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Clinical Pathology

2.1 Haematology and biochemistry

As rabbits are used extensively for toxicological and physiological studies, there is a lot of information about the effects of experimental infections, drugs and toxic substances on haematological and biochemical parameters. There is also information about diseases of commercial rabbits, primarily based on post-mortem examination. Information about the effects of clinical diseases on the blood picture of live pet rabbits or the use of blood tests as diagnostic or prognostic indicators has increased exponentially over the past decade with the increasing popularity of rabbits as pets.

2.1.1 Sample collection

The collection of blood and urine samples is covered in [Section 1.10.3](#). Parameters such as glucose, creatine kinase and aspartate aminotransferase (AST) can be altered by stress associated with handling and restraint, or tissue damage that has occurred during sample collection. For example, potassium results appear less reliable in samples taken with a plastic cannula as opposed to a hypodermic needle ([Robson *et al.*, 1981](#)).

Rabbit blood haemolyses easily and clots quickly ([Perry-Clark and Meunier, 1991](#)). Small clots affect haematology results and haemolysis affects certain biochemistry results, especially potassium and serum inorganic phosphorus that are released from erythrocytes. Rapid clotting can affect the performance of some analysers and heparinized syringes and needles are required to avoid this.

2.1.2 Fasting and other physiological considerations

It is not possible to take a guaranteed fasting sample from a rabbit because they ingest caecotrophs. Parameters such as blood glucose are affected by digestion. Some parameters such as bile acids, cholesterol and urea follow a diurnal rhythm that also affects the total and differential white cell count ([Fekete, 1989](#); [Fox and Laird, 1970](#); [Loeb and Quimby, 1989](#)). Stress associated with car journeys or a period in unfamiliar surroundings will increase blood glucose and alter haematological parameters such as the distribution of neutrophils and lymphocytes. Pregnancy affects parameters such as protein, haematocrit, cholesterol, alkaline phosphatase, triglycerides ([Viard-Drouet *et al.*, 1984](#)), glucose, sodium, calcium, phosphate and red cell indices ([Palm, 1997](#)). Serum cholesterol is the parameter most affected and can be up to 30% lower in pregnant than in non-pregnant animals ([Palm, 1997](#)).

Anaesthesia affects some blood parameters such as potassium ([Robson *et al.*, 1981](#)). The effect of anaesthesia on biochemical parameters is minimized by taking samples within 5 minutes of induction. Intravenous or intraosseous fluids will also affect haematological findings ([Ros *et al.*, 1991](#)) and blood samples should be taken prior to treatment.

2.1.3 Reference ranges

There are a number of published reference ranges for haematological and biochemical parameters in rabbits. Conversion factors for the variety of units used in reference ranges are given in [Table 2.1](#). Differences

Table 2.1 Conversion factors for SI to imperial units

| To convert | From SI units | To imperial units | Multiply by |
|-----------------------|---------------|-------------------|-------------|
| Albumin | g/l | g/dl | 0.1 |
| Bilirubin | µmol/l | mg/dl | 0.059 |
| Calcium | mmol/l | mg/dl mEq/l | 4 2 |
| Cholesterol | mmol/l | mg/dl | 38.7 |
| Creatinine | mg/dl | µmol/l | 88.4 |
| Globulin | g/l | g/dl | 0.1 |
| Glucose | mmol/l | mg/dl | 18 |
| Haemoglobin | g/dl | g/l mmol/l | 10 0.62 |
| Lead | µg/dl | µmol/l | 0.0483 |
| Packed cell volume | l/l | % or ml/dl | 100 |
| Phosphate (inorganic) | mmol/l | mg/dl mEq/l | 3.1 1.8 |
| Potassium | mmol/l | mEq/l mg/dl | 1 3.9 |
| Sodium | mmol/l | mEq/l mg/dl | 1 2.3 |
| Total protein | g/l | g/dl | 0.1 |
| Urea | mmol/l | mg/dl | 6 |

in analytical techniques between laboratories can lead to disparity in results. Historically laboratory data were often derived from populations of rabbits of the same breed, sex and age that are not genetically diverse. In contrast, the pet rabbit population is made up of a variety of breeds and cross-breeds, and is composed of rabbits of all ages. With the increased interest of rabbit owners in pursuing diagnostics for their pets, many commercial labs have built up a vast database of samples from a genetically heterogeneous population. Significant breed and sex differences have been noted

for some parameters in laboratory rabbits (Kozma *et al.*, 1974).

Published reference ranges for pet rabbits are derived either from sets of data from laboratory rabbits or from data collected by the author. Some reference ranges are an amalgamation of other reference ranges often without addressing the fact that the ranges have been determined using different methodologies. This results in ranges so wide that almost any result will fall within the normal range. For example, the reference range for serum albumin concentrations of pet rabbits is given as 27–46 g/L (Malley, 1996) based on four published sets of data. Gillett (1994) gives an even wider range of 27–50 g/L for laboratory rabbits based on five sets of data. For some parameters there are big differences between published reference ranges. An example is blood calcium. Gillett (1994) gives a range of 1.5–3.4 mmol/L, compared to 3.4–4.0 mmol/L given by Harkness and Wagner (1995). Differences in calcium content of the diet between the groups of rabbits or differences in analytical technique could explain these discrepancies. Different laboratory methods can result in large differences between reference ranges. An example is alkaline phosphatase. Collins (1988) gives a reference range of 4.1–6.2 IU/L, compared to 10–70 IU/L (Gillett, 1994) or 112–350 IU/L (Harkness and Wagner, 1995).

Automated flow cytometers are designed to measure numbers of human blood cells. Rabbit

Key Points 2.1 Physiological effects on blood parameters

- Digestion: blood glucose is elevated
- Diurnal variations: bile acids, cholesterol, urea, differential white cell count
- Age: neutrophil:lymphocyte ratio
- Stress: neutrophil:lymphocyte distribution
- Pregnancy: protein, haematocrit, cholesterol, alkaline phosphatase, triglycerides, glucose, sodium, calcium, phosphate, red blood cell indices
- Anaesthesia: potassium

erythrocytes are smaller in diameter than human erythrocytes and also vary in diameter. These differences cause problems with some automated analysers. Automated differential white cells counts cannot be relied upon for rabbit blood and accurate results can only be obtained using manual counting methods (Kabata *et al.*, 1991).

2.2 Haematology

2.2.1 Morphological characteristics of blood cells

Some morphological characteristics of rabbit blood cells are different from other species. The red blood cells vary in diameter within a range of 5.0–7.8 μm (Sanderson and Phillips, 1981). This variation in diameter (anisocytosis) is a feature of blood smears from rabbits and is not a significant finding (see Figure 2.1). The red cell distribution width (RDW) is a measurement of the variation in size of

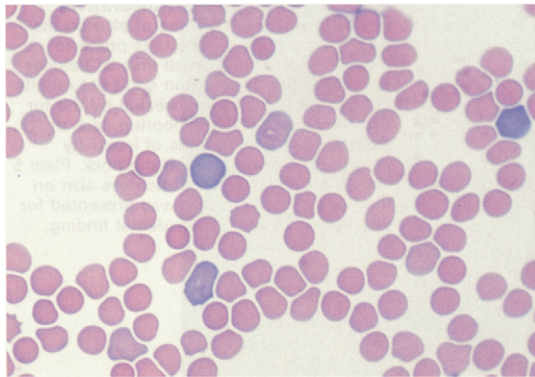


Figure 2.1 Anisocytosis and polychromasia. The red blood cells of rabbits vary in diameter within a range of 5.0–7.8 μm . This variation in diameter (anisocytosis) is a feature of blood smears from rabbits and is not a significant finding. There is also variation in erythrocyte colour (polychromasia) in normal rabbits. The blood smear illustrated was from a normal rabbit and was stained with Rapi-diff. Interpretation of haematology results is discussed in Section 2.2.2. Photograph taken by Dr Joan Duncan, IDEXX Laboratories, Wetherby.

erythrocytes and is higher in rabbits (11–15, IDEXX reference range) than in dogs and cats (8–10; Bush, 1991). In dogs and cats, anisocytosis is indicative of the presence of reticulocytes and a regenerative anaemia. In rabbits, 1–4% of circulating erythrocytes may be reticulocytes. Polychromasia and reticulocytes in rabbit blood smears have been attributed to the short life span and high turnover of erythrocytes (Kraus *et al.*, 1984). Nucleated red cells and Howell–Jolly bodies can also be found occasionally (McLaughlin and Fish, 1994).

Rabbit neutrophils have an almost colourless cytoplasm and contain two types of granules. The smaller granules stain pink, giving a pinkish colour to the cytoplasm. Larger granules stain a deeper pinkish-red. The overall colour of the neutrophils varies according to the proportion of large and small granules. The granular appearance of the cytoplasm has led to different nomenclature. Rabbit neutrophils may be called heterophils, pseudoeosinophils, acidophils or amphophils, depending on the text (Benson and Paul-Murphy, 1999; Sanderson and Phillips, 1981). Neutrophils measure 10–15 μm in comparison with eosinophils, which measure 12–16 μm (Sanderson and Phillips, 1981).

Small lymphocytes are seen more commonly than large lymphocytes. The average cell diameter for small lymphocytes is 7–10 μm (Cooke, 2000). The lymphocytes are round cells with the typical morphology described for other species. An occasional large lymphocyte may be seen in the circulation or normal rabbits. These cells have a few azurophilic granules in the cytoplasm (Jain, 1986). Activated lymphocytes (immunocytes) typically have a more intensely stained cytoplasm.

Monocytes are large nucleated cells measuring 15–18 μm (Cooke, 2000). The nucleus has a diffuse lacey chromatin pattern that lightly stains a purple blue. The vacuolar cytoplasm stains light blue.

Eosinophils can be distinguished from neutrophils by their greater size and large acidophilic granules.

In contrast to other laboratory species, basophils are frequently found in the circulation of rabbits in small to modest numbers (Jain, 1986).

2.2.2 Interpretation of haematology results

A reference range for haematological parameters is given in Table 2.2. The haematological picture gives an indication of the general health status of a rabbit. Stress and a range of diseases will alter haematological parameters. Hinton *et al.* (1982) analysed the haematological findings in 117 healthy and diseased rabbits and found that blood cellularity was a good indicator of disease especially with regard to erythrocytes (these tended to be depressed) and lymphocyte

counts (the counts were variable; however, heterophils tended to become dominant in acutely ill animals). These findings are in agreement with studies of experimental infections in rabbits (Toth and Krueger, 1988, 1989) and in a clinical study by Harcourt-Brown and Baker (2001). In this study, significantly higher red cell counts, haemoglobin values, haematocrits and lymphocyte counts were found in rabbits kept outside with unlimited access to grazing and exercise. A comparison was made with rabbits kept in hutches and those suffering from dental disease.

Table 2.2 Haematological reference range

| | Laboratory reference range (Gillett, 1994) | Comment |
|---------------|--|---|
| Erythrocytes | $4-7 \times 10^{12}/L$ | Anisocytosis, polychromasia, small numbers of nucleated red blood cells and Howell-Jolly bodies can be normal findings in rabbit blood films. |
| Haemoglobin | 10–15 g/dL | |
| PCV | 33–48% (0.33–0.48 L/L) | 31–40% usual range for pet rabbits |
| MCV | $60-75 \mu\text{m}^3$ (fL) | |
| MCH | 19–23 pg | |
| MCHC | 34.5 g/dL | |
| Reticulocytes | 2–4% | Small numbers normal, increased with regenerative anaemia. |
| Platelets | $250-600 \times 10^3/L$ ($10^9/L$) | |
| White cells | $5-12 \times 10^3/L$ ($10^9/L$) | Total white cell count usually fairly stable. Increase indicates significant insult. |
| Neutrophils | 30–50% | Neutrophil:lymphocyte ratio is approximately 1:1 in a healthy adult; this increases in acute infection. |
| Lymphocytes | 30–60% | Lymphocyte numbers are depressed in acute infection, thereby increasing the neutrophil:lymphocyte ratio. Small numbers of large lymphocytes are normal in the circulation; activated lymphocytes are not. |
| Eosinophils | 0–5% | Not consistently elevated with parasitism. |
| Basophils | 0–8% | Significance of a decrease is not known. |
| Monocytes | 2–10% | Often found elevated in animals with chronic disease. |

Sanderson, J.H., Phillips, C.E. (1981). Rabbits. In *An Atlas of Laboratory Animal Haematology*, p. 6. Oxford University Press.

From: Gillett, C.S. (1994). *Selected drug dosages and clinical reference data*. In *The Biology of the Laboratory Rabbit*, 2nd edn. (P.J. Manning, D.H. Ringler, C.E. Newcomer, eds). pp 467–472. Academic Press.

2.2.3 Red cell parameters

Reference ranges for packed cell volumes (PCV) vary between sources, with values between 30 and 50% (Malley, 1996). Pet rabbits tend to have PCV values at the lower end of the range, typically between 30 and 40% (Harcourt-Brown and Baker, 2001). Values greater than 45% are indicative of dehydration, especially in rabbits suffering from gut motility problems. Values of less than 30% indicate anaemia that can be classified into non-regenerative and regenerative in a manner similar to that of other species.

Regenerative anaemia is associated with chronic blood loss, where compensatory mechanisms are attempting to redress the balance. Common causes include heavy flea infestation or from sites that intermittently bleed such as a uterine endometrial aneurysm or uterine adenocarcinomas, a common finding in middle-aged unneutered does. Lead poisoning can result in a regenerative anaemia with the presence of nucleated erythrocytes, hypochromasia, poikilocytosis and cytoplasmic basophilic stippling (Fudge, 2000). Non-regenerative anaemia is caused by diseases such as lymphoma or chronic renal failure. Autoimmune haemolytic disease has not been described as a clinical phenomenon in pet rabbits although there are reports that it occurs. Autoimmune haemolytic anaemia has been reported in laboratory rabbits in association with lymphosarcoma (Weisbroth, 1994). Chronic debilitating disease such as dental disorders or abscesses often cause a mild anaemia in pet rabbits (Harcourt-Brown and Baker, 2001).

Nucleated red blood cells can be associated with acute infectious processes although a few may be present in normal blood films. Experimentally, infections with *Escherichia coli* and *Staphylococcus aureus* cause an increase in number of circulating nucleated red blood cells during the septicaemic phase of the disease (Toth and Krueger, 1988, 1989).

2.2.4 White blood cells

2.2.4.1 Total white blood cell count

There is a natural diurnal variation in total white cell count, with lowest counts occurring during the late

afternoon and evening (Fox and Laird, 1970). The white cell count also varies with age (Jain, 1986). It is higher in young rabbits of approximately 3 months of age and in adults over 1 year old. The first peak in leucocyte count is due to an increase in the number of lymphocytes. The second peak is due to an increase in the number of neutrophils.

In other species, an increased white blood cell count is seen in response to bacterial infection or in response to endogenous or exogenous corticosteroids. Rabbits do not develop marked leucocytosis after either acute infectious challenge or the intramuscular injection of cortisone acetate (Toth and January, 1990). If an elevated total white cell count is found, this should be confirmed, and if consistent, it should be assumed to be significant, as it is likely that a significant disease process is occurring. In two studies by Toth and Krueger (1988, 1989) controlled experimental infections with *S. aureus*, *Streptococcus pyogenes*, *E. coli* and *Candida albicans* resulted in fever, increased plasma cortisol concentrations, neutrophilia and lymphopenia but no significant increase in total white blood cell count. High white cell counts can be found in rabbits suffering from lymphosarcoma (McLaughlin and Fish, 1994). Low white blood cell counts can be found in association with chronic disease (Hinton *et al.*, 1982).

2.2.4.2 Differential white cell counts

Although the total white cell count of rabbits seldom alters in diseased rabbits, the differential white cell count may show a number of changes due to the redistribution of white blood cells. A feature of many diseases in rabbits is an alteration in the ratio of neutrophils to lymphocytes and a reduction in blood cellularity (Hinton *et al.*, 1982). The neutrophil:lymphocyte ratio has been suggested as a method of predicting whether a rabbit is normal or abnormal (McLaughlin and Fish, 1994). Jain (1986) described a physiological variation in the neutrophil:lymphocyte ratio according to the age of the rabbit. The ratio changes from 33:60 in the second month of life to 45:45 in rabbits over 1 year of age. Stress and increased cortisol levels, as well as disease, can affect this ratio.

2.2.4.3 Effect of stress on the differential white cell count

Stress alters the differential white cell count in any species. Rabbits are particularly susceptible to the effects of stress. A car journey to the surgery, a period in the waiting room next to a barking dog or the excitement of handling can be reflected in the blood picture. Adrenaline and cortisol affect the distribution of lymphocytes throughout the body. Administration of exogenous adrenaline to rabbits results in redistribution of lymphocytes from spleen and bone marrow to peripheral blood, lungs and liver (Toft *et al.*, 1992a). Conversely, exogenous corticosteroid administration results in a redistribution of lymphocytes from the peripheral blood, bone marrow and spleen to the lymphatic tissue in rabbits (Toft *et al.*, 1992b). Prolonged periods of stress cause neutrophilia and lymphopenia. Marked changes in white cell distribution with a relative neutrophilia and lymphopenia were found in a study of cortisol levels and haemograms of rabbits after transport, either by air or by lorry. The changes in white cell distribution lasted for 24–48 h and were correlated with increased cortisol levels (Toth and January, 1990).

Disease is stressful as well as having a direct effect on the production and distribution of white cells.

Rabbits with experimental infections exhibit a neutrophilia and lymphopenia in comparison with control rabbits handled and sampled in exactly the same manner but inoculated with heat-killed cultures (Toth and Krueger, 1989). Rabbits inoculated with a heat-killed culture do not experience the same rise in plasma cortisol concentrations as those inoculated with a live culture, indicating that the stress response is initiated by disease rather than by handling. Therefore the stressful effects of a long car journey to the surgery or a morning spent in a kennel next to a barking dog is more likely to affect the neutrophil:lymphocyte ratio than the excitement response of taking blood.

2.2.4.4 Neutrophils

Neutrophils function primarily as phagocytes and are important in infectious conditions and in

inflammation. Rabbit neutrophils are often called heterophils in the literature; this is due to the staining characteristics of the cytoplasmic granules, and is not due to a difference in function. In other species, a neutrophilia occurs in response to inflammation, especially bacterial infection. An increase in the number of circulating neutrophils causes a rise in total white blood cell count. This response is not marked in rabbits. However, a change in the distribution of white cells can occur in response to infection with a relative neutrophilia and lymphopenia but no alteration in total white cell count (Toth and Krueger, 1989). A mature neutrophilia accompanied by an increase in plasma cortisol can also be associated with stress (Toth and January, 1990).

2.2.4.5 Lymphocytes

Lymphocytes are involved in immunological responses and are distributed throughout the body in various tissues, including blood, bone marrow, lymph nodes, spleen and gut-associated lymphoid tissue. The number of lymphocytes in the blood reflects a balance between cells leaving and entering the circulation and does not necessarily reflect a change in lymphopoiesis. Increased cortisol levels cause a lymphopenia and increased adrenaline levels cause lymphocytosis (Toft *et al.*, 1992a,b).

In rabbits, lymphopenia is a feature of a variety of clinical diseases, including vestibular disease, gut impaction and abscessation (Hinton *et al.*, 1982). Marked lymphopenia has been reported as a feature of differential white cell counts of pet rabbits, especially those suffering from dental disease (Harcourt-Brown and Baker, 2001).

Lymphoma is a relatively common tumour of rabbits and atypical lymphocytes can be found in the peripheral blood of some of these patients. These lymphocytes contribute to an elevated total white cell count in some cases.

2.2.4.6 Eosinophils

The main function of eosinophils is detoxification by either inactivation of histamine or histamine-like

toxic materials. Eosinophils are important in the allergic response and are capable of phagocytosis (Kerr, 1989). Chronic eosinophilia can be seen in diseases of tissues that contain large numbers of mast cells such as the skin, lungs, gastrointestinal tract and uterus. Eosinophilia can be associated with parasitism, especially when parasites are migrating through tissue. Mild eosinophilia has been associated with experimentally induced chronic ascarid parasitism in rabbits (Gupta and Trivedi, 1981). However, heavy worm burdens are rare in pet rabbits. Encephalitozoonosis does not appear to cause an eosinophilia. Slight to moderate elevations in eosinophil counts can be observed after traumatic wound repair in rabbits (Fudge, 2000).

Although eosinopenia can be a significant finding in other species, low eosinophil counts or a zero count are not unusual in rabbits.

2.2.4.7 Basophils

Basophils are similar to neutrophils but have dark blue cytoplasmic granules. Although basophils are rare in blood films from species such as the dog, they may be seen commonly in rabbit blood (Kerr, 1989). Basophil counts as high as 30% have been reported in clinically normal animals (Benson and Paul-Murphy, 1999).

2.2.4.8 Monocytes

In other species, monocytosis is associated with chronic disease, particularly chronic inflammatory conditions. In rabbits, increased monocyte counts can be associated with chronic bacterial infection. Hinton *et al.* (1982) noted increased monocyte counts in rabbits with subcutaneous abscesses, mastitis and 'labyrinthitis'. However, monocyte counts within the laboratory reference do not signify the absence of chronic infection. Rabbits with chronic osteomyelitis due to dental disease can have monocyte counts within the laboratory reference range (Harcourt-Brown, unpublished data).

Key Points 2.2 Clinical haematology

- Rabbit erythrocytes vary in diameter and anisocytosis can be a normal finding in rabbit blood films.
- Polychromasia and small numbers of reticulocytes and nucleated red cells may be seen on normal blood films.
- Rabbit neutrophils have a granular cytoplasm and may be mistaken for eosinophils.
- There are several different terms for the rabbit neutrophil. Some authors use terms such as heterophil, pseudoeosinophil, acidophil or amphophil instead of neutrophil, based on the cytoplasmic granules
- Basophils are frequently found on blood films from rabbits.
- Low blood cellularity, i.e., anaemia and lymphopenia, is a non-specific feature of disease in rabbits.
- High numbers of nucleated red blood cells may be associated with infectious disease.
- The neutrophil:lymphocyte ratio should be approximately 1:1 in adult rabbits. Alterations in the ratio can be associated with stress or disease.
- Adrenaline causes a shift of lymphocytes from the spleen and bone marrow to blood. Cortisol causes a shift of lymphocytes away from the bloodstream to the spleen and lymphatic tissue.
- An increase in total white cell count is unusual in rabbits even in the presence of infection.
- A neutrophilia with a left shift occurs in response to infection.
- Monocytosis can be seen in association with chronic infection.

2.3 Biochemistry

A reference range and discussion of relevance of abnormal results for biochemical parameters is given in Table 2.3.

2.3.1 Glucose

Herbivores, such as rabbits, differ from carnivores in their carbohydrate metabolism. Carnivores eat

Table 2.3 Biochemistry

| Ref. source ^a | Parameter | Reference range | Increase | Decrease |
|--------------------------|----------------------|--|--|--|
| b | Albumin | 27–50 g/L | Dehydration | Starvation Parasitism Reduced liver function |
| b | Alkaline phosphatase | 10–70 IU/L | Young animals Wide distribution so not tissue specific Biliary obstruction | Unknown |
| b | ALT | 25–65 IU/L | Low activity; cf. other species also not very tissue specific so less strongly correlated with degree of liver cell damage Elevations seen in hepatic lipidosis and hepatic coccidiosis | Unknown |
| b | AST | 10–98 IU/L | Widely distributed so poorly tissue specific Indicates tissue cell damage Muscle damage and hepatocellular disease can cause elevations | Unknown |
| b | Amylase | 200–500 U/L | Pancreatic disease Renal disease Also found in caecotrophs | Unknown |
| b | Bilirubin | 3.4–8.5 µmol/L | Biliary obstruction due to hepatic coccidiosis or neoplasia, VHD | Unknown |
| g | Bile acids | < 40 µmol/L | Reduced liver function | Unknown |
| a f | Blood lead | 3.7–5.3 µg/100 mL (dL) 2–27 µg/dL (0.002–0.027 mg/dL) | Lead intoxication | |
| c | Calcium (total) | 3.2–3.7 mmol/L (anecdotal range of 3–4.2 mmol/L for pet rabbits on a varied diet) | May be physiological or diet-related. Paraneoplastic syndrome Chronic renal disease | Dietary restriction |

Table 2.3 Biochemistry—cont'd

| Ref. source | Parameter | Reference range | Increase | Decrease |
|-------------|---------------------|---|--|---|
| h | Calcium (ionized) | 1.71 (+ 0.11) mmol/L | | |
| b | Cholesterol | 0.3–3.00 mmol/L | Hepatopathy, impaired biliary excretion Pregnancy Experimentally induced renal failure | Impaired liver function |
| b | Creatinine | 44.2–229 µmol/L | Renal disease | |
| b | Triglycerides | 1.4–1.76 mmol/L | Post feeding Fatty diet | |
| d | Gamma GT | 0–7.0 IU/L | Hepatobiliary disease Renal disease (increased in urine also) | |
| b | Globulin | 15–27 g/L | Acute or chronic inflammation or immune stimulation, immune-mediated disease, myeloproliferative disease | ?depressed immune function |
| b | Glucose | 4.2–7.8 mmol/L | Stress Terminal stages of gut stasis ?Diabetes mellitus | Anorexia Starvation Problems with carbohydrate absorption |
| b | Inorganic phosphate | 1.28–1.92 mmol/L | Renal disease | Renal disease caused by <i>E. cuniculi</i> |
| b j | Potassium | 3.5–7 mmol/L 3.2 + 0.1 mmol/L (NB. Values can be affected by anaesthesia) | Renal disease Haemolysis | Floppy rabbit syndrome |
| b | Sodium | 138–150 mmol/L | Water deprivation Loss of low sodium fluids | Chronic renal failure |

Continued

Table 2.3 Biochemistry—cont'd

| Ref. source | Parameter | Reference range | Increase | Decrease |
|-------------|---------------------------------------|--|--|---|
| a | T ₄ | 6.4–8.3 µg/100 mL (dL) or 82.37–106.82 nmol/L | | |
| b | Total protein | 54–75 g/L | Dehydration Elevated globulin levels (see above) | Starvation Reduced liver function |
| e | Urea | 6.14–8.38 mmol/L | Dehydration Renal disease | Depressed liver function |
| b | Vitamin A (plasma) | 30–80 µg/ml ± < 10 µg/mL indicates deficiency (liver levels of < 10 µg/g liver denote deficiency) | | |
| b | Vitamin E (plasma κ-tocopherol) | > 1 µg/ml (< 0.5 µg/ mL indicates deficiency) | | |

NB: A fasting sample cannot be guaranteed by withholding food from rabbits as they ingest caecotrophs.

^aReference sources: a, Jones (1975); b, Gillett (1994); c, Goad et al. (1989); d, Okerman (1994); e, Collins (1988); f, Swartout and Gerken (1987); g, Kerr (1989); h, Warren et al. (1989); j, Robson et al. (1981).

periodically and have sudden large intakes of nutrients that must be stored for utilization during the fast between meals. Herbivores graze for long periods of the day and are continually absorbing nutrients from the digestive tract. In rabbits, volatile fatty acids are produced from bacterial fermentation in the caecum and are continually absorbed as an energy source. A fasting sample is difficult to obtain from a rabbit. Withholding food does not prevent caecotrophy and the digestion of caecotrophs provides a source of glucose. Blood samples taken after 96 h of food deprivation may show no alteration in blood glucose levels (Kozma et al., 1974).

Hyperglycaemia is a relatively common finding in rabbits and can be accompanied by glycosuria. Handling alone can cause an increase in blood glucose to the order of 8.5 mmol/L experimentally (Knudtzon, 1988) and 15 mmol/L or more anecdotally. Diabetes mellitus has not been described in pet rabbits and

there is some difference of opinion about its importance as a clinical disease (Hoefer, 2000; Jenkins, 2000; Rosenthal, 2000). Herbivorous animals withstand the absence of insulin more readily than carnivorous ones (Bentley, 1998) and are therefore not so susceptible to diabetes mellitus. Diabetes mellitus has been induced in laboratory rabbits by the administration of alloxan. It has also been described as a hereditary disease in rabbits. A laboratory strain was selectively bred as an animal model of human diabetes mellitus (Roth and Conaway, 1982). Affected animals were polydipsic, polyuric and polyphagic with severely impaired insulin release. Elevated glycosylated haemoglobin values of 12.2% were observed in the overtly diabetic animals in comparison with 3.9% in normal animals. Increased glycosylated haemoglobin levels did not correlate with plasma glucose concentrations (Cannon and Conaway, 1981). Histologically there was hypergranulation of β-cells

of the islets of Langerhans. Obesity and ketoacidosis were not features of diabetes mellitus in the laboratory rabbits. The hyperglycaemia was in the region of 540–590 mg/dL (30–33.4 mmol/L) and there was marked glycosuria. Roth and Conaway (1982) described the maintenance of one diabetic individual on insulin at a dose of up to 8 units per day for 3 years. Ketonuria was not observed.

In pet rabbits, a diagnosis of diabetes mellitus cannot be made on a single blood sample and requires serial blood and urine sampling to confirm the diagnosis, as well as an evaluation of fructosamine levels (performed using methodology validated for rabbits). In view of the physiological factors that can increase blood glucose levels it is advisable to take repeat blood samples from hyperglycaemic rabbits with time of day, phase of digestion, anaesthesia, influence of handling or car journeys in mind. Mild glycosuria is not a significant finding.

Hyperglycaemia can be seen in the terminal stages of gut stasis and is a poor prognostic sign (Harcourt-Brown, 2011). It is associated with fatty degeneration of the liver at post-mortem examination. Marked hyperglycaemia is also seen in association with painful conditions such as acute intestinal obstruction. Blood glucose levels can rise to 20–25 mmol/L and return to normal once the condition is resolved (Harcourt-Brown, 2011). Experimental haemorrhagic or traumatic shock results in hyperglycaemia proportional to the severity of the condition. Hyperthermia also results in hyperglycaemia (McLaughlin and Fish, 1994). Diseases that elevate serum glucose levels in other species, such as hyperadrenocorticism or acute pancreatitis, have not been reported in pet rabbits, although they could occur.

Hypoglycaemia is a significant finding in rabbits and is associated with anorexia, starvation or disturbances in the digestion and absorption of carbohydrates. It can be a sign of hepatic dysfunction. A drop in blood glucose leads to mobilization of free fatty acids from adipose tissue and contributes to the development of ketoacidosis and fatty degeneration of the liver. Measurement of serum glucose is of value in moribund rabbits as a basis for the selection of appropriate fluid therapy. Septicaemia is another potential differential diagnosis for

hypoglycaemia. Other causes of hypoglycaemia such as Addison's disease or insulinomas have not been reported in pet rabbits although such conditions could occur.

2.3.2 Total protein

Interpretation of total protein concentrations is similar to other mammals. Artefactual increases in protein concentrations can result from excessive venous stasis during blood collection (for example, when it takes a long time to get a blood sample). Fluid and small molecules leave the plasma, resulting in a relative increase in proteins. This situation can occur in rabbits, especially in miniature breeds with small veins.

An increase in total protein indicates dehydration and chronic and immune-mediated disease. In rabbits dehydration due to water deprivation or gastrointestinal disturbances commonly occur. Examination of the haematocrit and albumin and globulin fractions can assist differential diagnosis.

Liver disease, chronic enteropathy, starvation or malnutrition may result in reduced protein levels. Glomerulonephropathy or protein-losing enteropathy are uncommon conditions that could cause low total protein levels in rabbits. A decrease in both albumin and globulin may be associated with haemorrhage or exudative skin lesions such as fly strike.

2.3.3 Albumin

The liver is the sole site of albumin synthesis and hypoalbuminaemia is a feature of advanced liver disease in all species. In rabbits, heavy parasitism can be a cause of liver disease. *Eimeria stiedae* causes hepatic coccidiosis (see Section 8.10.1.2). *Cysticercus pisiformis*, the larval stage of *Taenia pisiformis*, migrates through the liver and results in the development of fibrous tracks and necrotic foci (see Section 14.3.2). Severe infestations can result in low albumin levels. Non-hepatic causes of low serum albumin include glomerulonephropathy, protein-losing enteropathy, malabsorption and cardiogenic ascites.

Laboratory reference ranges for serum albumin levels in rabbits can be wide and vary between sources. Sex differences have been reported in laboratory rabbits. One study showed that female New Zealand white rabbits had higher serum albumin levels than males, although other studies have found no sex differences (Kozma *et al.*, 1974).

In rabbits, hypoalbuminaemia is most likely to be associated with nutritional factors such as abnormal caecotrophy, incorrect diet, starvation or malnutrition associated with dental disease. Primary or secondary hepatic neoplasia occasionally occurs in pet rabbits. Hepatic coccidiosis is a cause of low serum albumin levels, especially in young rabbits that have been kept in colonies.

A high serum albumin concentration is not a feature of any specific disease, although an increased albumin level in conjunction with a raised PCV is indicative of dehydration. In a study by [Harcourt-Brown and Baker \(2001\)](#), pet rabbits kept under free-range conditions had significantly higher serum albumin concentrations than rabbits suffering from advanced dental disease. They were also significantly higher than rabbits kept in hutches that were not suffering from dental problems. The difference in albumin levels was attributed to differences in diet and husbandry. Caecotrophs are a source of amino acids for rabbits and normal caecotrophy is an important element of their protein metabolism. Low fibre diets, obesity, dental disease or skeletal abnormalities can prevent rabbits ingesting caecotrophs from the anus and reduce the available amino acids for protein synthesis. Rabbits kept in hutches are more likely to be eating a low fibre diet and to suffer from obesity and skeletal problems than rabbits living outside with unrestricted access to natural vegetation and exercise.

2.3.4 Globulin

Plasma globulins are made up of a range of proteins including carrier proteins and immunoglobulins or antibodies. The types of globulin can be separated into five fractions by electrophoresis. The γ -globulin fraction is almost entirely composed of

immunoglobulins. Some globulins can be synthesized in the liver but immunoglobulins are synthesized exclusively in lymphoid tissue. Acute inflammation, chronic disease or immune-mediated disease can cause an increase in globulin levels. Myeloproliferative disease results in abnormal levels of immunoglobulin production.

There are few published data on the significance of globulin concentrations in rabbits. Lipaemia can artefactually elevate protein levels with some analytical methods. Experimental infections with rabbit coronavirus result in hypergammaglobulinaemia. Analogies have been made between coronavirus infection in rabbits and feline infectious peritonitis in cats ([DiGiacomo and Mare, 1994](#)). Coronavirus occurs in laboratory rabbits but is an unlikely diagnosis in the pet rabbit. In cases where an elevated globulin level is found, more information can be gleaned from performing serum protein electrophoresis. This will determine whether there is a monoclonal gammopathy (often associated with neoplastic processes in other species) or polyclonal changes. These can help in defining the type of inflammatory changes present and can give supportive information for a presumptive diagnosis. For example, an increase in the gamma globulin range, in association with a positive IgG and IgM titre is strong evidence for active encephalitozoonosis (Varga, unpublished data).

2.3.5 Cholesterol and triglycerides

Cholesterol is synthesized in the liver or absorbed from the diet. It is a metabolic precursor of steroid hormones. Cholesterol is broken down in the liver and excreted in bile. In other species, elevated cholesterol levels are indicative of a variety of metabolic disorders such as hypothyroidism, hepatopathy, diabetes mellitus and hyperadrenocorticism. Low levels can occur in association with impaired hepatic function. Changes in serum triglyceride levels reflect a similar range of diseases. Blood levels of triglycerides increase after a meal, especially if it is a fatty meal.

In rabbits, there are some physiological factors that affect cholesterol levels. Male rabbits have lower cholesterol levels than females and there is a diurnal

variation, with higher levels occurring during the late afternoon (Loeb and Quimby, 1989). Large variations in blood cholesterol and triglyceride values can occur between individual rabbits (Yu *et al.*, 1979). A fasting blood sample is required for cholesterol and triglyceride assay. In rabbits, it is difficult to obtain a fasting sample because of caecotrophy. Abnormal cholesterol or triglyceride levels are most likely to be associated with dietary factors or hepatic impairment. In anorexic rabbits, especially obese ones, a lipaemic sample is a poor prognostic indicator as it signifies impaired fat metabolism and the presence of hepatic lipidosis (see Section 8.3). A rise in triglyceride levels has been found in association with experimentally induced chronic renal failure in rabbits (Tvedegaard, 1987).

2.3.6 Amylase

In other species, amylase is found in the pancreas and to a lesser extent in the salivary glands, liver and small intestinal mucosa. Amylase has a short half-life and is rapidly removed from the circulation. It is excreted by the kidney. Elevated levels indicate pancreatic disease or renal insufficiency.

In rabbits, amylase is present in pancreatic tissue in high concentrations. Low concentrations are found in the salivary glands and none is produced by the liver (Jenkins, 2000). Amylase is also produced by caecal micro-organisms and is present in caecotrophs, aiding conversion of glucose to lactic acid during digestion in the stomach and small intestine. Serum amylase levels are lower in rabbits than in other species (McLaughlin and Fish, 1994). Pancreatic duct obstruction or pancreatic disease can result in a rise in blood amylase values. Rabbits can survive experimental ligation of the pancreatic duct (Brewer and Cruise, 1994).

2.3.7 Bilirubin

The rabbit secretes a large amount of bile, approximately seven times as much as a dog on a weight basis (Brewer and Cruise, 1994). The rabbit also differs from other species in the excretion of breakdown

products of haemoglobin. The rabbit has low biliverdin reductase activity (Fekete, 1989) and only 30% of biliverdin is converted to bilirubin. Bilirubin values can be affected by fasting. Glucose administration to rabbits lowers serum bilirubin concentrations by modifying hepatic conjugation and increasing biliary secretion (McLaughlin and Fish, 1994).

In rabbits, biliary obstruction results in jaundice and raised serum bilirubin values. In young rabbits, hepatic coccidiosis is the most usual cause of jaundice. In older rabbits, bile duct obstruction from neoplasia is more likely. Aflatoxicosis from the ingestion of mouldy feed can result in hepatic fibrosis and jaundice (Krishna *et al.*, 1991). Viral haemorrhagic disease (VHD) causes acute hepatic necrosis with elevated bilirubin concentrations in association with dramatic increases in AST and ALT concentrations. VHD is invariably fatal, although some rabbits may survive long enough to develop jaundice before they die.

There is little information on haemolytic disease as a cause of jaundice in pet rabbits. Haemolytic anaemia has been reported in association with lymphosarcoma in laboratory rabbits (Weisbroth, 1994).

2.3.8 Alanine aminotransferase (ALT)

In other species, ALT is used as an indicator of hepatocellular damage, especially in dogs and cats. ALT is also found in other tissues such as muscle and red blood cells. An increase in ALT signifies cell damage, although the degree of the increase does not correlate with the severity of hepatic disease and is not a prognostic indicator (Willard *et al.*, 1999).

In rabbits, liver ALT activity is lower than in other species and there is less organ specificity (Rosenthal, 1997). ALT is also present in cardiac muscle. The half-life of ALT in the rabbit is approximately 5 h. In the dog the half-life is 45–60 h (Jenkins, 2000). The lack of specificity and the short half-life limit its diagnostic usefulness in rabbits.

Hepatic coccidiosis due to *E. stiedae* is a cause of increased blood ALT concentrations, especially in conjunction with an increase in alkaline phosphatase, bilirubin and γ -glutamyl transferase (GGT). Elevated ALT values have been found in asymptomatic

house rabbits and have been attributed to the effects of organic solvents in wood shavings used as litter material. Other liver diseases such as neoplasia can cause a rise in ALT but sometimes not until the condition is advanced (McLaughlin and Fish, 1994). Low doses of aflatoxin caused a significant rise in ALT concentrations in a group of laboratory rabbits (Fekete and Huszenicza, 1993). Hepatic lipidosis will elevate ALT levels.

2.3.9 Aspartate aminotransferase (AST)

In other species AST is widely distributed throughout the body. In particular, it is found in skeletal muscle, cardiac muscle, liver and erythrocytes. Like ALT, AST is an indicator of tissue damage. It is sometimes used as an indicator of liver disease, especially in horses in which ALT is not liver specific.

In rabbits, AST is found in liver, heart, skeletal muscle, kidney and pancreas, with the highest activity in the liver and skeletal muscle (Benson and Paul-Murphy, 1999). Physical exertion or tissue damage during blood collection can elevate results. Raised AST levels can be found in association with liver disease. Raised AST in combination with normal CK levels is good evidence to suggest the AST is not of muscular origin.

2.3.10 γ -Glutamyl transferase (GGT)

GGT is found in liver and kidney tissue. In other species, GGT is used as an indicator of hepatobiliary disease especially in horses and ruminants where it is associated with long-term liver damage. Although GGT is found in high concentrations in renal tubular cells, kidney disease does not lead to elevated blood levels, probably because the enzyme is excreted in the urine (Bush, 1991).

In the rabbit GGT is located predominantly in the renal epithelium, with low activity in the liver. Liver GGT is present primarily in bile duct epithelial cells and is therefore an indicator of hepatobiliary disease rather than hepatocellular damage (McLaughlin and Fish, 1994). In cases where there is renal tissue damage, urine GGT may be increased in addition to plasma concentrations.

2.3.11 Alkaline phosphatase (AP)

Alkaline phosphatase consists of a group of several isoenzymes that hydrolyse phosphates at an alkaline pH (Kerr, 1989). It is one of the most widely distributed enzymes in the body. Alkaline phosphatase is found particularly in bone, liver and intestinal wall. Different isoenzymes are produced from each site. Increases in plasma activity are usually due to the isoenzymes derived from liver and bone. Higher concentrations are found in young animals with high osteoblastic activity.

In rabbits, alkaline phosphatase is present in nearly all tissues. It is found in association with cell membranes and especially in intestinal epithelium, renal tubules, osteoblasts, liver and placenta. The rabbit has three AP isoenzymes – the intestinal form as well as two isoenzymes present in both liver and kidney (McLaughlin and Fish, 1994). There is a wide variation between laboratory reference ranges for AP values for rabbits. Examples include: 4.1–16.2 IU/L (Collins, 1988); 10–70 IU/L (Gillett, 1994); 112–350 IU/L (Harkness and Wagner, 1995). Different analytical techniques could account for these variations.

In a survey of blood parameters relating to calcium metabolism in pet rabbits, serum alkaline phosphatase values varied widely even in apparently healthy individuals (Harcourt-Brown and Baker, 2001). Increased levels of alkaline phosphatase are seen in biliary obstruction, e.g., neoplasia or hepatic coccidiosis. Experimental ligation of the common bile duct results in increased levels of alkaline phosphatase up to 600 IU/L (McLaughlin and Fish, 1994). Enteric disease can also elevate alkaline phosphatase values (Jenkins, 2000).

2.3.12 Bile acids

Bile acids are derived from cholesterol and are secreted into the intestine to aid fat digestion. From the gut, they are reabsorbed into the circulation and transported to the liver to be re-secreted in the bile. Impaired hepatic function results in increased concentrations of bile acids in peripheral blood. There are physiological variations in circulatory bile acid

concentrations in association with the digestion of food and stimulation of the gall bladder to release bile into the small intestine. In most species, a fasting sample should have a low concentration of bile acids of less than 15 $\mu\text{mol/L}$ (Kerr, 1989). Impaired hepatic function can result in marked rises in fasting serum bile acid concentrations.

In rabbits, the production of bile acids shows a circadian rhythm (Fekete, 1989). There is a problem in obtaining a fasting sample from rabbits due to the ingestion of caecotrophs. Bile acids are not included in published reference ranges for rabbits at the present time. Bile acid levels in excess of 100 $\mu\text{mol/L}$ have been found in association with hepatic coccidiosis in comparison with levels that are generally less than 40 $\mu\text{mol/L}$ (Harcourt-Brown, unpublished data).

2.3.13 Urea

Urea is a nitrogenous waste product formed in the liver as the end product of deamination of amino acids. It is transported in the blood to the kidney where it is excreted in the urine. In other species, high blood urea concentrations are indicative of impaired renal function that may be due to renal disease or poor perfusion due to circulatory disorders or cardiac disease. Low blood urea levels can reflect hepatic dysfunction.

In rabbits, many physiological factors influence the concentration of urea in the blood. Dietary protein concentrations and quality, withholding food and natural diurnal rhythms can all affect blood urea concentrations. Higher levels occur in the late evening (Loeb and Quimby, 1989). The rabbit's urea metabolism is further complicated by urea utilization by caecal microflora during catabolism or during periods of dietary excess. Therefore, small fluctuations in serum urea concentrations are difficult to interpret. Laboratory reference ranges apply to animals that are fed a standard diet and have usually been fed at a specific time of day. Pet rabbits are subject to greater fluctuations in blood urea values due to the variation in diet and other factors, and can have values slightly higher than laboratory reference ranges. Prerenal azotaemia associated with

poor renal perfusion occurs during periods of dehydration. The rabbit has a limited capacity to concentrate urea and a greater volume of urine is required when urea load increases (Brewer and Cruise, 1994). Increased blood urea values were recorded in a study by Licois *et al.* (1978) of young rabbits with diarrhoea experimentally induced with coccidiosis. The authors suggested that the blood urea values rose as a result of intense nitrogen catabolism during weight loss associated with the disease. Water deprivation can lead to blood urea values as high as 40 mmol/L in association with creatinine values in excess of 200 $\mu\text{mol/L}$ (Harcourt-Brown, unpublished data). Water deprivation can be due to a lack of available drinking water, caused either by an oversight by the owner or by a faulty mechanism on the drinking bottle.

In rabbits, dehydration can cause urea and creatinine values that would signify renal disease in the dog and cat. High levels usually return to normal once the animal is rehydrated. Therefore, urea and creatinine values should be rechecked before making an absolute diagnosis of renal failure. As in other species, elevated blood urea values in rabbits are associated with renal insufficiency. Abdominal radiography is indicated in rabbits with raised urea and creatinine levels because nephrolithiasis is a potential cause of kidney disease in the rabbit (see Section 12.5). *Encephalitozoon cuniculi* can cause low-grade kidney disease in rabbits with mild elevations in blood urea. Most cases are subclinical. *Encephalitozoon cuniculi* infection causes granulomatous lesions in the kidneys that become pitted and scarred with fibrotic areas. The parasite has been associated with chronic renal failure, with blood urea values in the region of 152.7 mg/dL (25.45 mmol/L) and creatinine of 5.8 mg/dL (512.72 $\mu\text{mol/L}$) in a study by Ewingmann and Göbel (1999). Affected rabbits were anaemic, with low haemoglobin and red cell counts, and had elevated serum potassium concentrations. Neoplasia, interstitial nephritis and nephrotoxicity also occur in rabbits and cause renal disease.

Low blood urea values in association with impaired hepatic function and the use of anabolic steroids have been described (Benson and Paul-Murphy, 1999).

2.3.14 Creatinine

Creatinine is a nitrogenous waste product that, like urea, is transported in the blood to the kidney where it is excreted in the urine. Creatinine is not the product of amino acid breakdown but of creatine, which is a substance present in the muscle and is involved in high energy metabolism (Kerr, 1989). The slow catabolism of creatine results in a slow inflow of creatinine to the plasma at a rate that is directly proportional to the individual's muscle mass but is unaffected by any change in muscular activity or muscle damage. Any changes in blood creatinine concentrations are due to changes in excretion and are a reflection of renal function. Concentrations rise quickly at the outset of renal disease and decrease when an improvement of renal function takes place. Creatinine deteriorates in plasma, and readings from old samples (>24 h) cannot be relied upon. There is interference from a variety of other substances such as bilirubin (which decreases creatinine) or cephalosporins (which increase creatinine).

Key Points 2.3 Hepatic and renal parameters

- Stress of handling can cause a marked elevation of blood glucose levels in rabbits. Levels as high as 15 mmol/L can occur in association with handling. Higher levels (> 20 mmol/L) may be seen in association with stressful or painful diseases such as intestinal obstruction.
- There is debate about the occurrence of diabetes mellitus in domestic rabbits. It has been induced in laboratory rabbits and a genetically susceptible laboratory strain has been bred.
- Large individual variations in blood cholesterol and triglyceride values can occur in rabbits.
- Jaundice is unusual in rabbits but may be seen in association with cholestasis in diseases such as hepatic coccidiosis or neoplasia.
- There is a wide variation between laboratory reference ranges for alkaline phosphatase values.
- Blood urea and creatinine values can be high in cases of prerenal azotaemia in rabbits and do not always signify renal failure. Causes include dehydration or water deprivation.

2.3.15 Electrolytes

The rabbit's complex digestive physiology and the compromised renal capability of correcting acid–base disorders make the rabbit a prime candidate for electrolyte imbalances (see Sections 1.3.10 and 1.3.11). Dietary deficiency of electrolytes such as sodium and potassium is unlikely in the herbivorous diet of rabbits. Instead, electrolyte problems are more likely to be associated with abnormal losses. Although rabbits do not vomit, water and electrolyte absorption and secretion are affected by gastrointestinal disease. If facilities are available, electrolyte assays, especially potassium, can be a valuable part of the diagnostic work-up for critically ill rabbits.

2.3.15.1 Sodium

In general, changes in sodium concentrations reflect the osmolality of extracellular fluid rather than the total body sodium content. Increased blood sodium concentrations (hypernatraemia) can be the result of water deprivation or the loss of low sodium fluids. Failures in food formulation quality control can lead to incorrect dietary levels and hypernatraemia. Decreased sodium concentrations (hyponatraemia) may occur as a result of chronic renal failure when the kidney cannot concentrate urine and fast urine flow through the renal tubules prevents effective sodium/potassium exchange. Lipaemia or hyperproteinaemia can artefactually reduce sodium concentrations if certain laboratory methods are used.

At the present time, there are few data available on clinical conditions that affect sodium concentrations in rabbits.

2.3.15.2 Potassium

About 95% of the total body potassium is intracellular, so measurement of extracellular potassium in blood samples does not give a true reflection of the potassium status of the patient. The balance between intracellular and extracellular potassium is regulated by aldosterone, insulin and catecholamines and is affected by blood pH. Aldosterone stimulates renal excretion of potassium. Insulin promotes the

movement of potassium into cells. The effects of these hormones prevent large diet-induced changes in plasma potassium concentrations. Potassium is an important ion in the maintenance of membrane potential. Abnormally high or low potassium concentrations can have life-threatening consequences due to impaired electrical activity of cells. High blood potassium concentrations can result in cardiac arrest.

Alterations in blood potassium levels can be due to alterations in dietary intake and excretion, or redistribution across cell membranes. To maintain electroneutrality, potassium ions shift from intracellular to extracellular fluid in exchange for hydrogen ions. In other species, hypoadrenocorticism (Addison's disease) reduces the exchange of sodium and potassium ions across the cell membrane and results in increased serum potassium and decreased serum sodium concentrations. Hyperkalaemia can be the result of impaired renal excretion of potassium due to kidney disease or from tissue trauma such as crushing injuries that release large amounts of potassium into the circulation. Acidosis causes a redistribution of potassium across the cell membrane to maintain electroneutrality. This leads to more potassium in the extracellular fluid compared with body stores and therefore hyperkalaemia. Artefactually high levels of potassium can result from leakage from red cells in haemolysed samples or those that have not been separated until several hours after the blood was taken.

Low blood potassium can cause muscular weakness and depression. Hypokalaemia can be the result of dietary potassium deficiency or as a result of potassium loss from the gastrointestinal tract. Diuresis or the use of potassium-free intravenous fluids also causes hypokalaemia. Alkalosis can cause redistribution of potassium and sodium across the cell membrane and result in hypokalaemia. Artefactually low potassium concentrations are uncommon although they can occur secondarily to hyperlipidaemia or hyperproteinaemia (Willard *et al.*, 1999). Blood collection through a catheter that contains residual potassium-free fluids can lead to erroneously low results.

In rabbits, the effect of blood collection methods on plasma potassium levels has been investigated.

Discrepancies in results were found between blood collected from the ear and from the carotid artery when the blood was collected with a plastic catheter but not with a 21-gauge needle (Robson *et al.*, 1981). General anaesthesia with pentobarbitone depressed plasma potassium values but sedation with chlorpromazine did not affect results. Serum potassium concentrations were found to be higher than plasma and in venous rather than arterial blood (Robson *et al.*, 1981).

Low serum potassium values have been found in unanaesthetized rabbits in conjunction with signs of muscular weakness (Harcourt-Brown, unpublished data). Affected animals can still eat and drink but are unable to move. It is not known whether hypokalaemia is the cause of the muscular weakness. Possible causes of hypokalaemia are discussed in Section 10.6.1.1. Further investigations of serum potassium concentrations of rabbits are required to know the clinical significance of measured values and the influence of various physiological states. In horses, blood potassium concentrations can fall to 2.0 mmol/L after prolonged exercise due to potassium loss in sweat and to 2.5 mmol/L while eating hay due to potassium loss in saliva. During moderate exercise, concentrations can rise to 4.0 mmol/L due to potassium release from muscle cells (Kerr, 1989.) Similar physiological variations could occur in the rabbit.

2.3.15.3 Calcium

Calcium is an essential element that is involved in many body systems. Most of the body's calcium is stored in bone in conjunction with phosphate. Calcium is an essential part of the structure of bones and teeth. It is an important cation in intracellular and extracellular fluid where it is required for muscle metabolism, enzyme activation, blood coagulation and osmoregulation. Calcium is found in the blood in three forms: ionized, bound to other anions (especially phosphate) and bound to protein (especially albumin). Because of the protein-binding capacity of calcium, total serum calcium concentrations are proportional to albumin concentrations. Ionized

calcium is the physiologically active component of blood and is involved in the permeability of cell membranes. Hypocalcaemia is a life-threatening condition. In many species, a high demand for calcium during late pregnancy and lactation can result in hypocalcaemic tetany. There are also some metabolic disorders that can result in alterations in serum calcium concentrations in other species. Examples include renal, pancreatic and neoplastic diseases.

The rabbit has a different calcium metabolism from other domestic species (see Section 1.3.12). Dietary calcium is readily absorbed from the intestine and total plasma values reflect dietary intake. Total blood calcium levels are higher and can vary over a wider range than other species. An erroneous diagnosis of hypercalcaemia is often made because of the rabbit's high total serum calcium levels in comparison with that of other animals. Parathyroid hormone (PTH) regulates calcium metabolism in a manner similar to that of other animals, but a reduction in plasma PTH level occurs at a plasma calcium concentration higher than that in other species (Warren *et al.*, 1989). The kidney plays an important role in calcium regulation and has a high fractional excretion for calcium when blood levels are high. Calcium is excreted in the urine, in which it forms calcium carbonate precipitate. Some authors have suggested monitoring blood calcium concentrations as part of the protocol for treating 'sludgy urine' (see Figure 12.5). However, high blood calcium levels are not the sole cause of urinary tract disease in rabbits (see Section 12.4.1).

There are differences between published reference ranges for total serum calcium values in rabbits. Variations in dietary calcium intake could account for some of the discrepancies. The peak blood level obtained by increasing dietary calcium intake in laboratory rabbits was 5.42 mmol/L (21.7 mg/dL) in a study by Chapin and Smith (1967a). Experimental calcium restriction resulted in minimum serum concentrations of 3.22–3.5 mmol/L (13–15 mg/dL) before a rapid decline just before death in a separate study by Chapin and Smith (1967b).

A reference range of 3.2–3.7 mmol/L taken from eight laboratory reference sources has been made

by Goad *et al.* (1989). Values outside this range are encountered in otherwise healthy individuals and a range of 3.0–4.2 mmol/L is acceptable for pet rabbits on a varied diet.

Total blood calcium levels in rabbits are also affected by age and reproductive status. Kamphues *et al.* (1986) found that increased calcium intake only resulted in higher total plasma calcium concentrations in adult rabbits and not young ones of 5–19 weeks. Serum calcium concentrations in growing rabbits are fixed at a value of approximately 3.5 mmol/L (14 mg/dL) (Gilsanz *et al.*, 1991; Kamphues *et al.*, 1986). Blood calcium levels decrease during pregnancy (Assane *et al.*, 1993).

Because of the protein-binding properties of calcium, albumin levels can also affect total calcium concentrations. Albumin concentrations in pet rabbits are variable and appear to be affected by the manner in which they are kept (Harcourt-Brown and Baker, 2001). In dogs and man, total serum calcium values can be adjusted by using a mathematical formula that takes albumin concentration into account. (Adjusted calcium concentration = measured serum total calcium concentration – serum albumin (g/dL) + 3.5.) This formula is unreliable in cats (Flanders *et al.*, 1989) and has not been investigated in rabbits.

At present, most published reference ranges for pet rabbits refer to total serum calcium concentrations. Ideally, ionized calcium should be measured for an accurate assessment of calcium status but special sample handling and equipment that preclude its measurement in most practice situations is required. However, affordable equipment that can be used in the practice laboratory is now becoming available. Measurements of ionized calcium have been made during experimental investigations using rabbits. Warren *et al.* (1989) found a linear relationship between total serum calcium and ionized calcium values. A group of 29 non-pregnant female and male rabbits were found to have ionized serum calcium levels of 1.71 ± 0.11 mmol/L. Kamphues *et al.* (1986) reported ionized calcium values of 6.94 ± 0.21 mg/dL (1.73 ± 0.05 mmol/L) in adult rabbits on an average dietary calcium intake (0.85%).

Hypocalcaemic tetany has been reported in lactating does (Barlet, 1980). Hypercalcaemia is seen in rabbits with chronic renal failure and impaired calcium excretion (see Section 12.5.3.1). In a study by Tvedegaard (1987), experimentally induced chronic renal failure resulted in total serum calcium concentrations of 4.82 ± 0.77 mmol/L. Levels in excess of 4.25 mmol/L have been recorded in association with neoplasia (Voelkel *et al.*, 1978).

2.3.15.4 Phosphate

Inorganic phosphate is involved in many enzyme systems and is important in carbohydrate and muscle metabolism as well as forming a major component of bone. Phosphate is obtained from the diet. Vitamin D and PTH influence intestinal absorption of phosphorus in a manner similar to that of calcium. PTH stimulates renal excretion of phosphate and renal conservation of calcium. The pH of intestinal contents and the presence of cations such as calcium and magnesium can affect the availability of dietary phosphate.

Abnormalities of phosphate metabolism are complex and interdependent with many other factors. Blood phosphate values can be difficult to interpret and need to be examined alongside other parameters. In other species, physiological activities such as feeding and exercise reduce serum phosphorus concentrations (Aitken and Allen, 1994). There are drug interactions that alter serum phosphorus values. Examples include phosphate binders, anaesthetic agents, bicarbonate, parenteral glucose, anabolic steroids, diuretics and tetracyclines (Willard *et al.*, 1999). Phosphorus can shift between the intracellular and extracellular space in response to alterations in acid–base metabolism. In addition to these problems of interpretation, phosphate values are subject to artefactual error caused by poor sample handling. Haemolysis releases phosphate from erythrocytes, which results in elevated values.

In rabbits, there is little information about the clinical relevance of serum phosphate concentrations. Rabbit blood clots easily and good-quality non-haemolysed samples can be difficult to obtain. Hyperphosphataemia can result from impaired renal

phosphorus excretion due to kidney disease. Hypophosphataemia may result from dietary deficiency, impaired intestinal absorption or metabolic disorders.

2.4 Miscellaneous assays

2.4.1 Lead estimation

Blood lead concentrations are given in different units depending on the source. The conversion factor for $\mu\text{g/dL}$ to $\mu\text{mol/L}$ is 0.0483.

A study by Roscoe *et al.* (1975) evaluated three diagnostic tests for lead toxicity in rabbits. Whole blood lead concentrations of greater than 0.03 mg/dL ($1.45 \mu\text{mol/L}$) were considered a reliable indicator of lead ingestion. Measurement of urinary delta-aminolevulinic acid (δALA) was considered unreliable. Erythrocytes from rabbits that were given lead fluoresced red when exposed to light rays of 320–400 nm. The fluorescent erythrocyte test (FET) was considered a convenient and reliable test for lead ingestion.

Swartout and Gerken (1987) described two clinical cases of lead poisoning that had blood levels of 70 $\mu\text{g/dL}$ (0.07 mg/dL) and 40 $\mu\text{g/dL}$ (0.04 mg/dL). They gave a range of 2–27 $\mu\text{g/dL}$ (0.002–0.027 mg/dL) as a normal range for laboratory rabbits.

2.4.2 Parathyroid hormone (PTH)

PTH is released by the parathyroid gland in response to both a fall in blood calcium and low serum $1,25\text{-(OH)}_2\text{D}_3$ levels. It is responsible for the minute-to-minute regulation of calcium, due to its quick, short-duration response. PTH stimulates conversion of (25-OH-D) to the active form of vitamin D ($1,25\text{-(OH)}_2\text{D}_3$), which, in turn, stimulates intestinal absorption of calcium. PTH also stimulates osteoclastic resorption of bone to release calcium, phosphorus and magnesium into the circulation. PTH stimulates renal conservation of calcium but not phosphorus, which results in an increase in blood calcium without an increase in phosphorus concentrations.

In animals with disturbances in calcium metabolism that result in bone demineralization, PTH levels

are high, and PTH can be used to investigate metabolic bone disease that is often nutritional in origin. Dietary calcium deficiency and a failure to absorb calcium are reasons for metabolic bone disease. Failure to absorb calcium can be due to vitamin D deficiency (this is rare in rabbits and only occurs where dietary calcium levels are very low) or unavailability of calcium due to binding with substances such as oxalates, fats or phosphates in the gut.

PTH assays are available in commercial laboratories that specialize in endocrinological investigations. PTH assays have been performed on pet rabbits as part of an investigation of the possibility of metabolic bone disease as a cause of poor tooth and bone quality and the development of dental disease in rabbits (Harcourt-Brown and Baker, 2001). Sample handling is of paramount importance as the hormone is labile and haemolysis interferes with the assay. Samples require separating and freezing immediately after collection and must be shipped in the frozen state to a laboratory. Sufficient, non-haemolysed blood to harvest 1–2 mL of serum or plasma needs to be collected. As PTH is responsible for the minute-to-minute regulation of blood calcium, values can vary over a wide range, making interpretation of a single result difficult. Diet, age, pregnancy, lactation and diurnal rhythms cause physiological variations in results. Warren *et al.* (1989) reported PTH values of 59.6 + 41.2 pg/mL in a group of 29 non-pregnant farm and laboratory rabbits and Harcourt-Brown and Baker (2001) reported values of 40.3 + 10.7 pg/mL in a group of 12 pet rabbits kept outside under free-range conditions all year round. Values as high as 100–200 pg/mL have been recorded in baseline samples from laboratory rabbits (Warren *et al.*, 1989). Values in excess of 230 pg/mL have been found in pet rabbits, one of which was found to have a liver tumour on subsequent post-mortem examination (Harcourt-Brown, unpublished data).

2.4.3 Serology

In the UK, serological tests are available for *E. cuniculi*, *Toxoplasma gondii*, myxomatosis, viral haemorrhagic disease and *Treponema paraluis-cuniculi* as part

of the commercial health screening of laboratory rabbits. Commercial laboratories may accept individual samples from pet rabbits for serological screening. It is advisable to consult a veterinary laboratory in the first instance. In the USA, serology and a PCR test are also available to detect *Pasteurella multocida* infection (Sanchez *et al.*, 2000) but these tests are not available in the UK at the present time (January 2013).

Serological testing for *E. cuniculi* antibodies can be useful in the differential diagnosis of neurological diseases such as vestibular syndrome or paraplegia (Section 10.4) or uveitis (Section 9.7.3.1). It is also indicated in animals with mild renal insufficiency (Section 12.5.1). Serological and histological tests from naturally infected rabbits have demonstrated the presence of antibodies before the organism can be seen in the kidney. Lesions were not seen in the brain until at least 8 weeks after the first detectable antibodies, suggesting that serology is a sensitive procedure for early diagnosis (Cox and Gallichio, 1978). Therefore animals with clinical signs that are seronegative are unlikely to be suffering from encephalitozoonosis, although experimental infections with *E. cuniculi* have shown the presence of granulomas in the brain of animals that had become seronegative (Kunstyr *et al.*, 1986). Conversely,

Key Points 2.4 Electrolytes and serology

- Serum potassium values in rabbits can be depressed by anaesthesia. In unanaesthetized rabbits, low potassium values have been associated with generalized muscular weakness.
- Blood calcium levels in rabbits vary over a wide range and are higher than other domestic species.
- Total blood calcium values are correlated with serum albumin levels and are affected by diet, age and reproductive status.
- Parathyroid hormone (PTH) can be measured. Interpretation of a single result is difficult.
- In the UK, serological tests are available for *Encephalitozoon cuniculi*, *Toxoplasma gondii*, myxomatosis, viral haemorrhagic disease and *Treponema paraluis-cuniculi*.

asymptomatic rabbits can be seropositive. Therefore serology can only be used as a guide in the diagnosis of *E. cuniculi* infection. Antibody titres can be helpful in distinguishing between recent and chronic infection. Simultaneous detection of IgM and IgG suggests recent infection.

2.5 Urine examination

Urinalysis is summarized in Table 2.4. Urine collection is described in Section 1.10.3.3. In common with other herbivorous species, rabbits excrete alkaline urine. Urinary pH is usually 8–8.2. Rabbit urine is normally turbid due to the presence of calcium carbonate that can be seen as sediment in collected samples. The urine from anorexic, pregnant, lactating or young rabbits is often clear. The colour of normal urine can vary from pale yellow, to orange, brown or red, mimicking haematuria. Plant porphyrin pigments are the cause of red-coloured urine and can be distinguished from haematuria with urinalysis dipstick tests or microscopy. Alternatively, a Wood's lamp can be used, as urinary pigments fluoresce when exposed to ultraviolet light (Benson and Paul-Murphy, 1999). In addition to

Table 2.4 Urinalysis

| | |
|------------------|---|
| Specific gravity | 1.003–1.036 NB. Difficult to measure accurately due to mineral deposits. |
| pH | 7.6–8.8. |
| Protein | Small quantities of albumin may be a normal finding, especially in young rabbits. |
| Cells | A small number of leucocytes and erythrocytes are a normal finding in rabbit urine. |
| Crystals | Ammonium magnesium phosphate (struvite) and calcium carbonate deposits are a normal finding in rabbit urine. Oxalate crystals can also be seen. |

the presence of calcium carbonate crystals, oxalate or ammonium magnesium phosphate crystals can also be found in normal rabbit urine. The specific gravity of rabbit urine is difficult to evaluate accurately due to the presence of mineral deposits (Goad *et al.*, 1989) but is approximately 1.003–1.036. Traces of glucose and protein can be present in normal rabbit urine. As in other species, rabbit urine can be spun and the sediment examined microscopically for the presence of crystals, red cells, inflammatory cells and bacteria. Cultures can be taken to confirm bacterial infection and aid antibiotic selection. Examination of urine sediment stained with Gram stain can reveal *E. cuniculi* spores that are oval, strongly Gram-positive with a coiled filament inside (Patton, 2000; Pye and Cox, 1977). Ketones may be detected in the urine of anorexic rabbits and is a poor prognostic sign as it is associated with the development of hepatic lipidosis.

2.6 Faeces examination

Rabbits produce two types of faeces: hard dry pellets composed of compressed indigestible fibre and soft caecotrophs composed of a smooth paste rich in bacteria and other micro-organisms. The first step in faeces examination is to determine which type of faeces has been collected. It is often samples of soft faeces or caecotrophs that are collected for examination because their consistency is abnormally loose or the owner has mistaken uningested caecotrophs for diarrhoea. Microscopically the two types of faeces are completely different. Hard faeces contain indigestible fragments of plant debris and little else. Caecotrophic material contains a wide range of micro-organisms, including large Gram-negative bacilli, *Bacteroides*, large metachromatic staining bacilli and many other bacteria including oval and fusiform rods. In some types of diarrhoea, a mixture of indigestible fragments of plant material can be seen alongside a range of typical caecal micro-organisms. This signifies a failure of the proximal colon to separate the indigestible and digestible fractions of the diet. Coccidial oocysts or eggs of the

non-pathogenic oxyurid *Passalurus ambiguus* can be found in both hard and soft faeces of infected rabbits. Coccidial oocysts can be confused with a non-pathogenic *Saccharomyces*, a budding sporogenous yeast that can be present in large numbers in rabbit faeces (see Figure 2.2).

Clostridium spiroforme is a large Gram-positive, semicircular or spiral-shaped bacterium that may be seen in faecal smears from diarrhoeic rabbits or those that have died from enterotoxaemia. Centrifuging faecal material at 20,000 rpm for 15 minutes and Gram-staining the residue after the supernatant has been removed improves the chance of diagnosis (Langan and O'Rourke Schaeffer, 2000). Although the presence of semicircular bacteria in the faeces or caecal material is suggestive of clostridial enterotoxaemia, it is not a reliable diagnostic criterion. *Clostridium* spp. can be present in the normal caecal flora and proliferate after death. The demonstration of the toxin and anaerobic culture of the organism

is required for positive diagnosis (Carman and Borriello, 1983).

Clostridium piliforme, the causative organism of Tyzzer's disease, is not detected in faeces. A PCR test is available in the USA for detection of this organism. *Escherichia coli* is not a normal inhabitant of the rabbit gut flora, although small numbers may be present in some animals. Enteropathogenic strains can be found in association with diarrhoea in weanling rabbits. Pathogenic *Salmonella* spp. may be isolated, although post-mortem material is usually available in these cases. Infectious enteritis is rare in the individual pet rabbit.

2.7 Laboratory examination of hair

Plucked hair samples may be examined visually for the presence of mites that are just visible with the naked eye. Under good illumination, *Cheyletiella* mites can be seen in scale and skin debris that has been brushed out of the coat. After a few minutes, individual mites can be seen moving into the warmth of the illuminating light. Egg cases and cuticles from developmental stages of the fur mite *Leporacarus gibbus* (formerly known as *Listrophorus gibbus*) may also be seen on visual examination of hair brushings. They remain attached to hair shafts after hatching or moulting and give the fur a characteristic 'salt and pepper appearance' in heavy infestations. Visual evidence of *L. gibbus* is seen more easily on white or light-coloured areas of fur, especially if it is wet. Occasionally lice may be found.

Microscopic examination of acetate strips applied to the skin of alopecic areas can be used to detect *Cheyletiella parasitovorax*. All stages of the life cycle of *C. parasitovorax* can be seen by this method. Skin brushings can also be examined microscopically. Fleas, flea dirt, *C. parasitovorax* and *L. gibbus* can be seen in skin brushings examined under low magnification.

A trichogram is useful in differentiating between conditions that cause alopecia. For this technique a sample of hair is plucked from as close to skin as possible using forceps. The hair is placed on a microscope slide, taking care to ensure the hairs remain

Key Points 2.5 Clinical tips: faecal examination

- Rabbits produce two forms of faeces that differ in composition. These must be differentiated.
- Hard faeces are hard, dry and fibrous, composed mainly of undigestible fibre.
- Soft faeces (caecotrophs) are stronger smelling and pasty, and contain high levels of bacteria.
- In cases of diarrhoea, rabbits can excrete both types of faeces at once, suggesting that the proximal colon is unable to separate them.
- Faeces can be analysed for parasites using routine zinc sulphate flotation techniques.
- Coccidia and *Passalurus ambiguus* ova may be found in either type of faeces.
- A pseudoparasite *Saccharomyces guttulatus* may be seen in large numbers. It can mimic coccidial oocysts.
- Faecal PCR or smears can be helpful in the diagnosis of clostridial enterotoxaemia. *Clostridium spiroforme* can sometimes be identified on Gram-stained faecal smears.

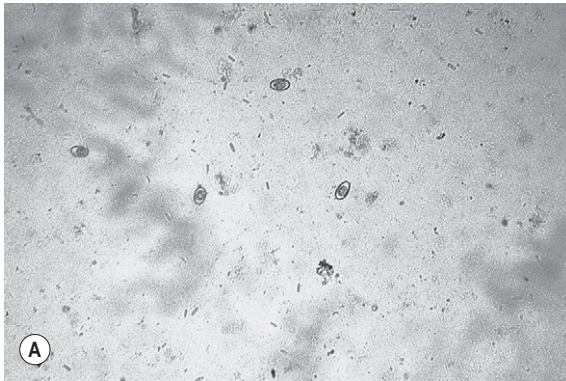


Figure 2.2 Coccidial oocysts in faeces samples from rabbits. Coccidial oocysts can be confused with non-pathogenic *Saccharomyces guttulatus*, a budding sporogenous yeast that can be present in large numbers in rabbit faeces. **(A) Coccidial oocysts and *Saccharomyces*** (low power). Large numbers of coccidial oocysts interspersed with *S. guttulatus*. The faecal sample was from a 14-week-old, thin rabbit suffering from diarrhoea. Faecal material was emulsified with water before being passed through a fine sieve to remove the coarse debris. The homogenate was then centrifuged and the supernatant discarded. The residue was mixed with saturated salt solution and centrifuged again. After the sample had been spun, more saturated salt solution was added to the test tube until a meniscus formed. A cover slip was placed over the meniscus and the sample left for approximately 30 min for the oocysts or worm eggs to float to the top of the tube. At the end of this period, the cover slip and surface film was removed from the test tube, placed on a microscope slide and examined under low power. **(B) *Eimeria stiedae*** (high power). Several *Eimeria* spp. affect rabbits and mixed infections occur. The species can be differentiated by the morphological characteristics of the oocysts. The most pathogenic species is *E. stiedae*, which causes hepatic coccidiosis (see [Section 14.4.1](#)). *Eimeria stiedae* invades the epithelial cells of the bile ducts and can cause severe liver damage. Oocysts may be seen in faeces from infected rabbits or in smears of bile collected from rabbits during post-mortem examination. The relative sizes of coccidial oocysts and *S. guttulatus* can be seen in (A) and (B). (Worm eggs are larger than coccidial oocysts. The most common helminth infestation of pet rabbits is *Passalurus ambiguus*. Adult worms or ova from *P. ambiguus* may be seen in rabbit faeces. The ova are ovoid, slightly flattened and asymmetrical with a cap at one end.) (Image kindly supplied by Dr Sheelagh Lloyd, Division of Animal Pathology, University of Cambridge.) **(C) *Saccharomyces guttulatus*** (oil immersion). *Saccharomyces guttulatus* is a budding yeast that is commonly found in the faeces of rabbits, chinchillas and guinea pigs. It is not believed to be pathogenic. Some texts call the yeast *Cyniclomyces guttulatus*. (Image kindly supplied by Idexx Laboratories, Wetherby.)

orientated in the same direction. A drop of mineral oil and a cover slip are applied before examining the shafts of hair under the microscope. Abrupt, fragmented, distal ends of the hair shaft suggest barbering by a companion. Egg cases, cuticles or adult mites, usually *L. gibbus*, may be seen attached to hair shafts. The presence of fungal spores in broken hair shafts plucked from a lesion is diagnostic of dermatophytosis.

Dermatophyte infection can be demonstrated by the presence of mycelia or ectothrix arthrospores in potassium hydroxide preparations of macerated scale. Asymptomatic infections can be detected by brushing the entire body with a sterile toothbrush and incubating the brushings at 25°C on dermasel agar (Oxoid). Plates that do not show fungal growth within 3 weeks can be considered negative (Vangeel *et al.*, 2000). Dermatophyte infections are usually due to *Trichophyton mentagrophytes*, which does not fluoresce under ultraviolet light. *Microsporum canis* infections, which are evident from the characteristic apple green spores that fluoresce under a Wood's lamp, can also occur.

Exudate from crusty lesions may be examined for the presence of mites. Although *Psoroptes cuniculi* normally inhabits the ear canal, the mite can be found in other areas of the body such as the perineal skin folds and can be seen on microscopic examination of the exudate from affected areas. Skin scrapings may be required to demonstrate sarcoptic mange mites in scabies cases characterized by intense pruritus and crusty lesions. Dark field microscopy can be used to look for *T. cuniculi* organisms in crusty lesions suggestive of rabbit syphilis (Section 14.5.9). The organism is a motile corkscrew-shaped spirochaete. Lesions are found on the mucocutaneous junctions of the anus and genitalia or on the nose, lips and eyelids (see Figure 7.16). The lesion is abraded with a sterile saline-soaked swab. Serum from the lesion is then expressed onto a slide and covered with a cover slip before being examined immediately (DiGiacomo *et al.*, 1984). The slide can be placed in a moisturized chamber. The differential diagnosis of exudative skin lesions in rabbits can be difficult and histopathological examination of biopsy specimens may be required.

Key Points 2.6 Clinical diagnosis of ectoparasites

- *Cheyletiella parasitovorax*, fur mites (*Leporacus gibbus*), lice (*Haemodipsus ventricosus*), fleas or flea dirt can be seen in skin brushings from affected animals.
- Acetate tape strips can be used to detect all stages of the life cycle of *Cheyletiella parasitovorax*.
- A trichogram can differentiate between dermatophytosis and barbering. Mites, cuticles and egg cases may be seen attached to the hair shafts.
- *Psoroptes cuniculi*, the rabbit ear mite, can sometimes be seen on microscopic examination of smears from skin lesions in other parts of the body such as the perineum.
- Dark field microscopy may be used to detect *Treponema pallidum* organisms.

2.8 Cerebrospinal fluid

Cerebrospinal fluid can be collected from the cisterna magna of rabbits in a manner similar to that of other animals. Some normal parameters are summarized in Table 2.5. Depressed glucose concentrations (< 56 mg/dL) may be indicative of purulent inflammation (Kusumi and Plouffe, 1980).

Table 2.5 Cerebrospinal fluid

| Ref. | Parameter | |
|------|---------------|---|
| b | Glucose | 56–135 mg/dL |
| a | WBC | 0–7 cells/mm ³ (up to 20 cells/mm ³ have been found in healthy rabbits) |
| a | Lymphocytes | 40–79% |
| a | Monocytes | 21–60% |
| b | Total protein | 16–66 mg/dL |

Reference sources: a, Curiel *et al.* (1982); b, Kusumi and Plouffe, (1980).

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