Veterinary Laboratory Medicine

CLINICAL BIOCHEMISTRY AND HAEMATOLOGY

Second Edition

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b
Blackwell Science

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To my mother:
in gratitude for the winter of the millennium

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Introduction

Laboratory medicine and the veterinary surgeon

Since the first edition of this book was published in 1989, there have been many changes in veterinary laboratory practice – some very much for the better, others less so.

The most striking change is the much greater volume of biochemistry and haematology investigation being carried out. To a large extent this is a good thing, though a note of caution has to be sounded against using blood tests as a substitute for thorough clinical examination and history-taking, and anyone who finds themselves paralysed to act in an emergency because blood results are unavailable really ought to be reconsidering their priorities. In general, however, the more relevant information which is available to the clinician the more likely it is that the correct diagnosis will be arrived at, and so long as the laboratory data is *in addition to* the clinical data then more widespread use of laboratory investigation is to be welcomed. Indeed, the much greater readiness of practitioners to embark on laboratory investigation of the more challenging cases and to seek laboratory confirmation of the presumptive diagnosis in the more straightforward ones has made laboratory medicine a very rewarding discipline.

Following on from that, a more recent development has been the emergence of more veterinary surgeons specializing in clinical pathology/laboratory medicine at postgraduate level. Twelve years ago only a minority of commercial veterinary laboratories were under professional veterinary direction, with the majority run by technicians (often trained only in analysis of human samples) providing a results-only service without any professional interpretation. Now only a few laboratories remain in the latter category, and practitioners have a good choice of professionally-run laboratories offering not simply a string of numbers but a full range of advice covering selection of tests, interpretation of results and recommendations regarding treatment. Practitioners now recognize the laboratory as a second-opinion referral service, made extremely convenient and accessible by the fact that only the blood (or other) samples have to be referred rather then the entire patient.

In parallel with this there has also been an enormous increase in the amount of laboratory work carried out within veterinary practices. This is a bit of a mixed blessing. A near-patient facility designed to complement the professional laboratory and enable quick (if sometimes approximate) results of appropriate tests to be obtained as an interim measure in emergencies and out-of-hours, and to allow simple monitoring of already-diagnosed patients on treatment is invaluable. Certain items (e.g. the pocket glucose meter, the refractometer, the microhaematocrit centrifuge and, of course, the microscope) are so easy for the non-technician to use, so cheap and so useful, that it really is a case of 'every home should have one'. On the other hand, what is sometimes not appreciated is the enormous gulf between this type of side-room facility and a professional laboratory. However conscientiously those concerned with teaching the subject at undergraduate level try to instil a few of the principles of analytical procedure into veterinary students, a veterinary course is far removed from the sort of training a laboratory technician receives, and although some laboratory component is included in the veterinary nursing syllabus, this again should be regarded as helping equip nurses to perform the near-patient type of testing competently rather than expecting them to run a full laboratory service in between setting up drips and monitoring anaesthetics.

The main driving force of the 'practice lab' has been, as expected, the dry-reagent biochemistry analyser. Twelve years ago these machines were just emerging, having been developed for near-patient testing of human samples. It was clear that there were substantial problems when non-human samples were analysed by these methods, apparently due to what is termed the 'plasma matrix effect', but the optimistic view was that these problems would be solved and that there was good cause to hope that a wide range of reliable biochemistry results might be available in the practice side-room. Unfortunately this hope proved to be unfounded. There have been very few published studies comparing results of dry-chemistry methods to standard wet-chemistry methods for animal samples (and most of those are, for some reason, in German), but it is quite clear that for most of the methods the correlation is far poorer than would be required for professional laboratory application. Thus, although some practices owning these machines still do rely on them for routine work-up of non-emergency cases, many now realize that their place, if they are used at all, is in the near-patient emergency testing category, confining their use to the tests which are less poor performers (such as urea), concentrating on gross deviations from normal and not trying to read subtleties into smaller abnormalities which the accuracy of the methods is not really good enough to support.

Thus the thrust of this edition, contrary to expectations of twelve years ago, is much more towards the practitioner in partnership with the professional laboratory, performing relevant side-room tests where appropriate, but relying on the referral laboratory for the bulk of the routine testing and non-emergency case work-up.

So, does that mean that the clinical student or the practitioner can put this

book down, sit back, and wait for the clinical pathologist to tell him or her what is wrong with the patient and what to do about it? Well, no. Two heads are always better than one: the person who has actually seen the patient has an insight into the case which cannot be replicated simply by reading even the best-expressed clinical history, even the smartest clinical pathologist occasionally misses the blindingly obvious, and really successful use of the laboratory relies on an intelligent dialogue between the clinical pathologist and a well-informed and interested practitioner.

The format of the book remains based on the lecture notes approach. Some sections of comparatively basic science have been included, but the rule has been to cover only those areas which are genuinely relevant to clinical use. The information is initially organized on a test-by-test basis as this is still the essential way into the subject for the student, and it is important to have some way of assessing all the possible clinical implications of a single result. However, the systematic reassembly of the data has been expanded to give more emphasis to the pattern recognition approach to interpretation of laboratory reports. Detailed information regarding treatment and case management is given for a few specific conditions, but in general, information which is easily available in other basic texts has not been duplicated. Very unusual and rare conditions have also been omitted, as have tests which are not likely to be available to the general practitioner, and for information on these subjects the reader is referred to more advanced textbooks such as those listed on p. 355.

Laboratory medicine in case management

The most common use of laboratory work in veterinary practice is as an ancillary diagnostic aid. Other applications such as assessment of severity of the disease, prognosis and response to treatment tend to be secondary to this. It is therefore useful to consider where this type of procedure fits into the general management of a case.

The first rule of laboratory medicine is, *first catch your differential diagnosis*. This is something which must be arrived at, at least to a first approximation, on clinical grounds, for the very simple reason that only when you have at least some theory about what is going on can you begin to decide which tests to carry out to prove it.

At the most basic level, one first has to decide whether laboratory investigation (blood analysis or microbiological investigation), or radiography or other diagnostic imaging, or electrocardiography or whatever, is the most promising initial route to pursue.

The second step is to try to *ask the lab a specific question*. The clearer you are in your own mind just what question you want answered the easier it will be to decide which tests to ask for, to interpret the results when you get them back, and to realize when your question is, in fact, not one which a laboratory can really answer. For example, to consider a dog with severe acute vomiting, you

may decide to ask 'Does this dog have acute pancreatitis or is it in renal failure?', which leads straightforwardly to one set of test requests (amylase, lipase and urea and creatinine), or you may want to know 'How dehydrated is this dog and which i/v fluid should I be giving?', which leads to a different set of requests (total protein, albumin and electrolytes). Both questions are quite valid, both questions can be answered by the laboratory, but only you can decide which one you want to ask or whether you want to ask both. Or to consider a different point, 'Is this cow hypocalcaemic?' is obviously a realistic question, but 'Does this cow have a fractured pelvis, or obdurator paralysis?' is not really something which a laboratory is going to be able to answer with any real certainty. Here the formulation of the question, as opposed to just writing 'downer cow', can help clarify both the extent and the limitations of the information which the laboratory can be expected to provide. It is important in this context to realize that while laboratory data can be highly revealing in a large number of areas, there are certain areas of medicine where general 'routine' blood tests are usually not particularly informative, at least in a diagnostic sense. These include respiratory disease, most orthopaedic conditions and the majority of neurological cases.

Next, translate your question into a *request for specific tests* to be done. In order to do this it is necessary to know what information can be gained from each of the available tests and what is its likely applicability to the situation under consideration. This aspect occupies the bulk of the scope of this book. However, in spite of this, it is probably the actual formulation of the question which requires the most clinical skill, and turning this into a specific request soon follows on naturally. A single result is seldom pathognomonic for a particular disease, however, and the judicious selection of the most appropriate range of tests for each case is very important. It is necessary to strike a balance between requesting dozens of tests (which can be very expensive and may even lead to the relevant information being overlooked in the deluge of results), and the often false economy of restricting requests to one or two tests per sample. As one becomes more familiar with the extent and limitations of the information available from each test this process of acquiring maximum information from a reasonably small number of tests becomes easier and easier (the approach to this is outlined in Chapter 15). In addition, many laboratories have now adopted the approach to profiling first outlined in the previous edition of this book, where profiles are designed around common major presenting signs rather than on an organ-by-organ basis. Profiles designed in this way provide a short-cut to the most rational selection of tests by ensuring that all the differential diagnoses are covered which should realistically be considered when that presenting sign is present – for example, the polydipsia profile for dogs will include calcium, as hypercalcaemia is an extremely important but uncommon cause of polydipsia which might otherwise be forgotten when selecting tests. Nevertheless, it is still good practice to 'engage brain before ticking boxes', as sometimes an extra test or two might be needed to cover particular circumstances, or you might be confident enough that

certain conditions are *not* on the cards to allow a less extensive range of tests to be requested. Once you have decided on what information you require from the laboratory and which tests you need to acquire it, you are ready to collect and submit your sample.

The fourth step is to *consider the results in the context of the whole clinical picture*. The conscious act of formulating your original question will make this step much easier, in that when you ask a specific question you tend to have some idea in mind of the answers you are likely to receive, and of your probable response to these answers. However, this stage is definitely the time for some lateral thinking. Even in cases where the answer to the original question seems fairly straightforward, it is well worth asking 'Is there any *other* explanation which could fit all the facts of this case?', and in cases where unexpected or even apparently inconsistent results appear then it is essential to consider the situation in some depth. There is a sort of laboratory 'cringe' which says 'where the clinical picture and the lab results disagree then you should always believe the clinical picture', but this view is misleading. Results from a *reliable* laboratory should never be ignored just because they don't fit your cosy little theory – and if you can't rely on your laboratory, you shouldn't be using it. When arriving at a diagnosis it is essential to look every single fact straight in the eye and to come to a conclusion which can be reconciled with *all* of them. A laboratory result, normal or abnormal, is a fact just like any other piece of clinical information and should be given its *due consideration*. Obviously in each case some facts will weigh more heavily than others, and the decision as to just how much importance to give to each item involves a great deal of clinical skill which takes time and experience to acquire. Unfortunately there are no easy generalizations like 'clinical facts are always more important than lab facts' (or vice versa!) to help here, and there is really no substitute for a *thorough* knowledge of the significance and implications of *all* your findings.

The final maxim to bear in mind is *sample before treatment whenever possible*. The rather desperate approach to laboratory medicine which views lab investigations as a last resort when all attempts at 'diagnosis' by response to treatment have failed causes some veterinary surgeons to come unstuck at this point. It is true that antibiotic treatment is not often a direct cause of trouble with haematology or biochemistry tests (though it can play havoc with any bacteriology you may subsequently decide to do) but the ubiquitous corticosteroids have a wide range of haematological and biochemical effects which can mask vital information of diagnostic significance. Other culprits are fluid therapy (especially when the fluid contains glucose) and mineral preparations such as calcium borogluconate. Clearly, it is difficult to avoid the situation where a farmer has administered every nostrum in his cupboard before you arrive, but it is good practice, whenever treatment is about to be instituted, to consider 'Am I likely to want any laboratory work done on this case, and if so, am I going to regret not having a pre-treatment sample?' Even in circumstances where treatment must be started before any results will be received – a fairly

frequent occurrence – a pre-treatment sample can be invaluable and can save a lot of time and trouble in the long run.

Basic principles of haematology and biochemistry

Haematology is the study of the cellular elements of the blood and the associated clotting factors, and can be extended to include cytology of non-blood fluids such as cerebro-spinal fluid (CSF). It is a subject which can provide a great deal of useful information, but, like all diagnostic tests, intelligent assessment of the results is vital. In some ways haematology can be easier to cope with than biochemistry, if only because the easy option of a 'full blood count' or 'general series' examination is available on all lab request forms. This means that it is actually quite easy to bypass the mental disciplines outlined above which lead up to the selection of individual tests. However, if you omit this prior consideration of *why* you are taking this sample and what conclusions you might expect to derive from the results, you must expect to compensate by a particularly thorough assessment of the findings once you receive the results. Remember also that haematology can only tell you what is happening, directly or indirectly, to a fairly small number of circulating cell types, and that the actual number of tests available is quite limited. For general metabolic investigations the wider range of tests and the more direct nature of the information offered by clinical biochemistry is at least as helpful, possibly more so, and normal practice should be to consider *both* disciplines side by side when deciding on the range of tests required for each case.

Clinical biochemistry is a very different subject from pure biochemistry and an antipathy to the latter acquired in early student days should not deter anyone from tackling the former. Basically, clinical biochemistry involves the analysis of samples of body fluids, principally plasma (though occasionally other samples are used such as urine, faeces, CSF and pleural and peritoneal fluids), and the use of the results to clarify the clinical picture. The nature of the subject and the much larger number of 'routine' tests on offer mean that, in general, a wider range of specific information is available from biochemistry than from haematology, but also that a single group of tests cannot be regarded as a basic 'profile' applicable to all (or nearly all) situations. Judicious selection of the appropriate tests for each individual case is therefore of particular importance in clinical biochemistry.

'Normal values'

Many publications quote apparently rigid 'normal values' for biochemical and haematological measurements, sometimes to an extraordinary number of significant figures. The fact that it is extremely rare to find two publications in absolute agreement on these numbers demonstrates clearly the artificiality of this situation.

The spread of values from 'normal' individuals for most constituents (excluding some enzymes) takes the form of a normal distribution curve (see Fig. A.1). If the limits of this curve are defined as the mean ± 2 standard deviations then very rigid values to any number of significant figures can be derived. However, these limits will of necessity exclude 2.5% of all *normal* individuals on each side of the curve – how can you know that your individual patient is not one of this 5%? In addition, it is important to realize that a value *within* these limits is not necessarily 'normal' for every individual animal – one which was towards the lower part of the range when healthy may have a genuinely pathologically evaluated value when ill, which is still within the statistically 'normal' limits. Thus on either side of every 'normal range' there is a grey area where a result may be normal or may be abnormal, and only statistical probabilities of its being one or the other can be quoted. In dealing with individual results in these grey areas it is particularly important to take other factors into consideration, both clinical signs and other laboratory results.

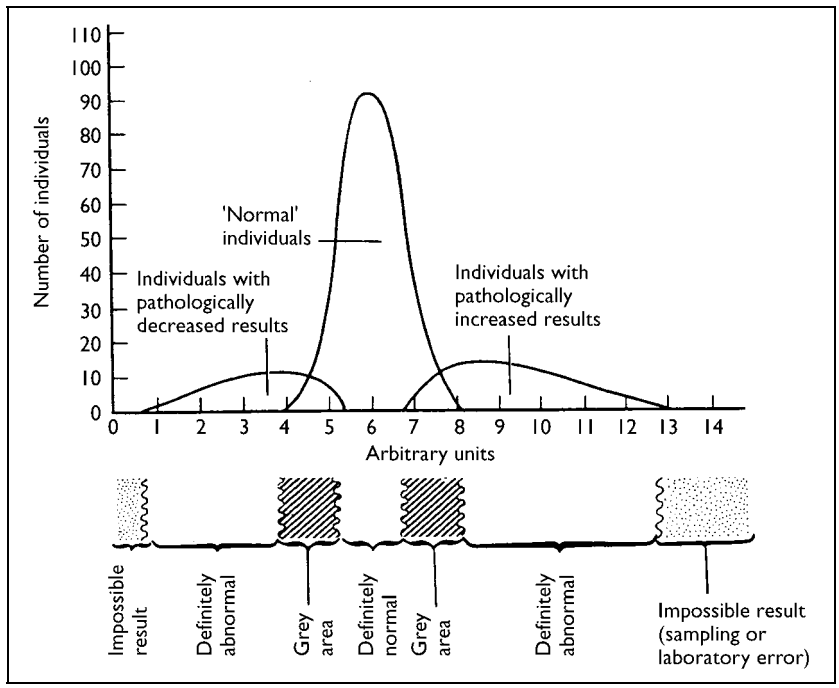

Fig. A.1 Schematic representation of the distribution of results for a figurative laboratory test showing overlaps of 'normal' and pathological ranges.

As a consequence of this, only approximate guideline values are given in this book for each constituent, and when interpreting actual results the modifying effects of species (only the very major species differences are highlighted), breed, sex, age, diet and management systems must be taken into account. It is this multiplicity of species, breeds and patient 'lifestyle' differences which make

veterinary laboratory medicine a bit of an art as well as a science, and there is no doubt that the best way to become proficient in interpreting laboratory data is to examine numerical results for as many actual cases as possible. In particular, remember that it is much more important to know what degree of weight to attach to a particular *level* of deviation from normal (e.g. insignificant–ill–dying) than to be able to quote glibly memorized 'normals'.

There is also the question of methodological variation. Since the advent of external quality assessment in NHS laboratories in the 1960s, great attention has been paid to uniformity of reference ranges and results between laboratories. This 'inter-laboratory precision' ensures that patients with chronic illnesses who move from one part of the country to another do not run into serious problems when their new consultant is faced with results from an unfamiliar laboratory with unfamiliar reference ranges. University, state and commercial veterinary laboratories have also benefited from these schemes and participated in them, and nowadays any discrepancies between laboratories' reference ranges should be minor and insignificant (with perhaps a few specific exceptions such as alkaline phosphatase (ALP), where method differences can still have an appreciable effect). Thus it is possible to quote general guideline values which are fairly universally applicable, and it should not be necessary either to completely relearn the subject when changing laboratories, or to be constantly enquiring 'what is *your* reference range for this analyte?'.

Units

The changeover from the old 'conventional' (mostly gravimetric in biochemistry) units to the modern 'SI' (mostly molar in biochemistry) units has created some considerable confusion, particularly among clinical users who just want to know what is wrong with the patient and don't want to be bothered with technicalities. This was probably inevitable at the time, but now that it is at least 25 years since the actual changeover it is about time things settled down.

In haematology there has been comparatively little trouble, in that the adoption of the litre as the standard volume of measurement has usually involved either a simple change in the name of the units (or in the power of 10 included in it) while leaving the actual number unaffected, or at the most there has been a shift in the position of the decimal point. So, mean corpuscular volume (MCV) has moved from cubic microns (μ^3 or cu.μ) to femtolitres (fl) with no change in the number (as they are actually the same thing), while packed cell volume (PCV) has changed from a percentage to a decimal fraction, which in effect moves the decimal point two places to the left (the decimal fraction is sometimes labelled 'l/l', but this is a non-unit in which the top and bottom cancel out – gallons/gallon would be equally valid, as PCV is in fact a v/v ratio). One place where care is required is where a unit of '$\times 10^3$/mm^3' or 'thousands/cu.mm' has been replaced by '$\times 10^9$/l', as with white cell and platelet counts. The numerical result has not in fact changed, but as some people were in the habit of quoting the figure as so many *thousand*, it is possible

to fall into the (sometimes potentially dangerous) trap of reporting a result as several thousand $\times 10^9$/l, which is of course out by three orders of magnitude.

Biochemistry unit changes have been more complex because the actual numbers involved have been affected. Historically, plasma constituents were measured by weight (usually mg/100 ml), but subsequently all branches of chemistry and pure biochemistry adopted molar concentration units as the only realistic way to describe reaction processes. In the early 1970s clinical biochemists also changed to molar (SI) units to describe concentrations of plasma constituents, as these are obviously much more meaningful in real terms. However, a few countries have lagged behind in this and the USA in particular has still failed to address the situation even at the beginning of the twenty-first century. This means that the old gravimetric units are still to be found not only in pre-1975 books and journals, but in modern American publications, and the table of conversion factors given below (Table A.1) should be used to convert these figures to the SI equivalents whenever they are

Table A.1 Conversion from old 'gravimetric' biochemistry units to SI units

Constituent	Gravimetric unit	SI unit	Conversion factor
Total protein, albumin, globulin	g/100 ml	g/l	10
Sodium	mg/100 ml*	mmol/l	0.435
	mEq/l		no change
Potassium	mg/100 ml*	mmol/l	0.26
	mEq/l		no change
Chloride	mg/100 ml*	mmol/l	0.28
	mEq/l		no change
Calcium	mg/100 ml	mmol/l	0.25
	mEq/l*		0.5
Magnesium	mg/100 ml	mmol/l	0.41
	mEq/l*		0.5
Phosphate	mg phosphorus/100 ml	mmol/l	0.32
Copper	µg/100 ml	µmol/l	0.16
Urea	mg nitrogen/100 ml (BUN)	mmol/l	0.36
	mg urea/100 ml		0.17
Creatinine	mg/100 ml	µmol/l	88.4
Ammonia	µg/100 ml	µmol/l	0.59
Glucose	mg/100 ml	mmol/l	0.056
Bilirubin	mg/100 ml	µmol/l	17.1
Cholesterol	mg/100 ml	mmol/l	0.026
Triglycerides	mg/100 ml	mmol/l	0.011
Tri-iodothyronine (T_3)	µg/100 ml	nmol/l	15.4
Thyroxine (T_4)	µg/100 ml	nmol/l	12.9
Cortisol	µg/100 ml	nmol/l	27.6
Urine protein/creatinine ratio	g/g	g/mmol	0.113

*Less commonly encountered units.

encountered. When doing this, take care to avoid acquiring extra, spurious, 'significant' figures which may be misleading. (This is another source of the unrealistic number of significant figures seen in some lists of normal values.) It is important to avoid trying to interpret results in gravimetric units as they stand. For one thing, it is quite enough work to become completely familiar with one set of units and probably impossible to become fluently 'bilingual'. If, on the other hand, you persist in converting everything back into old units you will find yourself regarded as somewhat out of touch by the clinical biochemistry establishment in the UK, where SI units have been solidly established for at least 25 years now!

1

Haematology

Haematology is the study of the cellular elements of the blood, which can be divided into three categories:

(1) The erythrocytes or red blood cells.
(2) The thrombocytes or platelets.
(3) The leucocytes or white blood cells.

Occasionally other cells which are not normally present in circulation can also be detected in a blood sample, such as mast cells or plasma cells – usually because the cells are neoplastic.

The red cells are responsible for oxygen transport from the lungs to all the tissues of the body, the platelets are responsible for routine maintenance and repair of the blood vessels, and the white cells (at a wild generalization) are responsible in various ways for repelling foreign invaders. Haematological examination may in a sense be regarded as a 'biopsy' of these systems.

1 The Red Blood Cells (Erythrocytes)

The erythron

The 'erythron' is the name given to the organ of the body, technically classified as connective tissue, which comprises all the red cells plus all the red cell producing tissue – essentially the relevant fractions of the blood, the spleen and the bone marrow. In so far as the red cells are concerned, a blood sample can be thought of as a biopsy of this organ. The single function of the erythron is oxygen/carbon dioxide transport between the tissues and the lungs, with haemoglobin as the O_2/CO_2 carrier, and the main reason that the haemoglobin is contained within cells rather than being free in the plasma like all the other blood proteins is simply that the sheer amount of protein involved (100–150 g/l whole blood as opposed to only about 40 g/l whole blood of all other proteins) would cause massive disruption of the osmotic pressure. Functionally speaking, therefore, mature red cells are little more than very flexible bags of haemoglobin in the shape of a slightly biconcave disc.

Red cell production (erythropoiesis)

This takes place in the red (haemopoietic) bone marrow (not in the white fatty marrow). This haemopoietic bone marrow is much more extensive in young animals than in mature ones, where it retreats to the centres of the bones. This tends to make effective bone marrow biopsy rather more difficult in older animals. The stages of development of the red cells are shown in Fig. 1.1.

As the erythrocytes mature they become very readily deformable (necessary in order to pass through small capillaries) and when they are flexible enough they can slide into the circulation through openings in the sinusoidal walls. The total maturation time varies between species from about 4–5 days in cattle to about 1 week in the dog. Normally about 10–15% of developing red cells die before reaching maturity (ineffective erythropoiesis) and this percentage can increase in certain disease situations.

When there is an increased demand for red cells (e.g. haemorrhage, oxygen

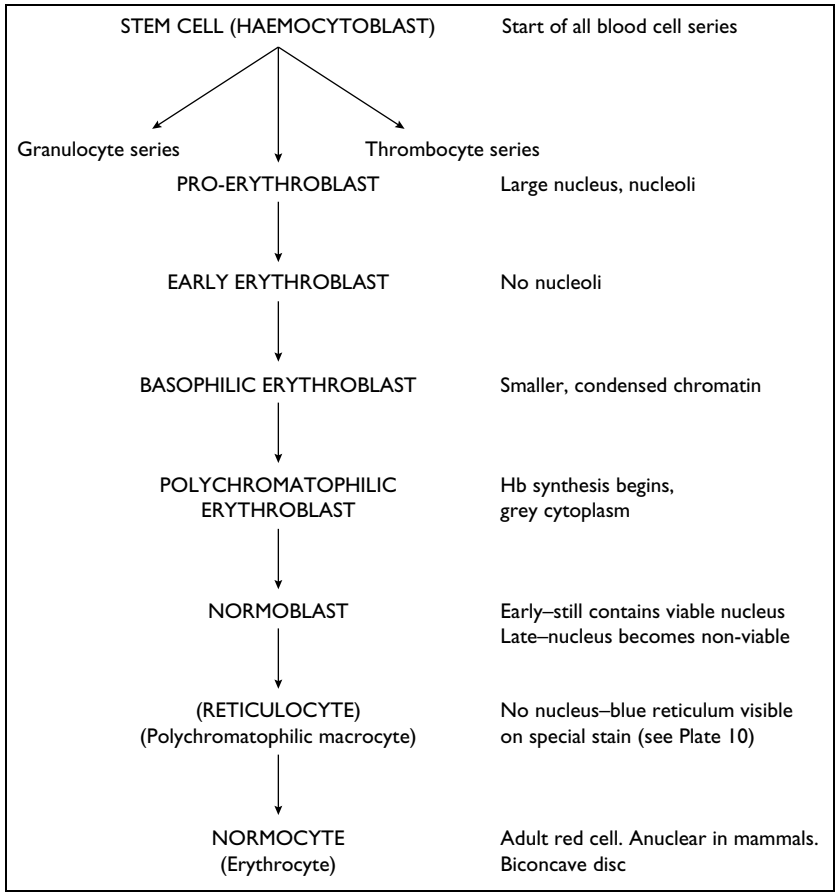

Fig. 1.1 Simplified representation of the stages of erythropoiesis.

starvation) production is increased firstly by allowing younger forms (reticulocytes, normoblasts) to enter the circulation, and secondly by allowing the maturation stages to merge and skip so that erythropoiesis speeds up. The former is not seen in all species – for example, dogs demonstrate reticulocytosis very readily, cattle only on extreme provocation such as severe acute haemorrhage, and horses never. The latter occurs in all species and sometimes leads to the appearance of a few imperfect erythrocytes in circulation, such as Howell–Jolly bodies, poikilocytes and leptocytes.

Erythrocyte lifespan

This varies between species from about 2 months in pigs to over 5 months in cattle. Sheep are unique in having two populations of red cells, one short-lived (70 days), the other long-lived (150 days). These differences mean that the rate of progression of a hypoplastic anaemia varies between species. In certain

disease situations the survival time of the erythrocytes is shortened, particularly some nutritional deficiencies (iron, vitamin B_{12}, folic acid), congenital porphyria in cattle and congenital pyruvate kinase deficiency in basenji dogs.

Erythrocyte breakdown

This occurs in three ways. The cell may be fragmented into pieces small enough for the reticulo-endothelial system to take up, or when the enzymes present in the cell membrane are used up the much more fragile cell breaks up and is phagocytosed, or the whole cell may be phagocytosed directly.

The haemoglobin from a defunct red cell is also broken down. The (protein) globin fraction is lysed into its component amino acids which join the general body amino acid pool, either being restructured into new proteins as needed, or being deaminated with the amino residue excreted as urea and the carbohydrate residue entering the fuel metabolism pathways. The haem fraction loses its iron atom, which is *not* excreted but is recycled into a new haemoglobin molecule. The remaining part of the haem complex becomes bilirubin which, in its original form, is non-water-soluble and so must be transported in the plasma bound to albumin. On reaching the liver it is conjugated to glucuronic acid or a similar substance, which renders it soluble so that it can be excreted in the bile. After some recycling round the hepatic circulation and further metabolism most of this is excreted in the faeces as urobilin and stercobilin – these give the faeces their characteristic colour. Some is also excreted in the urine as urobilinogen. Investigation of these metabolites can be useful in the differential diagnosis of hepatobiliary disease in man, but only bilirubin seems to be of any real clinical use in veterinary species.

Control of erythropoiesis

Normally, production and destruction of red cells are kept in balance so that total erythrocyte numbers (i.e. erythron size) are constant – in a 15 kg dog about 800 000 red cells die and are replaced every second!

The hormone responsible for the regulation of the rate of erythropoiesis is a glycoprotein with a molecular weight of about 60 000–70 000 daltons, called *erythropoietin* (EP; it is sometimes referred to as EPO, but this invites confusion with evening primrose oil). It is not species specific, but bird and mammal hormones are not interchangeable. Fetal and maternal EP are quite separate because the hormone does not cross the placenta. The principal site of EP production is the kidney – in dogs this is the only site and thus the hormone is totally absent in nephrectomized animals, but there is an additional extra-renal site in some species (e.g. rats) which has not been identified. The fundamental stimulus to EP production is tissue hypoxia, and so the concentration in plasma is related to the ratio of oxygen supply to oxygen demand.

Erythropoietin affects red cell production in four ways:

(1) More stem cells differentiate to red cell precursors.
(2) Stages of red cell development are speeded up.
(3) Transit time out of bone marrow is reduced.
(4) Immature red cells are released (depending on species).

(1) is the normal method of obtaining fine control over the size of the erythron. (2), (3) and (4) only occur in response to large doses of EP, usually because of an acute requirement for more erythrocytes. The actual mechanisms involved are not fully understood but may be connected to the rate of haemoglobin synthesis.

Measurement of plasma EP concentration is becoming increasingly used in human medicine to aid differentiation of the causes of anaemia, and has recently become available in the veterinary field. EP is also available as a therapeutic drug in human medicine, most importantly in long-term renal failure patients being maintained on dialysis. It has also become a drug of abuse among endurance athletes. Its high cost and restricted availability have meant that only a few small-scale trials have been carried out in animals. It certainly does increase the packed cell volume (PCV) in chronic renal failure cases, but this is of little clinical benefit if the excretory capacity of the kidneys continues to deteriorate. Effects of changes in EP concentration can often be readily appreciated on examination of routine haematology results, and certain other hormones which affect EP synthesis can be used to stimulate its production. The endocrine organs involved in the modification of EP production are the pituitary, adrenals, thyroid and gonads. The hormones in question actually affect cell metabolism and hence tissue oxygen requirements, and so have a feedback on EP synthesis.

(1) *Hormones which increase EP production:* androgens, cortisol, thyroxine, adrenaline, noradrenaline, angiotensin, prolactin, growth hormone, thyroid-stimulating hormone (TSH) and adrenocorticotrophic hormone (ACTH).
(2) *Hormones which decrease EP production:* oestrogens.

Resultant clinical effects are quite wide ranging. By these mechanisms apparently unrelated occurrences can have a marked and unexpected effect on an animal's erythrocyte status.

(1) Males tend to have a higher PCV than females – this is a hormonal effect abolished by castration and spaying.
(2) Excess of a hormone in the first group will lead to an increase in PCV – the most common example is excess cortisol in Cushing's disease (hyperadrenocorticism).
(3) Deficiency of a hormone in the first group will lead to a decrease in PCV, i.e. slight anaemia. Examples are hypothyroidism, Addison's disease (hypoadrenocorticism) and anterior pituitary insufficiency. However, note that patients with untreated Addison's disease are nearly always dehydrated, which can cause the PCV to rise back into the normal range.

The anaemia will only be apparent as such when rehydration has been achieved.

(4) Excess of oestrogens will lead to decreased erythropoiesis and in some cases to complete (and fatal) bone marrow aplasia. This has been recorded as a spontaneous occurrence in unmated ferrets in prolonged oestrus, but most cases are iatrogenic as a consequence of oestrogen treatment for misalliance incontinence or enlarged prostate (see true aplastic anaemia, p. 27).

Basic interpretation of red cell parameters

When investigating the red cells there are several different but related measurements which can be made, and these can be combined to produce several more figures which are descriptive of red cell status. It is important to be aware of the meaning of each of these different numbers and of their relationship to one another in order to make sense of a haematology report.

The primary red cell measurement which gives a basic assessment of the size of the (circulating) erythron is the *packed cell volume* (PCV) or haematocrit. This is simply a measurement of the fraction of the blood volume which is occupied by erythrocytes and is expressed either as a percentage or as a decimal fraction (35% = 0.35). Normal values vary slightly with species: about 0.30–0.40 in large animals, about 0.30–0.45 in cats and a wide-ranging 0.35–0.65 in dogs, with the greyhound/whippet/lurcher type breeds showing the highest values.

Next, information about the morphology of the red cells is provided by the *mean corpuscular volume* (MCV) and *mean corpuscular haemoglobin concentration* (MCHC) values, which are both calculated parameters in veterinary haematology.

The *MCV* is a measure of the size of the red cells and is obtained by simple arithmetic from the PCV and the total red cell count of the sample:

$$\text{MVC (fl)} = \frac{\text{PCV (\%)}}{\text{RBC } (\times 10^{12}/\text{l})} \times 10 \quad [1 \text{ femtolitre (fl)} = 10^{-15}\,\text{l}]$$

The '$\times\ 10^{12}$/l' does not enter into the actual calculation, e.g. a sample with a PCV of 0.35 (35%) and RBC count of 4.89 $\times\ 10^{12}$/l has an MCV of 71.6 fl. Normal values vary widely with species, and are completely independent of the size of the individual animal (Table 1.1).

Young animals tend to have rather smaller red cells than adults; in particular calves often have an MCV as low as 30 fl. Paradoxically, neonates actually have red cells at least as large as the adult.

The size of the red cells can also be assessed by looking at a well-made blood film. This is done partly by comparing the red cells with the white cells, which vary very little in size (see Plate 7), and partly by appreciating that where abnormal cells are present normal red cells are usually also present, and with practice the comparison is easy to make. In particular, abnormally large cells

Table 1.1 Erythrocyte size

Species	Approximate mean red cell volume (fl)
Man	90
Dog	70
Pig	60
Cow (adult)	50
Cat	45
Horse	45
Sheep	30
Goat	15

(macrocytes) are nearly always polychromatophilic (blue-mauve coloured) which makes them easy to spot (see Plate 8). Where a calculated MCV value disagrees with the appearance of the blood film, *believe what you see*, particularly if the red cell count was done manually. The manual method is not very accurate and often gives falsely high (or sometimes low) MCV values.

The *MCHC* is a measure of the haemoglobin concentration in the red cells and is obtained arithmetically from the PCV and the total haemoglobin concentration of the sample:

$$\text{MCHC (g/100 ml)} = \frac{\text{Whole blood haemoglobin concentration (g/100 ml)}}{\text{PCV (decimal fraction)}}$$

The normal value is about 35 g/100 ml irrespective of species or size of the red cells, i.e. for any given PCV the total amount of haemoglobin per unit volume of blood will be the same irrespective of species. In the sheep it is contained in a large number of small packets; in the dog it is contained in a smaller number of larger packets.

Corpuscular haemoglobin concentration can also be assessed by eye on a well-made blood film, with the hypochromic cells (low haemoglobin concentration) having noticeably pale centres (see Plate 9). Again, where a numerical value disagrees with the appearance of the blood film, believe what you see. Sometimes only a proportion of the cells are hypochromic, not enough to lower the MCHC value (which is, after all, a *mean*), but enough to be clinically significant.

An abnormally *high* MCHC is not possible as such; there is no such thing as a hyperchromic red cell. However, because of the way the figure is obtained, MCHC values of over 40 g/100 ml are sometimes obtained. There are three possible reasons:

(1) Haemolysed blood sample (either due to bad collection technique or, more rarely, genuine intravascular haemolysis). Since the calculation of MCHC assumes that all the haemoglobin is inside the cells, when in this case it is not, a falsely high value will be obtained.
(2) Other interfering substances in the plasma (e.g. lipaemic plasma) may cause an erroneously high haemoglobin reading and hence an erroneously high MCHC.

Table 1.2 Effects of sample artefacts on calculated RBC parameters.

	MCV	MCHC
Old sample	↑	↓
Lipaemia	—	↑
Haemolysis	—	↑
Underfilled tube	↓	↑
Autoagglutination	↑	—

(3) Excessive osmotic shrinkage of the red cells. This is rarely an *in vivo* phenomenon, but is common when an EDTA tube is underfilled leading to an excessive concentration of EDTA in the sample.
(4) Simple laboratory error in either haemoglobin or PCV measurements.

A third red cell parameter which can be calculated is the *mean cell haemoglobin* (MCH), measured in picograms (pg). This obviously varies with cell size and so with species, and is therefore not often used in veterinary medicine. It can be useful in assessing whether hypochromic macrocytic cells actually have the normal absolute amount of haemoglobin in them or not.

Total *red cell count* (RBC or RCC) and whole blood *haemoglobin concentration* (Hb) should *not* be interpreted clinically. Clearly, they vary almost exactly in parallel with the PCV and can tell you nothing more than the PCV result as they stand. Their function is to allow calculation of the MCV and MCHC, respec-

Case 1.1

A 6-year-old cairn terrier bitch was presented on a Friday afternoon with malaise and poor appetite. Rectal temperature was 39.2°C. By the time the results were received 4 days later, she had completely recovered. Can you explain the abnormalities?

PCV	0.61	raised
Hb	17.6 g/100 ml	
RBC count	7.35×10^{12}/l	
MCV	83.0 fl	raised
MCHC	28.9 g/100 ml	low
Total WBC count	12.4×10^{9}/l	

Film comment. RBCs: normal
WBCs: too degenerate to differentiate
Platelets: adequate

Comments

The haematology was in fact completely normal. The 'abnormalities' are artefacts caused by a 3-day delay in analysis due to weekend post. Erythrocytes swell, causing the MCV to increase and the PCV to rise, but as the haemoglobin content of the cells (MCH) remains unchanged the MCHC decreases. Leucocyte morphology degenerates, and unless a blood film made at the time of sample collection is sent with the specimen a differential WBC count will not be obtained.

tively, and these are the figures which should be interpreted. The only exceptions are where a sample is so badly haemolysed that the microhaematocrit simply cannot be read (but the haemoglobin result may still be valid), or perhaps where a very approximate side-room haemoglobin estimation may be all that is available.

Erythrocyte sedimentation rate (ESR) involves measuring how fast red cells will settle out on standing, a measurement which depends to some extent on plasma viscosity, which alters when inflammatory proteins are present. It is an old-fashioned test, but still favoured by some general practitioners as a general indicator as to whether a patient is actually ill or not. However, results are extremely species specific, and the test has no place in veterinary medicine. In particular, the tendency of equine erythrocytes to form rouleaux means that the cells sediment extremely quickly, and the result is almost entirely a factor of the PCV. Feline cells often behave in a very similar manner. In contrast, bovine cells barely sediment at all. Some attempts have been made to produce tables of correction for PCV to allow the test to be used on canine samples, but the results appear to have little clinical relevance.

Abnormalities of the erythron: polycythaemia (abnormally high PCV)

Polycythaemia can be divided into two fundamentally different classes:

Relative polycythaemia

This is defined as an increase in PCV *without* any increase in the actual size of the erythron as a whole, and is by far the more common type of polycythaemia. In veterinary species there are two possible causes.

(1) *Water deficiency (dehydration)*. In a dehydrated animal the plasma water content will be reduced, and as the red cells cannot escape from the circulation their concentration, and hence the PCV, will rise. Plasma proteins are also to a large extent (though not completely) trapped in the circulation and so in dehydrated patients the total plasma protein concentration will rise along with the PCV and by approximately the same percentage. However, as other smaller molecules are more or less freely diffusable into the interstitial fluid and tend to be under tighter homeostatic control, concentrations of these are of no use in assessing dehydration; attention should therefore be restricted to PCV, total plasma protein and albumin for this purpose.

(2) *Splenic contraction*. Excitement, apprehension or fright will cause the smooth muscle in the spleen to contract, expelling the stored red cells into the circulation. This is part of the adrenergic 'fight or flight' reaction. Horses, particularly hot-blooded breeds, show this response very readily (it can be very difficult to get a baseline PCV result on a highly-strung

racehorse), but it can occur in all veterinary species. During this occurrence the total plasma protein concentration remains unchanged.

In human medicine the PCV is frequently used alone as a measure of state of hydration, as human subjects do not have a contractile spleen and so apprehension will not affect the results. Also, the normal range for PCV in man is quite narrow. In veterinary medicine it is generally good practice to use both PCV and total plasma protein concentration in conjunction, as the contractile spleen can seriously influence results, especially in horses. In dogs, splenic contraction is usually less of a problem, but the very wide normal range can make interpretation of a single PCV result impossible so far as assessing dehydration is concerned.

The absence of a contractile spleen in human athletes is the reason for the presumed efficacy of altitude training (where the natural effect of the hypoxia of high altitudes is used to induce an increase in PCV which persists advantageously for several weeks after the athlete has returned to sea level), erythropoietin administration and 'blood doping' (where a unit of blood removed from the athlete a few weeks earlier is auto-transfused just before competition to boost the PCV, which has recovered to normal by then). These stratagems produce an artificially increased PCV which improves the oxygen carrying capacity of the blood and so should improve athletic performance, but doubt has been expressed as to whether these procedures have any real effect and they can be dangerous. Altitude training is legal, blood doping and erythropoietin administration are not. In the horse the contractile spleen acts as a natural, endogenous 'blood doping' mechanism, with the PCV of a racehorse commonly increasing from 0.35 at rest to over 0.60 during a race. This means that 'blood doping' as practised by human athletes is a complete waste of time in horses. It also means that assessment of red cell status (PCV, Hb or RBC) is totally useless for predicting either stage of fitness or performance potential of racehorses. This does not prevent it from being widely used for these purposes!

Absolute polycythaemia

In this case the increase in PCV is a consequence of a genuine increase in the absolute size of the erythron. Absolute polycythaemia is much less common than relative polycythaemia. There are several possible causes.

(1) *Polycythaemia vera* is a rare type of myeloproliferative disorder characterized by a marked overproduction of normal-looking, adult red blood cells. It may be thought of as a type of bone marrow tumour. Its diagnosis depends on finding a PCV of around 0.70 or more in a normally hydrated, non-excited animal in the *absence* of any demonstrable respiratory, cardiovascular or endocrine disorder (see secondary polycythaemia, below). Erythropoietin levels, if measured, are normal.

In the past this was treated by repeated phlebotomy, but recently hydroxyurea has come into use as an effective medical treatment.

(2) *Erythropoietin-producing neoplasm of the kidney* is a very rare condition which can be distinguished from polycythaemia vera by a more regenerative RBC picture and higher circulating levels of erythropoietin.
(3) *Secondary polycythaemia* is the term used where the increase in erythron size is a secondary consequence of disease in another organ system. Secondary polycythaemia can itself be divided into two groups, depending on whether or not it accompanies low tissue oxygen tension. Where lowered tissue oxygen is a consequence of disease (as opposed to altitude), cyanosis is usually present and the organs involved are either the respiratory system (e.g. obstructive pulmonary disease) or the cardiovascular system (heart defects involving right-to-left shunting of blood, e.g. tetralogy of Fallot). Blood gas measurements can be helpful in these cases. The causes of secondary polycythaemia unassociated with decreased tissue oxygen tension are mainly endocrine problems where the primary hormone abnormality has a direct effect on erythropoietin production, for example excess cortisol in Cushing's disease. These cases are not cyanotic. In general the PCV values measured in secondary polycythaemia are less spectacularly abnormal than those seen in polycythaemia vera.

Summary, differentiation of the causes of polycythaemia

Relative, erythron size not increased

(1) Dehydration (total plasma protein also raised).
(2) Splenic contraction (total plasma protein unchanged).

Absolute, erythron size increased

(1) Polycythaemia vera (primary disease of the erythron, no evidence of cardiac, pulmonary or endocrine disease. No cyanosis). Erythropoietin-producing tumour may be a differential diagnosis here, but the condition is extremely rare.
(2) Secondary polycythaemia (due to disease of other organ). The only class to consider if many immature cells in circulation.
    (a) Result of low tissue oxygen tension, usually respiratory or cardiovascular disease (cyanosis may be present).
    (b) Tissue oxygen tension normal, usually endocrine disease with hormonal stimulation of EP production (cyanosis absent).

Abnormalities of the erythron: anaemia (strictly oligocythaemia, abnormally low PCV)

Anaemia is almost always absolute. Overenthusiastic administration of i/v fluids may occasionally push the PCV down to abnormally low levels, some cases of

Case 1.2

A 10-year-old black cat was presented as vaguely unwell. Much of the clinical examination was unremarkable, but the mucous membranes were observed to be dark blue in colour. There were no observable cardiac abnormalities. The following haematology results were received. What is the likely diagnosis, and how might it be further investigated?

PCV	0.72		high
Hb	23.7 g/100 ml		
RBC	15.68×10^{12}/l		
MCV	45.9 fl		
MCHC	32.9 g/100 ml		
Total WBC count		5.8×10^9/l	
Band neutrophils	0%	0×10^9/l	
Adult neutrophils	76%	4.4×10^9/l	
Eosinophils	2%	0.1×10^9/l	
Basophils	0%	0×10^9/l	
Lymphocytes	19%	1.1×10^9/l	
Monocytes	3%	0.2×10^9/l	

Film comment. RBCs: normal
WBCs: normal
Platelets: adequate

Comments

Such an extremely high PCV should always arouse suspicions of polycythaemia vera, particularly if it is consistent over more than one sample collected on different days. Other causes of polycythaemia (heart disease with right-to-left shunt, obstructive pulmonary disease, dehydration) would be expected to show clinical signs by the time the PCV reached this level. Mucous membrane colour in polycythaemia vera is usually intense red, and the blue appearance in this cat did initially give rise to suspicions of a heart condition, but none could be demonstrated. Erythropoietin concentration was normal, which confirmed the diagnosis, and clinical response to hydroxyurea was good.

congestive heart failure do become a bit waterlogged now and again, and it can be surprising how low the PCV of a depressed horse with no splenic tone can sometimes go, but in general an anaemia means that the size of the erythron is reduced.

Causes of anaemia can be divided into three basic aetiological classes: haemorrhagic, haemolytic and aplastic (or hypoplastic). The primary aim when attempting to diagnose a case of anaemia is to ascertain which of these three basic causes is involved – only then can a more precise diagnosis be investigated. The very first step, however, is to decide whether the onset of the anaemia is acute or chronic.

Acute onset anaemia

Severe anaemia cases often appear to present as acute onset even when the progress of the disease is actually chronic. This is because in a sedentary animal a gradual insidious decline in PCV, causing a very gradual onset of lethargy and exercise intolerance, often goes unnoticed by the owner until the condition is severe enough to cause obvious distress and/or fainting fits. However, the genuine acute onset anaemia cases are quite easy to distinguish on clinical grounds.

Acute haemorrhagic anaemia

The usual clinical signs are pallor, tachycardia, hyperpnoea and possibly collapse. Diagnosis is nearly always very easy as most cases have clear external evidence of extensive haemorrhage. Only where the haemorrhage is into the abdominal cavity is diagnosis difficult, as the clinical signs can be difficult to distinguish from simple shock, for example, post road traffic accident (RTA). In these cases the presence of blood in the abdomen may be suspected on palpation and confirmed by paracentesis. If there is doubt as to whether the fluid obtained is frank blood or a bloodstained transudate, measure the PCV of the fluid. Frank blood will have a PCV at least as high as the circulating blood, probably higher, as the water is reabsorbed into the circulation before the cells. (Cases of acute haemorrhage into pleural or pericardial cavities do not present as anaemia unless there is concurrent haemorrhage elsewhere, as signs of pulmonary collapse or cardiac tamponade will develop first.) In the very early stages of acute haemorrhage haematological investigation is of little use: because when whole blood is being lost the haematology of what remains will be quite normal (even to a normal PCV) although the animal may be in acute hypovolaemic shock. Over the next few hours as plasma volume is restored the PCV will fall, but haematological evidence of regeneration (immature cells in circulation) will not appear for a day or two. Two aetiologies should be considered.

(1) *Trauma.* Usually due to a road accident; also severe cuts, gunshot wounds, etc. Evidence of haemorrhage is accompanied by signs of trauma – torn claws on cats, road dirt in coat, obvious wounds. Blood clotting is normal. Most of these cases are straightforward, but it is important to check for unseen intra-abdominal bleeding as described above (e.g. ruptured spleen). The first treatment priority is restoration of circulating volume. Plasma expanders (e.g. polygeline 3.5% with electrolytes (Haemaccel: Intervet)) are usually sufficient, as an animal can survive losing up to two-thirds of its blood volume without requiring blood transfusion so long as hypovolaemic shock is prevented. Anaemia due to surgical haemorrhage should be treated in the same way as that due to accidental trauma. However, if severe intractable haemorrhage occurs as a result of minor or routine surgery, particularly in young animals, a clotting defect should be suspected (see Chapter 2).

(2) *Ruptured neoplasm*. Certain neoplasms, especially haemangiomas and haemangiosarcomas, consist largely of blood-filled 'cysts'. When they grow large enough they are prone to rupture with little or no provocation, and it is possible for an animal to bleed out into the abdominal cavity when such a lesion on the spleen or liver suddenly breaks open. However, the first rupture is not often fatal, and the more usual clinical presentation is of intermittent collapse (see p. 20).

(3) *Warfarin poisoning*. Warfarin is an anticoagulant of the coumarin type which acts as an antagonist to vitamin K. Vitamin K is an essential cofactor for the synthesis of prothrombin and several other clotting factors in the liver, and warfarin essentially halts production of these factors, causing a severe clotting deficiency. It is used as a rodenticide and therapeutically to treat navicular disease in horses. Poisoning occurs in small animals due to the consumption either of the rat bait itself or of rodents poisoned by warfarin – the manufacturers claim that it is safe for pets because the irritant bait is supposed to induce emesis in non-target species, but poisoning cases are common. Horses become affected due to overdosage of the therapeutic drug.

Warfarin poisoning in *small animals* is characterized by widespread haemorrhage without any real signs of trauma, obvious wounds, etc. Petechiation of gums, subcutaneous bruising/haematoma formation and blood in faeces and urine are often seen. Bleeding points are usually numerous, and serious intra-abdominal haemorrhage without external evidence of bleeding is unusual. These cases can be distinguished from RTA victims by lack of evidence of trauma and marked clotting abnormalities. Observation of whole blood collected into a test-tube is a very poor guide, but a properly performed clotting time (see p. 296) will show an increase from a normal of under 5 minutes to 10 minutes or more. More specifically, plasma prothrombin time will be prolonged from about 8–10 seconds to several minutes. In *horses* the condition is often less severe, presenting as marked haematoma formation after minor bumps, but occasionally substantial intra-abdominal bleeding can occur without other signs of haemorrhage – these cases can present as colic. To prevent this, all horses on warfarin therapy should have their prothrombin times checked regularly and the dosage reduced if this goes above 16–20 seconds (normally 10–12 seconds in horses).

Treatment is by administration of vitamin K_1 (phytomenadione–Konakion: Roche), a synthetic vitamin which is as biologically active as the natural vitamin (K_2). Note: vitamin K_3 (menadiol, formerly marketed as Synkavit) a water-soluble form of the vitamin intended for oral administration in patients suffering from fat malabsorption, is *not* an effective treatment for warfarin poisoning. Dose rate of Konakion (contrary to the human information on the package insert) is at least 2 mg/kg, and the route of administration should be chosen according to the severity of the case. Intravenous administration will begin to reverse the hypopro-

thrombinaemia in about 4 hours while with i/m administration 12 hours are required. (It has been suggested that s/c administration may be at least as effective as i/m, which seems reasonable, as vitamin K is a fat-soluble vitamin.) If ongoing haemorrhage is severe enough to endanger life in less than 4 hours, whole blood transfusion is necessary. The dose is 10–20 ml/kg depending on need, from a donor of the same species. The primary reason for transfusion is to give the patient active clotting factors, so the blood must be *fresh*. (Stored blood, or even blood removed from the patient's own pleural cavity and auto-transfused via a filtered giving set, will provide emergency oxygen transport but will not aid haemostasis.) Chest drainage may be necessary to prevent respiratory failure, but blood in the abdomen should not normally be removed as it will eventually be reabsorbed into the circulation. Konakion treatment should be repeated at 12-hour intervals for several weeks as the poison tends to persist – once the prothrombin time has returned to normal oral administration is usually sufficient. The use of 'second-generation' coumarins such as bromodiolone is becoming more widespread. These are extremely persistent and dogs have been known to suffer sudden haemorrhage even months after the initial episode. When these agents are involved it is prudent to continue oral Konakion for two or three months – this can be expensive, but so is emergency drainage of a chest full of blood! It is best to check the prothrombin time 4–6 days after the last tablet and restart treatment if an abnormality is found.

Acute haemolytic anaemia

As with haemorrhage, these cases present as collapsing, hyperpnoeic animals with marked tachycardia and a haemic murmur. However, pallor may not be evident – instead, jaundice is often present. In these cases PCV is reduced even from the earliest stages of the condition as no plasma is being lost concurrently. Initially free haemoglobin is seen in the plasma (but great care must be taken to avoid causing haemolysis of the sample by poor blood collection and handling, or diagnosis may be misleading), and as the disease progresses this is replaced by bilirubin (unconjugated), which gives rise to icterus or jaundice. However, note that the degree of clinical jaundice is seldom so marked as that seen in liver disease. Both haemoglobin (red) and bilirubin (orange-yellow) can be seen in the plasma layer of a micro-haematocrit PCV tube. Where there is free haemoglobin in the plasma the calculated MCHC will appear higher than normal, as the calculation assumes that this haemoglobin is inside the cells. In addition, haemoglobinuria is often present and may be demonstrated in a urine sample by a dipstick test (free haemoglobin in urine can be differentiated from red cells on the strip if only small amounts are present, or by centrifugation where large amounts are present, see p. 304). While unconjugated bilirubin should not, theoretically, appear in the urine (as it is albumin-bound), animals which are jaundiced as a

result of haemolysis usually do show a positive urine bilirubin test. However, beware of false positives in this test (see p. 171).

In any one species the specific diagnoses associated with acute haemolytic anaemia are limited. Causes can be:

(1) *Infectious*, for example *Haemobartonella felis* (acute cases), *Leptospira icterohaemorrhagiae*, *Babesia* spp., bacillary haemoglobinuria (*Clostridium haemolyticum*), and others. In the UK, babesiosis does occur in cattle in tick-infested areas. Canine babesiosis has been reported in dogs entering the country under the Pet Passport scheme, and equine babesiosis is occasionally seen in imported horses. Ehrlichiosis and leishmaniasis have been rare in the UK but increased vigilance is wise following the relaxation of the quarantine laws. Feline infectious anaemia (FIA, caused by *Haemobartonella felis*) is occasionally seen in its own right. However, the other conditions are rare, and even FIA usually manifests secondarily to immunosuppression caused by such things as feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV).

(2) *Toxic*, for example copper poisoning (sheep) – due to chronic excess of dietary copper stored in the liver suddenly being released to cause massive acute haemolysis (see p. 98). Acute brassica poisoning (see p. 22) may also be included here.

(3) *Ag/Ab reactions*, for example haemolytic anaemia of the newborn. This is a condition of horses similar to the 'Rhesus baby' syndrome, in which the mare forms antibodies to the 'foreign' red cells of her foal. Like the Rhesus baby problem it does not affect the first pregnancy, but second and subsequent foals with maternally incompatible red cell antigens will be affected. Unlike the Rhesus babies, which are affected *in utero*, these foals are healthy until they are born and begin to drink the colostrum, due to the different mare placental structure which does not allow antibodies to cross. Affected foals must not be allowed to suck but should be fostered or hand-fed. A similar condition has been described in cats, usually associated with cross-suckling in households where more than one queen is nursing a litter at the same time. Transfusion reactions, due to a second transfusion of incompatible blood, are also included in this category.

Autoimmune haemolytic anaemia (AIHA), which is common in dogs and occurs occasionally in cats, can present as acute haemolysis. However, a more chronic presentation is more usual and so the condition is discussed under that heading (see p. 22).

Treatment is specific to the cause of the condition, plus blood transfusion (transfusion of packed red cells is even better) if the PCV falls dangerously low (below about 0.15 in acute cases). The safest transfusion for a foal with haemolytic anaemia is *washed* red cells from the mare (*not* whole blood, which contains the offending antibodies). Failing this, whole blood from a horse (preferably a gelding) which is not related to the foal's father can be used. As hypovolaemia does not occur, i/v administration of non-blood fluids is merely

supportive and may aid renal function where this is impaired by excessive free haemoglobin in circulation.

Gradual onset anaemia

In gradual onset anaemia the PCV falls gradually over a period of days or weeks, plasma volume expands concurrently to compensate, and patients are not presented in acute hypovolaemia. Before considering the differential diagnoses it is important to consider the severity of the condition as this will affect the presenting signs and the interpretation of the haematological findings.

Mild/moderate anaemia (PCV below normal but still above 0.20–0.25) is often found when a full haematological examination is performed on an animal presented with a history apparently related to something quite different. In these cases the low PCV should be considered together with all other clinical and laboratory findings when arriving at a diagnosis, and can often be very helpful in this. However, as most animals will not be particularly inconvenienced by a PCV which is over 0.25, the anaemia is unlikely to be the main presenting sign, and so its investigation will not necessarily be the first priority in assessment of the case.

Severe/very severe anaemia (PCV below 0.20–0.25 down to about 0.05–0.06 which is more or less fatal) often presents as sudden onset illness because the insidious deterioration of the animal as the anaemia progresses has not been noticed by the owners. However, once the PCV falls to about 0.12–0.15, collapse and fainting will occur. These cases are weak, have poor exercise tolerance, show marked tachycardia with a pronounced haemic murmur (perhaps also tachypnoea/hyperpnoea) and have a history of collapse. If the extreme pallor of the mucous membranes is overlooked these may be mistaken for signs of cardiac disease. When an animal presents with these signs and a PCV below 0.20 the investigation of the anaemia is usually the first priority.

In any investigation of anaemia the major aim is to discover which of the three possible aetiologies is involved – haemorrhage, haemolysis or bone marrow failure. This is done by a combination of the examination of the *morphology* of the red cells, which is different in each case, and the piecing together of a number of other haematological and biochemical tests. Once the aetiology has been discovered, the basic cause of the problem can be investigated.

Chronic haemorrhagic anaemia

In cases of chronic haemorrhage the loss of blood is not always easy to appreciate and it is often necessary to establish the fact of haemorrhage first by other methods, then look for the source.

Red cell morphology

In small animals, there will be evidence of regeneration: many polychromatophilic cells are present together with some nucleated red cells. In the

early stages the polychromatophilic cells will be macrocytes (large, i.e. MCV will be increased) and the adult cells will be normocytic and normochromic (see Plate 8). However, in long-standing cases the continuing loss of red cell constituents (iron, protein, etc.) leads to a secondary bone marrow exhaustion. This results in the cells becoming gradually more and more hypochromic (i.e. MCHC is reduced, due to iron deficiency) and smaller, and in very long-standing cases even the young cells, although still polychromatophilic, become hypochromic and microcytic (see Plate 9). Misshapen cells – poikilocytes, folded cells, cup/bowl cells and sometimes target cells – may appear. With the exception of extremes of starvation and some rather obscure malabsorption conditions, chronic haemorrhage is the only cause of iron deficiency anaemia seen in adult animals.

In large animals morphological evidence of red cell regeneration (i.e. young cells in circulation) is often absent, particularly in horses, but again as the condition progresses signs of bone marrow exhaustion will appear. This means that in these species diagnosis may have to be made on grounds other than erythrocyte morphology, and particular care must be taken to differentiate long-standing haemorrhage cases from primary bone marrow problems.

Other haematology

In cases where the haemorrhage is not caused by thrombocytopenia, the platelet count will often be raised (i.e. over 400×10^9/l); this is known as reactive thrombocytosis and is due to the consumption of platelets at the site of the lesion feeding back to step up production. Other coagulation tests (e.g. clotting time and prothrombin time) may be *slightly* abnormal due to excessive consumption of clotting factors. If the site of haemorrhage is infected, neutrophilia and/or monocytosis may also be present. In cases with a primary clotting defect the platelet count and/or coagulation tests should provide the diagnosis; see Chapter 2.

Biochemistry

As plasma is being lost along with the red cells, a progressive hypoproteinaemia, particularly hypoalbuminaemia, will develop. Plasma bilirubin will usually not be elevated unless liver disease is also present, but mild jaundice is occasionally seen when a large haematoma or intra-abdominal haemorrhage is being reabsorbed.

Site of haemorrhage

Possible sites of chronic haemorrhage where the bleeding can go unnoticed by the owners are gut, urinary tract and skin (bloodsucking ectoparasites).

Intestinal bleeding is the most common. There may be altered blood in the vomit ('coffee-grounds' appearance), and blood will always be detectable in the

faeces. If the lesion is low down in the large intestine this may be seen as obvious fresh blood, but more usually the lesion is higher up (stomach/small intestine) and so the blood is digested and appears in altered form as a black colour in the faeces, called melaena. This 'occult blood' can be specifically demonstrated by the guaiac acid paper test (see p. 173). Carnivorous animals can show false positives due to haemoglobin in the diet, and so ideally these should be put on a meat-free diet for 3 days before testing (although as the patient is often anorectic this is not always necessary). Licking of a superficial bleeding wound and swallowing coughed-up blood will also produce positive results. Lesions to look for are ulcers (single or multiple), bloodsucking endoparasites (e.g. hookworm), bleeding ulcerated tumours, etc. In addition, liver failure patients are frequently hypoprothrombinaemic, and this, combined with increased portal venous pressure, can produce diffuse intestinal bleeding.

Urinary tract bleeding is easy to demonstrate, as a urine sample will give a positive blood result on dipstix test. Where only small amounts are present it is possible to distinguish whether this is due to blood cells (i.e. haemorrhage) or free haemoglobin (as a result of *haemolytic* disease) simply by examining the reagent patch for a stippled appearance (blood cells). However, where large amounts are present it will be necessary to centrifuge the sample and examine the sediment microscopically (see p. 304). Clinical conditions involved include severe chronic cystitis with bladder ulceration, and chronic bracken poisoning in cattle (a carcinogen in bracken leads to numerous small haemorrhagic, neoplastic lesions in the bladder). It is, however, quite unusual for enough blood to be lost from the urinary tract to cause anaemia in small animals.

A heavy infestation of bloodsucking ectoparasites (particularly lice and ticks, but fleas may also be to blame) should not be difficult to detect, but the owner may have treated the animal before presenting it, and so this should be suspected if the coat is poor and suggestive lesions are visible. It is surprising how severe an anaemia can result from a heavy flea infestation in cats, especially young kittens.

Intermittent intra-abdominal haemorrhage

This is a type of haemorrhagic anaemia which often presents differently from those discussed above. The animal (usually a dog, often a German shepherd dog) is presented with a typical history of anaemia (pallor, weakness, etc.), but even if no treatment is given it may recover almost miraculously by the following day. Several episodes of this nature may occur before one is severe enough to be acutely fatal. A blood sample taken when clinical signs are evident will show the typical low PCV and low plasma protein concentration of haemorrhage cases, but the red cell picture is often not particularly regenerative. A blood sample taken the following day may be absolutely normal, again often without signs of excessive regeneration. This is because blood lost into the abdomen will be reabsorbed (cells, protein and all) back into circulation within a day or so of the haemorrhage; therefore the bone marrow does not need to put in any special