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# CHANGES IN WHOLE BLOOD AND SERUM COMPONENTS DURING *FRANCISELLA TULARENSIS* AND RABBIT POX INFECTIONS OF RABBITS

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**Summary.—Rabbits infected with virulent *Francisella tularensis* strain Schu S4 or rabbit pox virus (Utrecht strain) showed significant early changes in serum levels of trace metals, neutral fat and alkaline phosphatase activity. With *F. tularensis* infections a marked early leukopenia and a decrease in serum amino acids were also observed; the effect on amino acid levels was less pronounced in rabbit pox infections. In both diseases these changes preceded the appearance of acute phase globulins in the serum. Vaccination with the live vaccine strain of *F. tularensis* slightly increased survival times but did not delay the onset of metabolic changes in rabbits subsequently infected with the virulent Schu S4 strain.**

CATABOLIC processes leading to a negative balance in nitrogen and other body elements are a prominent feature of the febrile period of infectious disease (Beisel *et al.*, 1967). Before the onset of fever and other overt symptoms of illness, however, other changes that are often anabolic in nature occur. Studies in man and laboratory animals suggest that many early metabolic changes are interrelated and may be non-specific reactions initiated by circulating substances formed or excreted in response to inflammatory processes (Wannemacher *et al.*, 1972*a*).

Early decreases in serum total free amino acids were observed in human volunteers infected with *Salmonella typhi* (Feigin *et al.*, 1968), *Francisella tularensis* (Feigin and Dangerfield, 1967), attenuated Venezuelan equine encephalomyelitis virus (Feigin *et al.*, 1967) or sandfly fever virus (Wannemacher *et al.*, 1972*b*). Wannemacher *et al.* (1971) showed that in rats infected with *Diplococcus pneumoniae* there was a flux of amino acids from the serum to the liver. Similarly, Powanda *et al.* (1975) showed that there is movement of amino acids to the liver during tularaemia in rats. This amino acid flux is associated with a subsequent increase in the rate of synthesis and release of acute phase serum proteins from the liver (Wannemacher *et al.*, 1972*a*; Powanda, *et al.*, 1975; Powanda, Kenyon and Moe, 1976). In contrast to the decrease in total amino acids, increases in phenylalanine and the ratio of phenylalanine to tyrosine often occur during infection or inflammatory processes (Wannemacher *et al.*, 1976). Similar changes in the levels of serum amino acids and certain protein components occurred when rats were injected with "leucocytic endogenous mediator" (LEM) which is released from stimulated polymorphonuclear leucocytes (Eddington, Upchurch and Kampschmidt, 1971; Wannemacher, Pekarek and Beisel, 1972*c*).

Changes in the concentrations of certain trace metals in serum or plasma occur during various microbial infections and inflammatory conditions in man and animals (Wannemacher *et al.*, 1972*a*; Powanda *et al.*, 1973; Powanda *et al.*, 1975; Beisel, 1976; Powanda *et al.*, 1976). Generally, decreases in the concentrations of zinc and iron occur and these precede the onset of clinical symptoms, whereas

rises in serum copper concentrations occur during or following the peak of the illness. LEM also initiated infection-like changes in serum trace metals (Kampschmidt and Upchurch, 1970*a*, *b*; Pekarek, Wannemacher and Beisel, 1972; Wannemacher *et al.*, 1972*a*; Wannemacher *et al.*, 1975).

Changes in serum enzyme activities also occur during infections; for example, elevated lactate dehydrogenase and creatine phosphokinase activities in typhoid fever (Wannemacher *et al.*, 1972*a*), and increases in serum β-glucuronidase and lysozyme activities in tularaemic rats (Canonico *et al.*, 1975).

In this report, we describe a study of changes in blood components during experimental bacterial (*F. tularensis*) and viral (rabbit pox) infections in the rabbit, a natural host for both infections. Serum levels of trace metals, amino acids, proteins and neutral fats, and various enzyme activities were measured throughout the infection; in addition, microbiological and haematological examinations were made on whole blood samples. The overall objective of this work is to assess the feasibility of diagnosing infectious disease before the onset of overt illness.

The effect of vaccination with the live vaccine strain of *F. tularensis* on the changes induced by subsequent infection with the virulent Schu S4 strain of *F. tularensis* was also studied.

## MATERIALS AND METHODS

*Micro-organisms.*—*Francisella tularensis*, Schu S4 strain (Eigelsbach *et al.*, 1951) and the live vaccine strain (LVS; Eigelsbach and Downs, 1961; Tigertt, 1962) and rabbit pox virus (Utrecht strain; provided by Dr E. A. Boulter) were used.

*Growth of bacteria and preparation of washed suspensions.*—Bacteria were grown in the medium of Scharer, Klein and Lincoln (1968) in shaken flasks, or on blood agar slopes (Downs *et al.*, 1947) at 37°. Washed bacterial suspensions were prepared as described by Baskerville and Hambleton (1976).

*Determination of viable bacterial counts.*—Viable bacteria in washed suspensions or blood samples were determined as described by Strange *et al.* (1972).

*Preparation of virus suspensions.*—Rabbit pox virus was provided as a crude Vero cell lysate. Dilutions of the lysate were made in 199 medium (BDH Chemicals Ltd, Poole, Dorset) containing foetal calf serum (10%, v/v) and Gentamycin (50 units/ml; Nicholas Laboratories Ltd, Slough, Bucks).

*Titre of virus suspensions and heparinized virus-infected blood samples.*—Numbers of plaque-forming units were determined by dropping samples (1 ml), diluted if necessary in 199 medium containing calf serum and Gentamycin, on to each of 3 Vero cell monolayers ($2 \times 10^6$ cells) formed on 50-mm Petri dishes and incubating the cultures in an atmosphere of 5% (v/v) $CO_2$ in air at 37° for 16–18 h. Monolayers were then covered with 5 ml of 199 medium containing foetal calf serum (10%, v/v) and sodium carboxymethyl cellulose (1%, w/v) and re-incubated as before for a further 48 h. Excess fluid was poured off and the plaques visualized by staining the cell sheets with crystal violet (0·05% in saline phosphate buffer, pH 7·4).

*Animals.*—Cross-bred or New Zealand White rabbits of both sexes were used.

*Infection of animals.*—Rabbits were infected by i.p. injection of *F. tularensis* Schu S4 suspen- (1 ml; $10^4$–$10^5$ viable bacteria) or intradermal injection of rabbit pox virus suspension (0·2 ml; $5 \times 10^3$ pfu/ml). In some experiments, rabbits were infected by the respiratory route with aerosols of *F. tularensis* Schu S4 (Baskerville and Hambleton, 1976) or rabbit pox virus ($5 \times 10^3$ pfu/1; calculated inhalted retention dose, $2 \times 10^3$ pfu per rabbit). Control animals received injections or were exposed to aerosols of the appropriate diluting medium.

### *Determination of blood components*

*Trace metals.*—Serum Cu, Fe and Zn concentrations were determined with a Varian Techtron Model 63 carbon rod analyser attached to a Varian Techtron Model 1200 atomic absorption spectrophotometer (Varian Associates Ltd., Walton-on-Thames, Surrey). Samples were diluted 1 : 11, 1 : 33 and 1 : 110 in glass distilled water for Cu, Fe and Zn assays, respectively. Some types of plastic tips for automatic pipettes were found to be contaminated with zinc, so glass micropipettes were used for this assay. Usually, samples (5 μl) were inserted into the graphite furnace with an automatic pipette (Ultramicropipetting system; Oxford Laboratories Ltd) and analyses were carried out according to the Manufacturer's Instruction Manual.

*Other metals.*—The concentrations of K, Na, Ca and Mg in sera were determined by flame absorption spectrophotometry with the Varian instrument as described in the Varian Techtron

manual (*Analytical Methods for Flame Spectroscopy*).

*Amino acids.*—Samples (200 μl) of sera were mixed with cold sulphosalicylic acid (200 μl; 5% w/v) and precipitated protein was removed by centrifuging (12,000 *g*; 6 min). Serum amino acids were determined by Mr J. Slade (Analytical Section, MRE) with a Technicon amino acid analyser.

*Serum enzymes.*—Activities of alkaline phosphatase (AP), glutamine oxaloacetate transaminase (GOT), glutamine pyruvate transaminase (GPT), lactate dehydrogenase (LDH), α-hydroxybutyrate dehydrogenase (αHBDH), creatine phosphokinase (CPK) and γ-glutamyl transpeptidase (γGT) enzymes in sera were measured with Roche test kits (Roche Products Ltd, London) and an LKB 8600 Reaction Rate Analyser.

*Serum triglycerides.*—These were determined with a Boehringer test kit and an LKB 8600 Reaction Rate Analyser.

*Serum proteins.*—Total protein concentrations in sera were measured with a kinetic biuret method (LKB application note MLC/an-13; LKB Instruments Ltd, South Croydon, Surrey). Serum proteins were separated by cellulose acetate electrophoresis (apparatus from Gelman Hawksley Ltd, Lancing, Sussex).

*Alkaline phosphatase isoenzymes.*—Isoenzymes of alkaline phosphatase in sera and crude extracts of tissues were separated and visualized by electrophoresis and staining on polyacrylamide gels (Davis and Ornstein, 1961; Sargent, 1969) as described by Dingjan *et al.* (1973).

*Haematology.*—Heparinized blood samples (40 μl) were diluted in formol saline (0·85% NaCl, 0·3% formaldehyde, adjusted to pH 7·4; 20 ml) and held at 4° for 24 h. Total red and white cell counts, mean cell volume (MCV) and haematocrit (Hct) were measured with a Coulter counter (model ZB1 with MCV and Hct accessory; Coulter Electronics Ltd, Harpenden, Herts). Haemoglobin was measured with a Coulter Haemoglobinometer.

## RESULTS

### *"Normal values" and effect of severity of infection*

The concentrations of various blood constituents in "normal rabbits" usually varied considerably and the changes that occurred after infection depended on the severity of the disease. Therefore, values for a given constituent were expressed as percentages of the pre-infection values and

TABLE I.—*Changes in Rabbits Infected with* F. tularensis *Schu S4. Values Given are Means of Results for a Variable Number of Rabbits (up to 30) That Died 4 Days after Infection with doses of $10^4$ to $5\times10^4$ Viable Bacteria (i.p.). Figures in Parentheses are the Observed Ranges of Values*

| | Concentration of blood or serum constituents (per cent of value at time 0) Hours after infection | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 17 | 24 | 41 | 48 | 72 |
| *Blood* | | | | | | |
| WBC | 100 (6–14 × $10^3$/mm$^3$ | 113 | 101 | 55 | 47 | 35 (2–5·4 × $10^3$/mm$^3$) |
| *Serum* | | | | | | |
| Iron | 100 (1·8–3·0 μg/ml) | 91 | 62 | 52 | 34 | 29 (0·3–0·7 μg/ml) |
| Zinc | 100 (1·3–1·8 μg/ml) | 61 | 49 | 35 | 37 | 41·5 (0·29–0·92 μg/ml) |
| Copper | 100 (0·7–1·2 μg/ml) | 121 | 115 | 131 | 160 | 195 (1·23–3·05 μg/ml) |
| Alkaline phosphatase | 100 (268–480 U/I) | 89 | 84 | 50 | 42 | 29·6 (39–310 U/I) |
| Amino acids | 100 (4–5 μmol/ml) | 103 | 109 | 72 | 75 (2·8–3·2 μmol/ml) | N.D. |
| Neutral fat | 100 (0·42–1·38 mg/ml) | 151 | 90 | 378 | 522 (0·87–7·7 mg/ml) | N.D. |
| Bacteriaemia (c.f.u./ml blood) | 0 | 0–24 | 12–99 | 39–132 | 15–474 | 30–4,000 |
| Rectal temperature (°C ± standard error of mean) | 102·5 ± 0·24 | | 102·8 ± 0·15 | | 105·1 ± 0·21 | 105·2 ± 0·47 |

N.D. Not Done

the average of values for animals dying at about the same time is reported below.

*Changes during* F. tularensis *infections*

The earliest changes detected in the blood components of rabbits infected with *F. tularensis* strain Schu S4 by the i.p. route were decreases in alkaline phosphatase activity and the concentrations of iron and zinc at about 17 h after infection (Table I). These changes became more severe as the disease progressed. At about 41 h after infection, a decrease in the white cell count and an increase in serum copper were evident. Neutral fats showed a transient increase at about 17 h and high values after 41 h. The concentration of free amino acids decreased after 41 h, the levels of glycine, alanine and citrulline changing the most; the ratio of phenylalanine to tyrosine did not change significantly.

TABLE II.—*Changes in Rabbits Infected with Aerosols of* F. tularensis *Schu S4. Values Given are Means of Results from a Variable Number of Rabbits (up to 10) that died 4–5 Days after Infection with Estimated retained Doses of* $2\times10^5$ *Viable Bacteria (Aerosol Route;* see Materials and Methods.). *Figures in Parentheses are the Observed Ranges of Values*

| | Concentration of serum constituents (per cent of value at time 0) Days after infection | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 |
| Iron | 100 (1·5–3·32 μg/ml) | 76·8 | 42·4 | 47·3 (0·9–2·4 μg/ml) | N.D. |
| Zinc | 100 (1·8–4·3 μg/ml) | 80·8 | 42·4 (1·08–2·2 μg/ml) | N.D. | N.D. |
| Copper | 100 (0·63–1·4 μg/ml) | 101 | 152 | 288 | 516 (427 μg/ml) |
| Alkaline phosphatase | 100 (225–364 U/l) | 92·1 | 75·4 | 37·4 | 44 (124 U/l) |
| Amino acids | 100 (3·9–4·65 μmol/ml) | 101 | 83·8 | 83·1 (3·12–4·4 μmol/ml) | N.D. |

N.D. Not Done

TABLE III.—*Changes in* F. tularensis *LVS-vaccinated rabbits infected with* F. tularensis *Schu S4. Values given are means of results from a variable number of rabbits (up to 10) Vaccinated with* F. tularensis *LVS (4 Doses of* $5\times10^7$ *Viable Bacteria, i.p. Route) that Died 6–7 Days after Infection with Estimated Retained Doses of* $5\times10^5$ *Viable Schu S4 Strain Bacteria (Aerosol Route;* see Materials and Methods). *Figures in Parentheses are the Observed Ranges of Values*

| | Concentration of serum constituents (per cent of value at time 0) Days after infection | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Iron | 100 (1·75–3·3 μg/ml) | 66·3 | 43·6 | 34·8 | 31·3 | 27 | 23·8 (0·71–0·79 μg/ml) |
| Zinc | 100 (2·7–3·7 μg/ml) | 83·9 | 69·7 | 67·1 | 58·3 | 47·1 | 37·5 (0·88–1·3 μg/ml) |
| Copper | 208* (0·8–1·4 μg/ml) | 151 | 198 | 272 | 333 (1·65–2·4 μg/ml) | N.D. | N.D. |
| Alkaline phosphatase | 75·4* (111–338 U/l) | 75·3 | 63·2 | 53·2 | 39·6 | 20·2 | 30 (60–91 U/l) |
| Amino acids | 100 (4·15–5·7 μmol/ml) | 76·9 | 61·4 | 66·4 | 65·2 | 70·9 | 70·7 (2·83–4·3 μmol/ml) |

* During the course of vaccination with *F. tularensis* LVS serum copper increased and alkaline phosphatase activity decreased compared with pre-vaccination values.
N.D. Not Done.

Activities of the serum enzymes CPK, GOT, GPT, LDH and αHBDH usually remained fairly constant during the first 48 h of infection although, occasionally, GOT and GPT activities had increased by 48 h. Thereafter, marked increases (up to 300% or more of pre-infection value) in the activities of these enzymes were observed. The activity of γGT was generally low and did not change significantly.

Serum total protein concentrations changed little but cellulose acetate electrophoresis showed that, 41–48 h after infection, $\alpha_2$-globulin had increased and an additional component migrating just behind β-globulin had appeared.

No significant changes in the concentrations of K, Na, Ca or Mg were observed.

The changes in rabbits that survived for 6 or more days after infection were less rapid. Significant changes in the concentrations of iron, zinc and copper, and alkaline phosphatase activity in sera were not usually apparent before 41–48 h.

According to colony counts, the number of colony-forming units in samples of whole blood (incubated on blood agar plates) was very small 17 h after infection and did not exceed 500/ml blood after 48 h. Bacteria appeared to be in or associated with white blood cells and disruption of the latter sometimes led to an increase in bacterial counts (Harris-Smith, unpublished).

Rabbits exposed to aerosols of *F. tularensis* died about 4 days after infection and changes in blood constituents showed similar trends to these observed in animals infected by the i.p. route. Concentrations of iron and zinc had decreased, albeit to a lesser extent, by 24 h (Table II), copper did not increase until 48 h after infection and serum alkaline phosphatase was decreased at 48 h and thereafter fell rapidly.

Vaccination with repeated doses (4) of *F. tularensis* strain LVS ($5\times10^7$ per dose at 0, 9, 15 and 21 days) caused changes in the levels of serum copper (increased 100%) and alkaline phosphatase activity (decreased 25%) but no other changes were detected. Rabbits infected with *F. tularensis* strain Schu S4 ($5\times10^5$) 15 days after vaccination survived only 2 to 3 days longer than unvaccinated rabbits. Changes in blood constituents in the infected vaccinated animals showed similar trends (Table III) to those observed in unvaccinated rabbits (Tables I and II). Before infection, levels of serum copper were higher and alkaline phosphatase activity lower in vaccinated rabbits compared with those in unvaccinated rabbits. Three days after infection, serum copper levels increased further and alkaline phosphatase activity decreased after 2 days and fell progressively as the disease progressed.

After infection with *F. tularensis* strain Schu S4 (i.p. route), the rectal temperature of animals had increased at 48 h (Table I)—that is, after the earlier changes in trace metals and alkaline phosphatase activity.

*Changes during rabbit pox infections*

Rabbits infected with rabbit pox virus survived longer (6–14 days) than those with *F. tularensis* infections (4–6 days); the course of the viral disease was similar whether infection was by the respiratory or intradermal route and significant changes in blood constituents were not detected as early as with the bacterial infection.

Significant changes in the concentrations of iron, zinc and in alkaline phosphatase activity, and increases in those of copper and neutral fats occurred within 3 days after infection (Table IV). After an initial rise evident after 2 days, neutral fat levels decreased slightly then increased again rapidly. Usually, viraemias were not detected until the fourth day after infection, 1 day after rectal temperatures had increased. No significant early changes in activities of the serum enzymes CPK, GOT, GPT, LDH, αHBDH and γGT, amino acid concentrations or total white cell counts were detected. Sometimes, GOT and GPT activities increased 2 to 3 days before death of the animal. Total serum protein levels did not change significantly

TABLE IV.—*Changes in Rabbits Infected with Rabbit Pox Virus. Values Given are Means of Results for a Variable Number of Rabbits (10 to 28) That Died 6 to 9 Days after Infection with Doses of $10^3$ to $10^4$ p.f.u. of Virus (Aerosol or i.d. routes,* see Materials and Methods). *Figures in Parentheses are the Observed Ranges of Values*

| | Concentration of serum constituents (per cent of value at time 0) Days after infection | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| *Blood* | | | | | | | | | |
| WBC | 100 (3·9–12·2 × $10^3$/mm$^3$) | 121 | 141 | 106 | 89 | 89 | 98 | 113 | 137 (2·9–13·7 × $10^3$/mm$^3$) |
| *Serum* | | | | | | | | | |
| Iron | 100 (2·2–4·5 μg/ml) | 83 | 92 | 77 | 65 | 53 | 51 | 66 | 60 (0·43–2·1 μg/ml) |
| Zinc | 100 (1·1–3·5 μg/ml) | 60 | 61 | 67 | 58 | 54 | 42 | 47 | 34 (0·1–1·3 μg/ml) |
| Copper | 100 (0·6–1·7 μg/ml) | 113 | 115 | 133 | 158 | 192 | 253 | 309 | 289 (1·4–3·6 μg/ml) |
| Alkaline phosphatase | 100 (121–384 U/l) | 103 | 90 | 76 | 55 | 44 | 28 | 23 | 19 (20–152 U/l) |
| Amino acids | 100 (2·7–4·8 μmol/ml) | 86 | 90 | 89 | 91 | 105 | 124 | 98 | 104 (2–5 μmol/ml) |
| Neutral fat | 100 (0·1–1·6 mg/ml) | 146 | 234 | 154 | 169 | 178 | 455 | 800 | 1053 (3·2–11·2 mg/ml) |
| Viraemia (p.f.u./ml blood) | 0 | 0 | 0–50 | 0–75 | 10–125 | 80–580 | 30–2400 | 20–2900 | N.D. |
| Rectal temperature (°C ± standard error of mean) | 102·8 ±0·25 | 102·3 ±0·14 | 102·7 ±0·14 | 104·9 ±0·31 | 104·8 ±0·23 | 104·5 ±0·3 | 104·3 ±0·6 | N.D. | N.D. |

N.D. Not Done.

but cellulose acetate electrophoresis of samples taken 3 days after infection showed increased $\alpha_2$-globulin and an additional component migrating just behind $\beta$-globulin.

In a few cases, rabbits survived rabbit pox infection and, during their recovery, serum alkaline phosphatase activity and trace metal concentrations slowly returned to normal.

*Serum alkaline phosphatase isoenzymes in infected rabbits*

Early decreases in serum alkaline phosphatase activity were invariably observed in rabbits infected with *F. tularensis* or rabbit pox virus. On separation of alkaline phosphatase isoenzymes by polyacrylamide gel electrophoresis, usually 2 isoenzymes were detected in rabbit serum. The origin of these isoenzymes was not unequivocally identified but both migrated at rates similar to isoenzymes present in extracts of rabbit liver. After infections with both *F. tularensis* and rabbit pox virus, the relative activities of the 2 isoenzymes usually changed as the disease progressed; that of the major (slower moving) component decreased markedly after 24–41 h, whereas that of the minor (faster moving) component increased gradually throughout the course of the infection. In rabbits recovering from rabbit pox, the total enzyme activity returned to its initial level, mainly due to an increase in the concentration of the faster moving component.

## DISCUSSION

The finding that serum zinc concentration decreases during bacterial and viral infections in laboratory animals is in agreement with that of other workers (Pekarek and Beisel, 1971; Wannemacher

*et al.*, 1972*a*; Beisel, Pekarek and Wannemacher, 1974; Beisel, 1976). Associated with the decrease in zinc there was a significant decrease in free amino acids during *F. tularensis* infection but only a small change in the case of rabbit pox. The results with tularaemia are in accordance with the postulate that observed decreases in serum zinc and amino acids are related to the increased flux of these components to the liver where the amino acids are utilized for synthesis of acute phase proteins (Wannemacher *et al.*, 1972*a*; Powanda *et al.*, 1975; Berendt *et al.*, 1977). As observed here, decreases in plasma or serum zinc and iron precede the onset of clinical signs while an increase in serum copper, indicative of increased ceruloplasmin synthesis (Powanda *et al.*, 1976) usually occurs later on, although sometimes increased copper levels have been observed before changes occurred in zinc and iron levels (Powanda *et al.*, 1976).

A number of infectious diseases in man and animals are characterized by an increase in the serum phenylalanine: tyrosine ratio and Wannemacher *et al.* (1976) consider that an increase in this ratio may be a good indication of the onset of an inflammatory state, whether initiated by microbial infection or tissue injury. However, we observed no significant changes in this ratio during infections with *F. tularensis* or rabbit pox. The early fall in amino acids during tularaemia was due mainly to decreases in glycine, alanine and citrulline. Apparently, therefore, the rabbit differs in this respect from man and other laboratory animals.

Powanda *et al.* (1975) consider that during tularaemia in rats the onset of induced metabolic changes awaits the development of pyrogranulomatous hepatic lesions and other tissue damage. Rabbits infected with aerosols of *F. tularensis* strain Schu S4 showed changes in serum trace metals, amino acids, neutral fats and alkaline phosphatase 1 to 2 days after infection and, at this time, the animals were developing widespread hepatic, lung, spleen and lymphatic lesions (Baskerville and Hambleton, 1976). Similarly, in rabbit pox early changes in the levels of serum components correlate with the appearance of pyrogranulomatous hepatic lesions (Baskerville and Hambleton, unpublished).

Although increases in the activities of certain serum enzymes occurred, they were mostly observed during the later stages of infection. In contrast, serum alkaline phosphatase activity decreased early on in nearly all infected rabbits; this appears to be a significant finding. The effect is not confined to rabbits and similar early decreases were observed during tularaemia in mice and guinea pigs (Hambleton, unpublished). On the other hand, Sammons *et al.* (1976) reported decreased serum alkaline phosphatase activity in only 22% of *Macaca mulatta* monkeys infected with Rocky Mountain spotted fever and no overall decrease compared with uninfected control animals.

The electrophoretic mobilities of alkaline phosphatase isoenzymes (in rabbit serum) suggested they may originate from the liver. Histochemical staining of liver sections from infected rabbits showed that decreased alkaline phosphatase activity was confined to necrotic areas (Baskerville and Hambleton, unpublished) and it is unlikely that this accounts for the observed changes in the serum enzyme activity. The activity of one isoenzyme (slower migration on electrophoresis) decreased preferentially, suggesting the two isoenzymes originate from different sites in the liver, of which one is affected early during infection. Since in rabbits recovering from rabbit pox there was a preferential increase in the electrophoretically faster migrating isoenzyme, the selective early effect of infection may not be readily reversible. An alternative explanation for the fall in total alkaline phosphatase activity during infection is that a substance(s) is synthesized or released which preferentially inhibits the activity of the more slowly migrating component in serum.

The rates of changes in the concentrations of certain constituents in the blood of infected animals depended upon the number of microbes injected, the route of infection and the severity of the disease. Thus, changes were observed earlier in rabbits infected with *F. tularensis* by the i.p. route than in these infected by the respiratory route; rabbits infected with rabbit pox virus survived longer than those with tularaemia and the changes observed occurred later. Vaccination with the live vaccine strain of *F. tularensis* did not protect animals against subsequent challenge with the virulent Schu S4 strain (Nutter and Myrvik, 1966; Hambleton and Strange, unpublished), although it increased survival times by 1 to 2 days. Despite the increase in survival time, the onset of observed metabolic changes in vaccinated rabbits was not delayed compared with unvaccinated rabbits.

Clearly, changes in these blood components selected for analysis in the present study provide no indication of the nature of the infectious agent and, in fact, Wannemacher *et al.* (1972*a*) showed that a wide range of inflammatory processes induce similar changes. However, Powanda *et al.* (1976) reported a unique series of changes in guinea pigs infected with Rocky Mountain spotted fever. It is possible, therefore, that if changes in the concentrations of many other body components are studied, this may allow early diagnosis in a taxonomic sense—that is, whether the agent is of the viral, rickettsial, bacterial or fungal type.

## REFERENCES


BASKERVILLE, A. & HAMBLETON, P. (1976) Pathogenesis and Pathology of Respiratory Tularaemia in the Rabbit. *Br. J. exp. Path.*, **57**, 339.

BEISEL, W. R. (1976) Trace Elements in Infectious Processes. *Med. Clin. N. Amer.*, **60**, 831.

BEISEL, W. R., PEKAREK, R. S. & WANNEMACHER, R. W., JR. (1974) In *Trace Metal Metabolism in Animals—2*. Ed. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, & W. Mertz. Baltimore: University Park Press.

BEISEL, W. R., SAWYER, W. D., RYLL, E. D. & CROZIER, D. (1967) Metabolic Effects of Intracellular Infections in Man. *Ann. Intern. Med.*, **67**, 744.

BERENDT, R. F., LONG, G. G., ABELES, F. B., CANONICO, P. G., ELWELL, M. R. & POWANDA, M. C. (1977) Pathogenesis of Respiratory *Klebsiella pneumoniae* Infection in Rats: Bacteriological and Histological Findings and Metabolic Alterations. *Infect. Immun.*, **15**, 586.

CANONICO, P. G., POWANDA, M. C., COCKERELL, G. L. & MOE, J. B. (1975) Relationship of Serum $\beta$-Glucuronidase and Lysozyme to Pathogenesis of Tularaemia in Immune and Non-immune Rats. *Infect. Immun.*, **12**, 42.

DAVIS, B. J. & ORNSTEIN, L. (1961) In *Disc Electrophoresis*. Rochester, USA: Distillation Products Industries.

DINGJAN, P. G., JONGENEEL, J., POSTMA, T. & STROES, J. A. P. (1973) Evaluation of the Determination of Alanine Naphthylamidase Isoenzymes by Agar and Polyacrylamide Disc Electrophoresis for the Diagnosis of Pancreatic Diseases. *Clin. chim. Acta*, **49**, 215.

DOWNS, C. M., CORIELL, L. L., CHAPMAN, S. S. & KLAUBER, A. (1947) The Cultivation of *Bacterium tularense* in Embryonated Eggs. *J. Bact.*, **53**, 89.

EDDINGTON, C. L., UPCHURCH, H. F. & KAMPSCHMIDT, R. F. (1971) Effect of Extracts from Rabbit Leukocytes on Levels of Acute Phase Globulins in Rat Serum. *Proc. Soc. exp. Biol. Med.*, **136**, 159.

EIGELSBACH, H. T., BRAUM, W. & HERRING, R. D. (1951) Studies on the Variation of *Bacterium tularense*. *J. Bact.*, **61**, 557.

EIGELSBACH, H. T. & DOWNS, C. M. (1961) Prophylactic Effectiveness of Live and Killed Tularaemia Vaccines. I. Production of Vaccine and Evaluation in the White Mouse and Guinea-pig. *J. Immun.*, **87**, 415.

FEIGIN, R. D. & DANGERFIELD, H. G. (1967) Whole-blood Amino Acid Changes Following Respiratory-acquired *Pasteurella tularensis* Infection in Man. *J. infect. Dis.*, **117**, 346.

FEIGIN, R. D., JAEGER, R. F., MCKINNEY, R. W. & ALEVIZATOS, A. C. (1967) Live, Attenuated Venezuelan Equine Encephalomyelitis Virus Vaccine. II. Whole-blood Amino Acid and Fluorescent-antibody Studies Following Immunization. *Am. J. trop. Med.*, **16**, 769.

FEIGIN, R. D., KLAINER, A. S., BEISEL, W. R. & HORNICK, R. B. (1968) Whole-blood Amino Acids in Experimentally-induced Typhoid Fever in Man. *N. Engl. J. Med.*, **278**, 293.

KAMPSCHMIDT, R. F. & UPCHURCH, H. F. (1970*a*) A Comparison of the Effects of Rabbit Endogenous Pyrogen on the Body Temperature of the Rabbit and the Lowering of Plasma Iron in the Rat. *Proc. Soc. exp. Biol. Med.*, **133**, 128.

KAMPSCHMIDT, R. F. & UPCHURCH, H. F. (1970*b*) The Effects of Endogenous Pyrogen on the Plasma Zinc Concentration of the Rat. *Proc. Soc. exp. Biol. Med.*, **134**, 1150.

NUTTER, J. E. & MYRVIK, Q. N. (1966) *In vitro* Interactions Between Rabbit Alveolar Macrophages and *Pasteurella tularensis*. *J. Bact.*, **92**, 645.

PEKAREK, R. S. & BEISEL, W. R. (1971) Characterization of the Endogenous Mediator(s) of Serum Zinc and Iron Depression During Infection and Other Stresses. *Proc. Soc. exp. Biol. Med.*, **138**, 728.

PEKAREK, R. S., WANNEMACHER, R. W., JR & BEISEL, W. R. (1972) The Effect of Leukocytic Endogenous Mediator (LEM) on the Tissue

Distribution of Zinc and Iron. *Proc. Soc. exp. Biol. Med.*, **140**, 685.

POWANDA, M. C., COCKERELL, G. L., MOE, J. B., ABELES, F. B., PEKAREK, R. S. & CANONINCO, P. G. (1975) Induced Metabolic Sequelae of Tularaemia in the Rat: Correlation with Tissue Damage. *Amer. J. Physiol.*, **229**, 479.

POWANDA, M. C., COCKERELL, G. L. & PEKAREK, R. S. (1973) Amino Acid and Zinc Movement in Relation to Protein Synthesis Early in Inflammation. *Amer. J. Physiol.*, **225**, 399.

POWANDA, M. C., KENYON, R. H. & MOE, J. B. (1976) Alterations in Plasma Copper, Zinc, Amino Acids and Seromucoid during Rocky Mountain Spotted Fever in Guinea Pigs. *Proc. Soc. exp. Biol. Med.*, **151**, 804.

SAMMONS, L. S., KENYON, R. F., BURGER, G. T., PEDERSEN, C. E., JR & SPERTZEL, R. O. (1976) Changes in Blood Serum Constituents and Haematological Values in *Macaca mulatta* with Rocky Mountain Spotted Fever. *Amer. J. vet. Res.*, **37**, 725.

SARGENT, J. R. (1969) In *Methods in Zone Electrophoresis*. Poole, England: BDH Chemicals Ltd.

SCHARER, J. M., KLEIN, F. & LINCOLN, R. E. (1968) Growth and Metabolism of Live Vaccine Strain of *Pasteurella tularensis*. *Appl. Microbiol.*, **16**, 855.

STRANGE, R. E., BENBOUGH, J. E., HAMBLETON, P. & MARTIN, K. L. (1972) Methods for the Assessment of Microbiol Populations Recovered from Enclosed Aerosols. *J. gen. Microbiol.*, **72**, 117.

TIGERTT, W. D. (1962) Soviet Viable *Pasteurella tularensis* Vaccines. A Review of Selected Articles. *Bact Rev.*, **26**, 354.

WANNEMACHER, R. W., JR, DUPONT, H. L., PEKAREK, R. S., POWANDA, M. C., SCHWARTZ, A., HORNICK, R. B. & BEISEL, W. R. (1972*a*) An Endogenous Mediator of Depression of Amino Acids and Trace Metals in Serum During Typhoid Fever. *J. infect. Dis.*, **126**, 77.

WANNEMACHER, R. W., JR, KLAINER, A. S., DINTERMAN, R. E. & BEISEL, W. R. (1976) The Significance and Mechanism of an Increased Serum Phenylalanine-Tyrosine Ratio during Infection. *Amer. J. clin. Nut.*, **29**, 997.

WANNEMACHER, R. W., JR, PEKAREK, R. S., BARTELLONI, P. J., VOLLMER, R. T. & BEISEL, W. R. (1972*b*) Changes in Individual Plasma Amino Acids Following Experimentally Induced Sandfly Fever Virus Infection. *Metabolism*, **21**, 67.

WANNEMACHER, R. W., JR, PEKAREK, R. S. & BEISEL, W. R. (1972*c*) Mediator of Hepatic Amino Acid Flux in Infected Rats. *Proc. Soc. exp. Biol. Med.*, **139**, 128.

WANNEMACHER, R. W., JR, PEKAREK, R. S., KLAINER, A. S., BARTELLONI, P. J., DUPONT, H. L., HORNICK, R. B. & BEISEL, W. R. (1975) Detection of a Leukocytic Endogenous Mediator-like Mediator of Serum Amino Acid and Zinc Depression During Various Infectious Illnesses. *Infect. Immun.*, **11**, 873.

WANNEMACHER, R. W., JR, POWANDA, M. C., PEKAREK, R. S. & BEISEL, W. R. (1971) Tissue Amino Acid Flux After Exposure of Rats to *Diplococcus pneumoniae*. *Infect. Immun.*, **4**, 566.