Pitfalls in the Interpretation of Blood Chemistry Results*

P. PANNALL, *Department of Chemical Pathology, University of the Orange Free State, Bloemfontein*

SUMMARY

Apparently abnormal blood chemistry results may be caused by many factors other than disease. Incorrect specimen collection with excessive venous stasis and haemolysis, or undue delay in separating cells from serum can affect a number of tests. Medication may also influence results, notably the protein-bound iodine, as may procedures such as rectal examination and intravenous fluid administration. Interpretation of results requires full clinical information as well as a knowledge of the time of day that the specimen was collected. Normal ranges should be used intelligently and may vary with age, sex, race and diet. There is no clearcut distinction between values found in healthy persons and those with a particular disease. Difference between laboratories makes it imperative that results be compared only with the normal range issued by the laboratory performing the test.

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Most measured biochemical changes in the blood indicate deranged metabolism or disturbed function of one or more organs; few are diagnostic of a specific disease. Intelligent interpretation requires a full knowledge of the clinical picture. With the development of multichannel automation and the current craze for multiphasic screening it is not unusual for 15 or more chemical estimations to be performed on a single specimen of blood. Particularly in the case of well-population screening, these tests may be performed before the patient is examined. With the full clinical background and, regrettably, sometimes without it, an unexpected and apparently anomalous result is often ignored as being due to laboratory error. This suspicion is strengthened if on repeat testing the abnormality has disappeared. The magnitude of the problem is shown by an early series of 1 300 hospital admission profiles.[24] An unexplained abnormal result of a test that would not otherwise have been requested, was found in 38·5% of the profiles.

It is perhaps insufficiently appreciated that a wide variety of factors other than disease can produce results outside the quoted normal range. Such factors include incorrect specimen collection and handling, laboratory and physiological variation and the results of therapeutic procedures on the patient. An additional problem is introduced by the pattern of medical practice in South Africa where laboratories tend to be concentrated in the larger centres. Specimens from other areas may be in transit for several hours or even days and require special attention. The purpose of this article is to draw attention to these factors and indicate ways of minimizing their effects. The concept of the normal range is also examined. Discussion is confined to the tests most commonly performed in routine diagnosis or screening. Special investigations frequently require particular care in collection and handling and will not be considered here.

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LIMITATIONS OF THE NORMAL RANGE

For most constituents of the blood there is no clearcut distinction between values found in healthy and ill persons.[11] Any normal range that includes all healthy persons will contain an unacceptable number of pathological results. Published normal ranges, e.g. plasma urea of 15 - 40 mg/100 ml, usually include about 95% of the values found in apparently healthy people. This implies that 1 in 20 normal people may have a plasma urea outside this range. While a value of 200 mg/100 ml is clearly abnormal, no such confident decision can be made on an isolated observation of 43 mg/100 ml; nor does a figure of 35 mg/100 ml assure normal renal function. Apart from the relative insensitivity of plasma urea as an index of early renal disease, such a value may represent a rise from a previously constant level of 20 mg/100 ml and is a significant change.

The wide range quoted for many constituents of the blood is due to the inclusion of a heterogenous population. One of the first tasks of a multiphasic screening programme should be to establish accurate ranges[23] subdivided into sex and age groups. This, however, will raise the problem of 'desirable' as opposed to 'actual' normal ranges. For example the range of plasma cholesterol in males of 50 years has been given as 150 - 340 mg/100 ml.[9] This may include an undisclosed number of persons with subclinical atheromatosis or hypothyroidism and many would consider the desirable upper limit of cholesterol, for any adult, to be about 250 mg/100 ml.

Laboratory Factors and the Normal Range

The reproducibility of many estimations, even with rigid quality control, also affects interpretation. Urea, for example, by most methods has an error of about 5% and changes smaller than this may be ignored. For calcium, on the other hand, such variation is unacceptable and estimations should be accurate to within 2%. As a rough guide, the precision of most routine laboratory tests is about 3%. These factors are included in published normal ranges.

The type of specimen may also influence results. Some laboratories perform most estimations on plasma while others use serum and results are not necessarily comparable. Protein levels are higher in plasma because of the inclusion of fibrinogen, while potassium levels in serum are on the average 0·3 mEq/litre higher than those in plasma, probably due to the release of potassium from platelets during clotting.[18] These differences should, however, be allowed for in the issued normal range. A more important factor affecting comparison of results from different laboratories is the method of estimation used. A blood glucose level of 115 mg/100 ml by a method measuring reducing substances may be normal, whereas it is elevated if estimated as 'true glucose'. Other tests significantly affected by differences in methodology include plasma albumin, calcium and protein electrophoresis. Plasma enzyme methods and units are multiple and even with the introduction of International Units[21] differences in technique may give very different answers.

The clinician is not expected to assess these factors; it is incumbent upon every practising laboratory to issue the normal ranges for the method in use. It is, however, necessary for the clinician to appreciate that uncritical comparison of results with a normal range expressed in a textbook or by another laboratory is meaningless and possibly dangerous.

PHYSIOLOGICAL FACTORS AFFECTING LABORATORY RESULTS

Age

Many normal values in the newborn and infant differ markedly from those in the adult. As examples may be quoted the low plasma cholesterol at birth (± 70 mg/100 ml)[13] and the elevated protein-bound iodine[4] (PBI) which may be in the hyperthyroid range. Both of these reach adult levels at about 1 year. The blood glucose may be as low as 40 mg/100 ml for the first few days of life.[17] In the infant the low levels of IgG at 3 - 6 months are well recognized as a physiological phenomenon.[3] Throughout childhood the range of inorganic phosphate and alkaline phosphatase is higher than that in the adult;[6] these fluctuate with periods of active bone growth and may only reach 'adult levels' at 17 or 18 years.

Sex

The higher levels of plasma uric acid and iron in the male after puberty are well recognized and are probably of hormonal origin. It is likely that many constituents of the blood show small sex differences at present hidden in the wide 'normal' range.

Diet

Although variation in the cholesterol content of a normal diet has little effect on plasma cholesterol concentration,[12] levels are higher in an affluent than in a poorly nourished population. In an individual the plasma urea concentration varies with protein intake.[16]

Race

It is extremely difficult to separate differences due to ethnic factors from those due to environment. Normal ranges for various estimations in the Bantu have been published but figures are not easily available. The commonly elevated gammaglobulin levels in the Bantu may be due to a greater incidence of chronic disease although a similar observation has been made in North American Negroes as compared to Whites living in the same environment.[20]

Pregnancy

In pregnancy increased synthesis of several carrier proteins leads to raised levels of transferrin,[14] caeruloplasmin, cortisol-binding globulin[15] and thyroxine-binding globulin (TBG). The elevated TBG produces protein-bound iodine (PBI) values of 12 - 14 μg/100 ml[10] and low values for the T_3-resin uptake test while free thyroxine levels are normal. Plasma cholesterol, triglycerides and iron levels are also raised and should not be misinterpreted. In the last trimester of pregnancy there is an increase in plasma alkaline phosphatase due to production of the enzyme by the placenta.[19]

Diurnal and Random Variation

A number of blood constituents such as plasma cortisol,[15] iron[7] and inorganic phosphate[5] vary over a period of 24 hours. Most changes are relatively small and are included in the normal range. The variation becomes important, however, in assessing a series of estimations on specimens taken at different times of the day. The diurnal variation of plasma cortisol is used in the diagnosis of Cushing's syndrome. Plasma iron levels in the early morning may be twice those found in the late afternoon; in women iron may fall to sideropenic levels immediately before menstruation[27] and in both sexes large, apparently random, day-to-day variations occur. All these factors make an isolated plasma iron estimation particularly difficult to interpret.

EFFECTS OF MEALS, THERAPY AND DIAGNOSTIC PROCEDURES

Unlike the physiological factors discussed above, this group produces changes in blood constituents that, if misinterpreted, may lead to incorrect diagnosis.

Recent Meals

Ideally all routine chemical analyses should be performed on sera from fasting patients. This achieves standardiza-

tion of time of collection and avoids interfering effects such as turbidity of the specimen. In practice this is rarely possible and not always necessary. The concentrations of most blood constituents, including plasma cholesterol, are little affected by a recent meal. Variable elevation of glucose and triglycerides in the first few hours after food ingestion, however, makes interpretation difficult; for triglycerides particularly a fasting specimen is required. There is also a temporary lowering of plasma potassium and inorganic phosphate post-prandially, accompanying glucose uptake by cells.

Drugs

A large number of drugs produce changes in blood chemistry, some as a side-effect or extension of their primary action, e.g. the hypokalaemia and hyperuricaemia of thiazide diuretics, and others as a result of tissue damage, e.g. raised transaminase levels with liver involvement. These cannot strictly be called anomalous results but should not be misinterpreted as independent pathlogical processes. The relation to recent administration also has an effect; after an oral dose of potassium or iron, for example, the corresponding plasma levels are raised for a short while.[25]

Some medications strongly influence results and the test most subject to this is the PBI.[4] Markedly raised levels are seen for weeks or months after administration of iodine-containing X-ray contrast media. If the result is above about 25 μg/100 ml it is obviously due to some form of interference. Smaller rises may not be so apparent and may mask hypothyroidism or mimic thyrotoxicosis. Important causes of moderate elevation are the prevalent cough mixtures containing potassium iodide and certain amoebicides much valued by travellers. The problem is of such magnitude that the PBI is becoming a suspect test and is being replaced by methods measuring thyroxine other than by its iodine content. Low PBI values in euthyroid subjects may be due to displacement of thyroxine from TBG by drugs such as salicylates (usually only in large doses) and diphenylhydantoin. Mercurial diuretics or gold injections interfere with certain methods of analysis and may produce falsely low values of PBI.

The main confuser in the drug field at present is the oral contraceptive. The metabolic and biochemical effects are many, and are briefly reviewed elsewhere.[26] Several mimic the changes seen in pregnancy, with plasma iron and lipid values approaching those of males. Increased carrier-protein synthesis leads to raised levels of cortisol, transferrin, caeruloplasmin and PBI. The latter is the most troublesome as values may be in the thyrotoxic range. T_3-resin uptake is however low, and free thyroxine levels are normal.

Rectal Examination and Acid Phosphatase

Although the rise in prostatic acid phosphatase after rectal examination, catheterization or with acute urinary retention has been recognized for a long time,[2] specimens are still frequently received for acid phosphatase estimation after such a procedure. The elevation is usually moderate but may persist for up to a week and the test should be repeated after this interval of time.

Intravenous Infusions

An eminently avoidable error that nevertheless occurs from time to time is to take blood from a limb into which intravenous fluid is being administered. Such a specimen, even if taken distal to the site of infusion, reflects the composition of the fluid. The results of electrolyte estimation, for example, may be ridiculous and the cause realized but, depending on the fluid being infused, apparently normal values may induce a dangerous complacency.

ARTEFACTS PRODUCED BY INCORRECT HANDLING OF SPECIMENS

This is a very important but frequently ignored cause of abnormal results. If the discrepancy is gross, the cause may be obvious and no harm is done other than the need for a repeat test. The danger lies in the production of feasible though erroneous results, normal or abnormal, upon which treatment may be based. Most of the factors to be described are avoidable. The examples in Tables I and II were produced in the laboratory for illustration.

TABLE I. EFFECT OF PROLONGED VENOUS STASIS ON RESULTS

Stasis for	0 min	2 min	4 min	6 min
Calcium (mg/100 ml)	**9·5**	**9·8**	**10·1**	**10·3**
Total protein (g/100 ml)	**7·2**	**7·4**	**7·7**	**8·0**
Albumin (g/100 ml)	**3·9**	**4·0**	**4·2**	**4·3**
Haemoglobin (g/100 ml)	**14·7**	**14·8**	**15·1**	**15·5**

TABLE II. EFFECT OF DELAYED SEPARATION OF BLOOD ON LEVELS OF GLUCOSE AND POTASSIUM

Blood separated after	0 h	4 h	8 h	24 h
Potassium (mEq/litre)	**4·0**	**4.3**	**4.8**	**6·4**
Glucose (mg/100 ml)	**87**	**71**	**54**	**35**

Venous Stasis During Collection

It is usual to apply some form of venous constriction to facilitate venepuncture. In the case of 'difficult' veins a tourniquet may be left in place for several minutes before the specimen is obtained. Local anoxia causes releases of intracellular components such as potassium and plasma levels are raised. In addition, water and small molecules pass out of the vascular compartment with resultant haemoconcentration, and raised levels of large molecules such as protein and cellular elements. As calcium is partly bound to albumin its levels also increase (Table I).[5] This is the commonest cause of a mildly elevated plasma calcium. If a tourniquet is used it should be released once the needle is

in the vein and blood for calcium, protein or haemoglobin estimation withdrawn only after normal flow is established. Elevations of PBI of up to 2 μg/100 ml have also been described as due to this cause.[4]

Haemolysis

Even minor degrees of haemolysis result in raised concentrations of substances normally contained largely within red cells. Such specimens give falsely elevated values for potassium, inorganic phosphate, lactate dehydrogenase, aspartate aminotransferase (SGOT) and total acid phosphatase. The presence of haemoglobin in serum may interfere with some estimations, e.g. certain methods for cholesterol and bilirubin. Haemolysis may be due to difficult venepuncture or, more commonly, to avoidable causes such as use of a wet syringe or forcibly ejecting the specimen into the tube through the needle.

Delay in Separating Cells from Serum

The normal concentration gradient of electrolytes across the red cell membrane requires energy derived from glucose metabolism. If metabolism is slowed by refrigerating the unseparated blood, or as glucose is consumed by the erythrocytes, intracellular ions diffuse into the plasma, as in haemolysis, but with the important difference that the separated serum or plasma appears clear so the artefact is less obvious. This results in elevated potassium levels in both cases and low glucose values in the latter instance (Table II).

Specimens for glucose estimation must be taken into special containers with fluoride as a preservative which will inhibit glycolysis. For all other specimens, if there is a delay of more than a few hours, the serum should be separated from the cells before transfer to the laboratory. Unseparated specimens should never be refrigerated.

Age of Specimen

Even with separated serum, certain estimations must be completed without delay. Both creatine phosphokinase and tartrate-labile acid phosphatase are unstable enzymes and specimens for these investigations must reach the laboratory within a few hours if reliable results are to be obtained.

Contamination of the Specimen

This is not a common source of trouble but can give extremely bizarre results. An error that occurs from time to time is contamination by ammonium or potassium oxalate or sequestrene. These are used as anticoagulants in containers for haemotology specimens and act by precipitating or chelating calcium. Such tubes are usually prominently marked to indicate the volume of blood required and, in his enthusiasm to comply with this, the collector tips any excess into a tube for chemical tests. This may result in (depending on the method used) low or absent calcium and raised potassium or urea results.

A source of contamination for calcium is the use of cork stoppers.[1,22] If blood is left in contact with the stopper for any length of time spurious high calcium values may be obtained. The effect is unpredictable but may introduce an error of 1 - 1·5 mg/100 ml. Such tubes should be kept upright.

CONCLUSION

This article has attempted to bring to the attention of users of a chemical pathology laboratory a number of factors, other than disease, that may affect results. The list is not complete and only major examples have been mentioned. It will have succeeded in its purpose if it causes the clinician to consider the possibility of anomalous, though genuine, results in the interpretation of a laboratory report.

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