significant differences between groups.

(b) Correlations between Values of Blood Characters on Days 0, 33, and 70

There were correlations between the initial values and the values on day 33 and day 70 for amylase ($P < 0{\cdot}001$), alkaline phosphatase ($P < 0{\cdot}05$), total cholesterol

concentration ($P < 0{\cdot}001$), and free cholesterol concentration ($P < 0{\cdot}01$). Haematocrit values and haemoglobin concentration on day 33 were correlated ($P < 0{\cdot}01$) with their respective values on the initial day but those on day 70 were not.

(c) *Relationships between Tick Selection Counts and Blood Composition*

Tick scores of the 21 animals in the pretreatment period, i.e. the selection counts, were negatively correlated with initial concentrations of haemoglobin ($r = -0{\cdot}496$, $P < 0{\cdot}05$), total cholesterol ($r = -0{\cdot}550$, $P < 0{\cdot}01$), and free cholesterol ($r = -0{\cdot}561$, $P < 0{\cdot}01$). The correlations between the selection count of the 14 animals in the pair-fed and control groups and total cholesterol concentration on days 0, 33, and 70 were negative, but not significant.

(d) *Relationships between Tick Load and Blood Composition*

On day 33 lactate dehydrogenase was negatively correlated ($P < 0{\cdot}05$) and serum globulin concentration was positively correlated ($P < 0{\cdot}05$) with the total number of ticks carried by each animal during treatment. On day 70 the total number of ticks carried during treatment was negatively correlated with lactate dehydrogenase ($P < 0{\cdot}05$), transaminase ($P < 0{\cdot}05$), albumin concentration ($P < 0{\cdot}01$), albumin : globulin ratio ($P < 0{\cdot}01$), and free cholesterol concentration ($P < 0{\cdot}05$) and positively correlated with globulin concentration ($P < 0{\cdot}05$). Total tick numbers carried during treatment were negatively correlated ($P < 0{\cdot}01$) with monocytes at the same total leucocyte count on day 70.

IV. Discussion

The specific effect of tick produces hypoalbuminaemia and an increase in serum globulin concentrations. While tick removes protein from the blood of its host (Springell *et al.*, unpublished data), serum albumin concentration changes could also be produced by altered catabolism or synthesis or both as a result of either reduced protein intake or liver damage. The tick-free pair-fed animals showed that protein intake was not limiting, unless of course, tick infestation caused pathological changes which result in inefficient absorption of nutrients. The negative correlations between tick numbers and serum albumin concentrations could be explained by toxin secretion of the tick as suggested previously (O'Kelly and Seifert 1970). Since serum albumin is formed exclusively in the liver (McFarlane 1957) a large number of ticks on a susceptible animal would produce more toxin, and hence interfere with albumin metabolism to a greater extent, than a small number of ticks on a more resistant animal. While there are indications of the immunological nature of tick resistance (Roberts 1968) there is no evidence of specific circulating antibodies against ticks or their metabolic products to explain the positive correlation between tick numbers and serum globulin concentrations. Furthermore, the changes observed in serum albumin and globulin concentrations are relatively non-specific and are associated with stress including many parasitic conditions and infections (e.g. see Leland 1961; Herlich and Merkal 1963).

Many papers describe depressed haematocrits in tick-infested cattle (e.g. see Riek 1957; Francis 1960). More recent studies demonstrated that haematocrit and haemoglobin levels could increase significantly in Shorthorn × Hereford steers fed an adequate diet *ad libitum* and subjected to light tick infestations (O'Kelly and Seifert

1969); heavy tick infestations, however, depressed these parameters (O'Kelly and Seifert 1970). Poor nutrition reduces haematocrit and haemoglobin levels in cattle (Meacham *et al.* 1964; Springell 1968) but the role of depressed feed intake in contributing to the anaemia of tick-infested cattle has not been evaluated in previous work. The experiment reported here has clearly shown that on a high-quality diet the specific effect of tick caused the depression in haematocrit and haemoglobin concentration. A reduction in plasma iron concentration was associated with the lowered haematocrit and haemoglobin values. Reticulocytosis is usually observed in cattle only after severe blood loss (e.g. see Schnappauf *et al.* 1967). The absence of reticulocytosis in the tick-infested animals is then not very informative in explaining the mechanism of the anaemia.

Leucocyte values reported in the literature for various breeds of cattle in many parts of the world show wide variations. The values reported here generally agree with those found by Granzien (1968) in Queensland cattle. Infection in cattle is frequently accompanied by a neutropenia without alteration in total leucocyte count (Schalm 1961) and eosinophilia is common in animals with parasitic infestations. The neutropenia and eosinophilia produced by the specific effect of tick may then be considered as agreeing with a common response of host to attack by parasite. The full significance of the lymphocytosis due to the specific effect and the negative correlation between tick load and monocytes must await the results of immunological studies.

Amylase is synthesized by the liver as well as by the pancreas and other tissues (McGeachin, Potter, and Despopoulos 1960; Arnold and Rutter 1963) and the liver is an important source of the amylase activity of plasma in some animal species (McGeachin, Potter, and Lindsey 1964). Little is known about the origin of amylase activity in ruminants but it is dependent on the nutritional status of the animal (O'Kelly and Seifert 1970). It is not possible, then, to say by what mechanism tick depresses the serum amylase activity, but reduced production of the enzyme by the liver is a likely explanation since other protein metabolism is also influenced by tick. Similarly, serum alkaline phosphatase is derived from various organs such as liver and intestine and it is not possible to speculate which specific organs are acted upon by the tick in depressing serum enzyme activity.

The progressive rise in lactate dehydrogenase found in the control animals may be related to such factors as nutrition or age; season also influences the change of enzyme activity in the blood serum of cattle (Roussel and Stallcup 1967). Serum lactate dehydrogenase activity was depressed by the specific effect and contrasts with the elevated activity used as a criterion of a wide range of diseases (e.g. muscular dystrophy and haemolytic anaemias) in human clinical biochemistry. There were significant negative correlations between tick load and serum enzyme activity for both lactate dehydrogenase and glutamate–oxaloacetate transaminase. Both enzymes are widely distributed in animal tissues and transaminase forms a link between carbohydrate and protein metabolism. Thus, depressed enzyme activities may reflect reduced body metabolism under high tick loads.

The synthesis of cholesterol is dependent upon hormone action and the presence of adequate precursors such as acetate. Total plasma cholesterol concentration was depressed in the tick-infested animals and the results from the pair-fed group indicate that exogenous precursors were not limiting unless rumen metabolism was altered.

The initial specific effect of tick was a reduction in the concentration of esterified cholesterol with the free cholesterol fraction unaltered so that the ratio of free : total cholesterol was significantly increased. These results suggest that the enzymes esterifying cholesterol are depleted or inhibited in some manner. However, the transport of the major lipid constituents in plasma occurs in the form of large lipoprotein particles. The mechanisms involved in the synthesis of the plasma lipoproteins are not well understood but the liver is considered to be a major site of formation. Thus, an interference with either the synthesis or secretion of lipoprotein may be part of the mechanism by which tick affects the concentration of plasma cholesterol. At the end of the treatment period plasma concentrations of free cholesterol as well as the esterified fraction were reduced by the specific effect, a result analogous to that obtained in tick-infested cattle on a low plane of nutrition (O'Kelly and Seifert 1970).

The large doses of tick larvae used in this experiment may have overemphasized some effects. Total cholesterol, for example, was negatively correlated with tick numbers when the animals were lightly infested in the pretreatment period (agreeing with previous findings of O'Kelly and Seifert 1969) but not when they were heavily infested in the treatment period. However, this investigation has demonstrated that the specific effect of tick directly influences many of its host's metabolic processes. Several of the biochemical results, such as reduced albumin level and increased free : total cholesterol ratio, suggest that the functional capacity of the liver is reduced. The results from the pair-fed animals eliminate the reduced intake of nutritional components as a cause of liver dysfunction. It is considered that the metabolic derangement observed in tick-infested cattle on an adequate diet is due to a toxin secreted by the parasite.

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