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EDITORIAL

With this issue of "Clinical Biochemistry Reports" we note the arrival of Mr. M. J. Golbey who will assume responsibility for Clinical Biochemistry at Makerere, and who consequently will be pleased to discuss any clinical Biochemistry problems.

Since the first edition of the "Reports", the member of samples analysed by this laboratory has been slowly increasing, but we have by no means reached our capacity as yet, and invite those who wish to do so to make full use of our service.

We are also expanding our range of tests and as well as the original tests offered (serum Na, K, GOT) we are now able to offer serum lithuim, creatinine, alkaline phosphatase and total protein estimations, with bilirubin to be added in the near future.

Our plans for the future expansion of the service include the introduction of serum calcium, phosphate, urinary steroid assays, but we would very much like to receive your suggestions of the analyses you would like to see performed by this laboratory.

As new assays are introduced, a "normal range" will be quoted for each test based on published figures. This is not very satisfactory, but will have to suffice until the laboratory has analysed sufficient samples to determine its own "normal range" which will then be published.

Quality control procedures are becoming well established in the laboratory and we are able to quote our analytical results with confidence. We hope to be able to publish quality control results in the next issue of the "Reports".

Finally, we still invite comment and criticism and will be pleased to use these "Reports" as a forum for your views if you so wish.

The Editor

S.- I. UNITS

by T. R. C. BOYDE

The S.- I. (Systeme-Internationale) system of units is now being widely adopted throughout the world of Science, under the active sponsorship of national scientific bodies. There seems no reason other than inertia why this change should not be made in Clinical Biochemistry, and, indeed, in this laboratory it is intended to report in S.-I. units from the outset. The International Federation of Clinical Chemistry (I.F.C.C.) has advised that the change should be made and is arranging for the publication of explanatory articles in many medical journals throughout the world (see references). As far as we know, however, no major hospital laboratory has yet dared to convert wholly to the new system, although there is little remaining to be done as far as really common tests are concerned, most being already reported in compatible units. Significant changes will be required for urea, glucose, cholesterol, iron, calcium, phosphate, creatinine, and uric acid. There will undoubtedly be some difficulty at first. There was difficulty over reporting electrolyte results in mEq./l. instead of "mg.NaCl/100 ml." and "vols% CO_2", but no one now doubts that the change was worthwhile. Even if these present proposals were to be accepted at once for world-wide use, clinicians would undoubtedly go on thinking in the old units for several years, and results quoted in the old literature will still require conversion. It is therefore desirable that tables of conversion factors should be easily available, at all times, in the form of local publications as well as in the regular scientific journals.

The essential features of the S.-I. system are:-

a) The adoption of a standardized and simplified set of symbols for basic units of mass, length, time, etc., and for some derived units. Note also that it is recommended to omit the stop indicating an abbreviation - g and not g. for gram, ml and not ml. for millitre.

b) The adoption of a standard set of prefixes to indicate multiples and submultiples, and that these should be at intervals of 10^3 (see table 1).

c) The exclusion of all other units and symbols if the quantities involved could be expressed in S.-I. units. Taken by themselves, these proposals do not exclude the use of weight concentration units, but the I.F.C.C. have recommended in addition that molar units should be used whenever possible.

It should be stated that the I.F.C.C. proposals (see references) are expressed in rather difficult and philosophical terms which may deter many would-be students, and include a number of suggestions extraneous to the question of the introduction of S.-I. units. The essence is to grasp the simplifying principle of the S.-I. system and in addition:
1) to distinguish mentally between units of quantity (mass, moles) and units of intensity (concentration, density, rate);
2) to carry at all times a mental picture of the physiological and technical processes which lead up to a given analytical result. Most of the additional suggestions of I.F.C.C. then become simply unnecessary as well as potentially confusing.

There is so much to be said in favour of these proposals that we have no doubt of the value of adopting them as soon as practicable. The whole world is going this way, if slowly. The number of different kinds of units and symbols in medical papers will become less, and those in use will be much easier to manipulate. The possibilities of misunderstanding arising from incorrect terminology will be diminished. All or nearly all quantities in Clinical Biochemistry will be expressed in molar terms - proportional to the actual numbers of molecules present. This is important, first because conducive to clarity of thought, but perhaps more practically because directly related to osmolarity and rather simply to ionic strength and buffering capacity.

Some of the traditional units can be defended on the basis that they represent actual manipulations in making up standards for analysis, thus minimizing the chance of error (e.g., glucose, urea - mg/100 ml). This is fair comment, although in many cases the direct relationship of standards to unknowns is lost to the technician in the mumbo jumbo of analytical procedure. What, however, of cases like calcium - expressed in mg/100 ml although it is usual to employ calcium salts in making up standard solutions (i.e. calcium itself is not weighed out directly)? Other, worse, examples are dealt with below, but perhaps the most glaring case is that of phosphate. The most-used traditional unit is mg P/100 ml, which might be expressed fully as "mg per 100 ml of phosphorus atoms present in the form of inorganic phosphate ions". The unit was adopted presumably to evade facing the difficulty that phosphate was present as two ions, $H_2PO_4^-$ and HPO_4^{2-}, in varying proportions according to pH, and that it was, therefore, going to be difficult to express results in terms of the mass of phosphate ions. The difficulty vanishes when one uses molar units, since both forms are included.

Electrolytes

In reporting electrolytes results mM(mmol/l) will replace mEq./l. and mmol replaces mEq. There is no change in the numbers reported for Na^+, K^+, Cl^-, HCO_3^-; i.e. the conversion factor is 1.0. It should be explained that there is no fundamental objection on chemical grounds to regarding an ion as a molecular species and reporting its concentration in molar terms.

Certain laboratories have reported calcium results in mEq./l. This has the particular disadvantage that about half the calcium in plasma is not present as free ions, as might be thought from the use of mEq. terminology. The new proposals eliminate this source of ambiguity.

Faecal Fats.

Faecal fats are commonly estimated by titration of the liberated fatty acids. This result, determined in millimoles, is then laboriously converted to g stearic acid - although much of the acid is not in fact stearic acid. The results would now be reported in millimoles/24 hours. If a gravimetric technique is used, it would be appropriate to continue reporting in g/24 hours.

Difficult cases.

Molar units cannot be so readily used where several substances are estimated together (for example, total protein, 17-oxosteroids) although there can be no objection where the reaction of one particular chemical group is employed in the determination and it is known that all the substances concerned give the same molar yield under the conditions of assay employed. Alternatively, one standard substance can be used to represent all others and results expressed in terms of the standard - as is already commonly done for both the above examples. (The fact is not usually published to clinicians although it accounts for some of the recorded discrepancies between methods and between laboratories.) For the time being, weight units will continue in use for the above and similar cases. Albumin, and other well-characterized proteins such as haemoglobin could readily be reported in molar terms, but it is felt better for the time being to retain uniformity with the units used for total protein.

Enzymes.

In many hospital laboratories, enzyme results are already quoted in S.-I. units, but this is not yet the case at Mulago. A warning note may be given here. The international unit of enzyme activity is not immutable since it is not possible to lay down sensible standard conditions of assay for every conceivable enzyme. Variations in methods thus lead to different results, even when expressed in I U, and hence to changes in the normal range. In addition, the relationship between certain conventional enzyme units and the corresponding I U values is largely notional, and different assay methods may respond differently to varying proportions of isoenzymes in the samples tested. Thus, detailed interpretation of enzyme results can only be carried out when in full knowledge of the pitfalls in each particular case. At a slightly lower level the laboratory should always be able to say exactly what assay and calculation procedures are used, so as to allow a comparison with other laboratories, and at a lower level still one should be able to judge the individual result against the laboratory's own "normal range".

Enzymes will perhaps eventually be reported in terms of molar units of enzyme molecules themselves - but this is certainly many years off.

Symbols.

Some relevant S.-I. symbols and their verbal equivalents are given in Table 1.

Local variations in symbols.

The units and symbols employed here will always be in accordance with the S.-I. system, but in a few instances a form of symbol will be used which differs slightly from that recommended by I.F.C.C. (Strictly speaking, the I.F.C.C. recommendations are a sub-set of S.-I.)

For concentration, I.F.C.C. has recommended the form mol/l ("moles per litre"), instead of M ("molar"). This is firstly because the fuller form expresses directly that we are dealing with a concentration term, not a simple measure of quantity and secondly because the symbol M has been sadly misused in the past. It is extremely common in otherwise respectable medical journals to find M used for "mole", mM for "millimole", and so on. This is a quite unforgivable misuse of chemical terminology, which even goes to the extent of confusing concentration and quantity terms. Nevertheless, it has occurred and the fact must be lived with. A wish for brevity, plus chemists' prejudice, inclines us to use the older form but it will be abandoned if it appears that any confusion results.

To emphasize the break with convention, enzyme concentrations will be expressed in international units per litre (IU/l). This is because if the form U/l is used it is necessary to specify on each occasion what units are employed as well as what method. (It is possible to run a phosphatase determination by the King-Armstrong method and express the results either in King-Armstrong units or in international units - see above).

In expressing gas pressures or partial pressures we will for the time being use both "newtons per sq. metre" (N/m^2) and the slightly more familiar "millibar" (mbar). These differ only by a factor of 100 (1 mbar = $100N/m^2$). N/m^2 is preferred by I.F.C.C. as being closer to the fundamental units; we may note, however, that the same can be said of the unit of volume, the litre now being redefined as $0.001m^3$. In this case, it is presumably thought not politic to make the change immediately and the same may be argued about units of pressure.

Conversion factors.

A sample table of conversion factors is given below. Its use may be illustrated by examples. If the blood glucose is reported as 10 mM this may be converted to conventional units as follows:- 10 x 18.0 = 180 mg./100 ml. Conversely a serum calcium result of 11.7 mg./100 ml. may be expressed in S.-I. units thus:- 11.7 x 0.25 = 2.92 mM.

References:

Dykbaer, R., (1969) American Journal of Clinical Pathology, 52, 637

Dykbaer, R., (1968), Annals of Internal Medicine, 69, 621

Dykbaer, R., and Jorgensen, K., (1967) "Quantities and Units in Clinical Chemistry", Williams and Wilkins Co., Baltimore.

TABLE 1

BASIC SYMBOLS

Symbol	Meaning
g	gram
l	litre
mol	mole (gram molecular weight)
m	metre
N	newton (force)
bar	bar (pressure)
s	second (time)
h	hour

PREFIXES

Symbol	Factor	Prefix
p	10^{-12}	pico
n	10^{-9}	nano
µ	10^{-6}	micro
m	10^{-3}	milli
k	10^{3}	kilo
M	10^{6}	mega

TABLE 2

Substance	Conventional Units	Proposed Units (S.-I.)	Conversion Convention-al to S.-I.	Factors S.I. TO Conventional
Na^+, K^+ Cl, HCO_3^-	mEq./l mEq.	mM mmol	1.0 1.0	1.0 1.0
Ca^{2+}	mg./100ml. mEq./l	mM mM	0.25 0.50	4.0 2.0
Mg^{2+}	mg./100ml. mEq./l	mM mM	0.412 0.50	2.42 2.0
Fe	µg./100ml.	µM	0.179	5.60
Zn	µg./100ml.	µM	0.153	6.54
Pb	µg./100ml.	µM	0.0483	20.7
Inorganic Phosphate	mg.P/100ml.	mM	0.323	3.10
Glucose	mg./100ml.	mM	0.0556	18.0
Urea	mg./100ml.	mM	0.167	6.0
Urate	mg./100ml. (as uric acid)	µM	59.5	0.0168
Creatinine	mg./100ml.	µM	88.5	0.0113
pH	"pH"	pH	no change	no change
H^+	mEq./l	nM	1.0	1.0
P.B.I.	µg./100ml.	nM(as I)	79.8	0.0127
Phenylalanine	mg./100ml.	µM	60.6	0.0165
Cortisol	µg./100ml.	nM	27.7	0.0361
Cholesterol	mg./100ml.	mM	0.0258	38.7
Bilirubin	mg./100ml.	µM	17.0	0.0587
NH_4^+	µg./100ml.	µM	0.555	1.80
Respiratory gas pressure	mmHg	N/m^2 mbar	133.3 1.333	0.007 0.750
Pyruvate	mg./100ml. (as pyruvic acid)	µM	114	0.0085
Lactate	mg./100ml. (as lactic acid)	µM	111	0.0090
HMMA	mg./24h.	µmmol/24h	5.05	0.198
NEFA	µEq./l	µM	1.0	
Faecal fats	g./24h. (as stearic acid)	mmol/24h	2.88	0.348
GOT	Karmen Unit/ml. =Reitman Frankel units	IU/l	0.483	2.07
LDH	Wroblewski-LaDu units/ml.	IU/l	0.483	2.07
Acid & Alkaline Phosphatase	King-Armstrong Units	IU/l	7.1	0.141
Inorganic Sulphate	mg.S/100ml.	mM	0.312	3.20

DETERMINATION OF SERUM SODIUM, POTASSIUM AND LITHIUM USING EPPENDORF FLAME PHOTOMETER

SUMMARY OF PROCEDURE

1. Place a 250ml beaker under the drain and a 100ml beaker full of deionised water under the atomising tube. Immerse atomising tube into water.

2. Connect the appropriate flame photometer electric cable to electricity supply. Switch supply to on.

3. Depress push-button labled "Mains" (Netz) on the front panel. (Air compressor should instantaneously start, manometer by the compressor should indicate a constant value of between 1 and 2 atomospheres, a light spot should appear along the calibrated scale and the atomising chamber should be illuminated). The manometer on the main instrument should record 0.5 atmospheres; if not, correct by turning knob labelled "air pressure" till indicator points to 0.5 atmospheres.

4. Open the valve on propane gas cylinder.

5. Depress push-button with red dot in centre labelled "Gaswahl". This will let propane gas into the instrument.

6. Ignite gas by depressing switch labelled "ignition" at short intervals until a flame appears.

7. Vary gas pressure by turning knob labelled "gas pressure" till the flame is non-luminous and separate blue cones in the flame are distinctly visible.

8. Pipette accurately 5ml of deionised water using a chemically clean bulb pipette into a chemically clean bijou bottle.

9. Using a Beckman micropipette add 50 microlitres of test solution (standard or serum) to 5ml of distilled water in bijou bottle; for lithium add 200 Microlitres of test solution to 5ml of water (i.e. dilution 1:100 for Na, K ~~1:10~~ 1:25 for Li)

10. Mix

11. Turn element selector knob till the element to be determined is illuminated. (This brings appropriate filter into light path).

12. Remove atomizing tube from water, pull out compensation knob I and adjust the light spot to zero (i.e. on a dry flame). Depress compensation knob I and return atomizing tube into deionised water.

13. Reset zero with compensation knob II

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14. Recheck gas and air pressure and ensure that atomizing rate is between 2 and 3ml of solution per minute.

15. Turn "sensitivity" knob to 100.

16. Spray the most concentrated calibrating standard solution and adjust the range by turning "range" knob till the light spot reaches 80 and remains stationary.

17. Return atomizing tube into water for a brief period.

18. Spray other standard and test solutions noting readings and rinsing with deionised water between each sample.

19. Draw a standard calibrating curve and read concentration of test solutions off the curve.

Potassium and Lithium calibrations are done in the same way except that concentration of standardising solution differs as described in reagents preparation section. (For lithium determination, random normal sera must be analysed to provide a serum blank level - see "background notes").

Preparation of Sodium, Potassium and Lithium standards.

Dry NaCL, KCL and Li_2CO_3 in oven at 100°C overnight. Store dessicated over $CaCL_2$ thereafter. Use dry powder for making standards as below.

Stock sodium solution: "A"

1000mM; 58.5 grams of sodium chloride per litre of solution.

Stock potassium solution: "B"

100mM; 7.46 grams of potassium chloride per litre of solution.

A series of stock standard solutions can be prepared from these as follows:

For sodium

mM. Na	100	110	120	130	140	150	160
ml stock soln. A	50	55	60	65	70	75	80
ml stock soln. B	25	25	25	25	25	25	25

Made up to 500ml with water

For Potassium

mM. K	2	3	4	5	6	8
ml stock soln. B	10	15	20	25	30	40
ml stock soln. A	70	70	70	70	70	70

Made up to 500ml with water

Stock lithium Standard

10mM; dissolve 0.3695 grams into a litre of distilled deionised water. Prepare series of standards as follows:

Concentration of Lithium in mM	0.1	0.2	0.4	0.6	0.8	1.0	1.5	1.8	2.0
Stock solution 10mM Lithium in Mls	1.0	2.0	4.0	6.0	8.0	10	15	18	20
Final Volume with distilled water	100	100	100	100	100	100	100	100	100

These solutions are then diluted and analysed as described in summary of procedure.

Store all standard solutions in polyethylene bottles, and when not in use keep standards tightly capped in refrigerator to avoid evaporation.

Check performance of analyses by using quality control sera and solutions obtainable on request from quality control officer.

Background Notes on Lithium Sodium and Potassium

Estimations by Eppendorf Flame Photometer

Elements consist of a nucleus containing protons and neutrons with electrons arranged in orbits of different energy levels around the nucleus.

Electrons may be transferred from one orbit to another and this transfer of electrons involves a definite acquisition or loss of energy. The energy level change is characteristic of a given element and can be used to qualitate the element.

Flame photometry deals with flame emission, (i.e. the energy change in the form of light emission resulting from flame induced atomic excitation). The intensity of light emitted is proportional to the number of participating excited atoms. This makes quantitation of elements possible Quantitation of elements like Na, K and lithium is made possible as follows:-

A small volume of solution containing the element to be determined is sprayed under controlled conditions into the flame of the photometer.

Light emitted is localised by an appropriate light filter, and is optically directed on to a photosensitive cell which measures arbitrarily the intensity of light falling on it. A sensitive galvanometer is connected to the photosensitive cell. The galvanometer responds to the photosensitive cell and registers the resulting deflections on to a calibrated scale. By ploting responses of the galvanometer (in scale units) vs concentration of standard solutions, concentration of the unknown can be worked out. The results thus obtained are, however, only valid when flame temperature (exciting energy) is adequate for excitation and other conditions are as listed in summary of procedure. Interference of other ions with sodium and potassium analyses has been found to be neglible.

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Determination of Serum Lithium.

Normal serum contains no lithium but lithium salts in trace quantities have been widely used in treatment of a number of clinical conditions particularly in control of patients suffering from manic depressive disorders. Since elevated concentration of serum lithium (over 2.0 mM) produces undesirable side effects on patients, and too low a level is not effective, it is important to perform routine serum lithium determinations in order to satisfactorily control lithium therapy.

The flame photometer technique described in summary of procedure is both accurate and rapid, but serum lithium results are biased by the presence of interference of other serum ions (particularly Na and K present in physiological concentrations).

Techniques to eliminate interference have been developed as follows:-

(i) A constant volume of a series of random sera was added to a series of calibrating lithium standard solutions in the following proportions:
volume of serum 200 microlitres;
volume of lithium calibrating solution 200 microlitres;
volume of deionised water 4.8 ml.

(ii) 100 Random samples in batches of 10 - 15 each diluted 1 : 25 in deionised water were read and scale deflection noted.

In (i) plot of meter deflection vs concentration of standard solution produced a straight line which did not go through the origin.

In (ii) although readings differed from batch to batch, the readings of individual samples within a batch were similar. Differences in individual batch readings can be attributed to different photometer settings (gas-air mixture and scale range setting made routinely before each batch reading.)

It has been shown that sample readings within a batch are similar under suitable and constant conditions but results differ from batch to batch.

It is reasonable therefore to use the deflection (scale units) of normal serum samples within a test batch as blank. The difference between reading of each test sample and blank is consequently the true deflection due to lithium present in serum. True deflection is used to work out results from the aqueous lithium calibration standard curve.

Possible Sources of error for Na, K and Lithium Estimations

1. Use of wet syringe, needle or container.
2. Use of container, syringe or needle contaminated with elements to be determined.
3. Delay in despatch of sample to Laboratory.
4. Sample contaminated with cigarette ash or smoke.
5. Over-enthusiastic use of tourniquet

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A Method for Measuring True Creatinine in Serum or Plasma Using Adsorption onto Fullers Earth (purified for adsorption purposes).

1) SUMMARY OF PROCEDURE

(i) As serum allows set up each test in duplicate as follows:-
To 0.8 ml distilled water in a centrifuge tube add 0.2 ml serum using a wash out micropipette or a Beckman automatic pipette.
Add 0.4 ml H_2SO_4 (0.33M)
Then add slowly with shaking 0.2 ml sodium tungstate 10%
Mix thoroughly and allow to stand for 30 mins. at room temperature.

(ii) From the stock standard creatinine solution at room temp. (40mM), prepare a working standard solution by making a 1 in. 100 dilution, (400μM).

(iii) Further dilute the working standard to form the following strengths of standard:-

	S_1	S_2	S_3	S_4	S_5	S_6
Strength of Std.	10μM	20μM	30μM	40μM	50μM	60μM
Amt. Working Std.	2.5ml	5.0ml	7.5ml	10ml	12.5ml	15ml
Made up to ..... with distilled water	100ml	100ml	100ml	100ml	100ml	100ml

(iv) Centrifuge the tubes containing the test solutions for 10 mins. at high speed and then take off 1ml of the supernatant taking care not to disturb the precipitate. Transfer to another labelled centrifuge tube.

(v) Set up tests and standards as follows:-

C	T_1	T_2	S_1	S_2	S_3	S_4	S_5	S_6
1 ml distilled water	1 ml super. from T_1	1 ml super. from T_2	1ml 10μM std.	1ml 20μM std.	1ml 30μM std.	1ml 40μM std.	1ml 50μM std.	1ml 60μM std.

(vi) To each of these tubes add 0.1 ml saturated oxalic acid + 0.2 ml 10% suspension Fullers Earth for adsorption purposes. Shake at intervals for 10 mins. Centrifuge at high speed for 10 mins. Then decant the supernatant carefully and drain for about 10 mins.

(vii) To all these tubes then add 2.5 ml Alkaline Picrate solution. (made by adding 5.5 ml of 2.5M NaOH to 27.5 ml of saturated picric acid solution and dilute to 100 ml with distilled water).
Mix the alkaline picrate solution with the Fullers Earth by means of a thin glass rod. Wash down the glass rod with 0.5 ml of distilled water. Shake at intervals for 10 mins. centrifuge at high speed for 10 mins. Take off the supernatant into another test tube using a Pasteur pipette and taking care not to include any Fullers Earth.

(viii) Read the colour of the supernatant at 520 nm using the SP 600 or SP 500 Spectrophotometer. (If the reading of the test is above OD 0.110, dilute with a blank consisting of 2.5 ml alkaline picrate reagent and 0.5m distilled water. Then multiply the final creatinine concentration X 2).
Read the colours against a blank consisting of 2.5 ml alkaline picrate and 0.5 mls distilled water and subtract the control reading from all the other readings.

(ix) Draw a graph of standard concentrations of creatinine against optical density readings and read the value of the test off the graph.
Value of True Creatinine in test = Reading of concentration from graph x $\frac{1.6}{.2}$

(original Dil of test)

= <u>Reading of conc. x 8 μM</u>

<u>NOTES</u>: Difficulties and Precautions.

1. The SP 600 or SP 500 must be allowed to warm up and become stable in its use before any readings are taken.
2. If some of the Fullers Earth is included in the solution to be read, this will cause spurious results - the test needs to be recentrifuged and then read.

3. The stock standard is stable for a few weeks at 4°c. Therefore, a standard curve must be set up each time to make sure its value has not decreased.

4. The test measures true creatine and can be done on whole blood, plasma or serum. The method in this Laboratory, however, is set up for serum. The normal range for whole blood or plasma will differ from the normal range given here.

2. REAGENTS, PREPARATIONS AND STORAGE

(i) Sodium Tungstate, 10 per cent. $Na_2 WO_4, 2H_2O$.W./V in water.

(ii) Sulphuric Acid 0.33M

(iii) LLoyds Reagent. Fuller's Earth for adsorption purposes. Each batch must be tested for satisfactory adsorption.

(iv) Oxalic Acid (saturated aqueous solution)

(v) Sodium Hydroxide, 2.5 M.

(vi) Picric Acid (saturated aqueous solution) - must be recrystallized twice from water unless of pure chemical grade. Made up freshly fairly often e.g. once a month and kept in a dark bottle.

(vii) Alkaline Picrate Solution. Prepare freshly immediately before use. As in method.

(viii) Stock Standard creatinine (40 mM). Dissolve 452 mg of creatinine in 100 ml of 0.IM Hydrochloric Acid. This solution may be kept at 0°C for up to 4 weeks.

(ix) Working Standards (as in the method).

3. QUALITY CONTROL PROCEDURES

As for SGOT method (Clinical Biochemistry Reports, Vol. 1, No. 1, 1971)

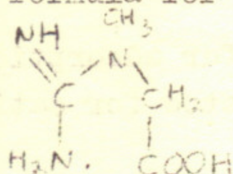
4. BACKGROUND AND INTERPRETATION

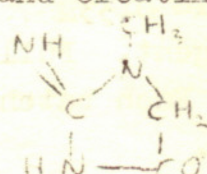
In 1886 Jaffe described the formation of a red colour by reaction of creatinine with picrate in an alkaline solution. In 1904, Folin applied the Jaffe reaction to the quantitative determination of creatine and creatinine in urine. Then Greenwold studied the nature of the red substance formed and concluded that it was a salt of creatinine picric acid and NaOH - Archibald did some further work and concluded that at temperatures below 30°C and within 15 mins. the colour formed is due almost exclusively to the red tautomer of creatinine picrate.

As the reaction time lengthens and especially when the temperature is elevated, methyl guanidine and a reduction product of picrate are formed. This and "chromogen" formed by other substances in blood estimated and expressed as creatinine also have clinical significance however.

The Workers, Roxoe (1953) and Owen (1954) studied methods for determining true creatinine; by the use of NC bacterium which removes creatinine; and by adsorption of the creatinine on to LLoyds reagent. They found that in the case of serum up to 20% of the total chromogens could be non-creatinine substances but only up to 5% in the case of urine.

The formula for creatinine and creatine are as follows:-





creatine creatinine

Creatinine is thus the internal anhydride of creatine, being formed when water is removed. This can be effected by boiling with hydrochloric acid or picric acid.

It now appears that with the method for true creatinine given above, at least 80 per cent of the colour given in the case of plasma is that due to creatinine. In the case of whole blood, however, it may be little more than half.

Normal theoretical values for this method have been reported by Tierney and Peters as 18μM to 53μM in men and 31μM to 80μM in women for serum and plasma.

Plasma creatinine increases in renal disease; values of up to and even occasionally exceeding 1800μM have been seen in the later stages of renal failure. It has been reported that the pre - renal factors which increase blood urea have little influence on the blood creatinine.

The determination of true creatinine concentration in plasma and urine is used to calculate the clearance of endogenous creatinine from plasma by the kidneys, which represents the glomerular filtration rate.

Accuracy:

The reproducibility of the creatinine determination in the normal range is about ±5%.

Chosen Technique - Summary:

The method chosen is that of Owen J.A., Iggo, B., Scandrett, F.J. and Stewart, C.P. Biochem J. 1954, 58 - 426. The only variation being the method of clearing the precipitated protein from the diluted test, i.e. by centrifugation and pipetting instead of filtration and the use of proportionately lower volumes of protein free filtrate, reagents and standards (Hirst 1971). This is to enable the test to be performed on 0.2 ml of serum instead of 4 ml or 2 ml as in the original method.

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