Cleaning the Apparatus

After each series of examinations the reaction chamber is cleaned with a 1% solution of triethanolamine stained with phenol red; this is followed by lactic acid water.

When the mercury becomes dirty and begins to form little balls in the reaction chamber 1 to 2 ml. of 20% ferricyanide solution is introduced into the reaction chamber and shaken well. Lactic acid and water follow as above.

Improvements

In a later model of the apparatus we introduced a magnetic stirrer,* which permitted vigorous mixing of the contents of the reaction chamber without the need of manual shaking. A twisted piece of stainless steel wire was enclosed in the reaction chamber and set into violent motion by means of a vibrator.

Summary

The apparatus described permits accurate determination of the CO_2 content of 0.05 ml. and 0.1 ml. samples of plasma. The calibration of the apparatus is so as to permit direct reading of the results from Van Slyke's nomogram.

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Sixth International Blood Transfusion Congress and Sixth International Hematology Congress

This meeting will be held from Monday, September 3, to Wednesday, September 5, 1956, in Boston, Mass. The Sixth International Hematology Congress will meet from Monday, August 27, until Saturday, September 1, 1956. Joint programmes are planned for September 1 and 3. All meetings will be held at the Hotel Somerset, Boston, Mass.

International European Congress on Clinical Chemistry

The Second International European Congress on Clinical Chemistry will take place in Stockholm, Sweden, from August 19 to 23, 1957. Free papers will be read, but some topics will be reviewed by invited specialists. The Congress will be combined with an international exhibition of laboratory instruments.

Organizing committee : Bertil Josephson, chairman, St. Erik's Hospital, Stockholm ; Kjell Agner, general Secretary, Serafimer Hospital, Stockholm ; Bo Norberg, treasurer, Sabbatsberg's Hospital, Stockholm ; Ingemar Jungner, exhibition manager, Epidemiologic Hospital, Stockholm. The address of the Congress is Box 12024, Stockholm 12.

European Society of Haematology

The sixth congress will be held in Copenhagen from Sunday, August 26, to Friday, August 31, 1957. Enquiries should be sent to Dr. A. Videbaek, Blegdamsvej 11, Copenhagen ø, Denmark.

* Messrs. Thomas and Co. are now marketing the normal Van Slyke apparatus with a magnetic vibrator.

The Improved Determination of Iron in Serum

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Three main difficulties are encountered in the colorimetric determination of iron in serum as follows.

(1) Owing to the minute amount of iron present, a large sample of serum must be used and, even so, the sensitivity of most of the available methods is low. Using 2′ dipyridyl, 1 : 10 phenanthroline, thiocyanate, or thioglycollic acid to develop the colour, it is usual to obtain an optical density of 0.03 to 0.04 on analysing a sample containing 100 µg. of iron/100 ml. using a final dilution of 1 in 4 and employing 10 mm. cells and the most favourable wavelength or filter. The range of optical densities between which the relative error is least is 0.2 to 0.7 (Archibald, 1950), and below an optical density of 0.1 the relative error increases rapidly. Recently 4 : 7 diphenyl 1 : 10 phenanthroline has been used for the determination of iron in serum (Peterson, 1953). This reagent gives a red colour with ferrous iron, having an optical density two to three times as great as that obtained using conventional reagents, and the iron complex is insoluble in water and can be extracted and concentrated from an aqueous medium by means of amyl alcohol or hexanol. Some of the reagents used in the determination can be rendered iron-free by preliminary extraction with this colour reagent. These advantages are offset by the tedium of extraction, which introduces an extra stage in an analysis which is already sufficiently complicated.

(2) It is difficult to determine whether all the iron has been extracted from the serum by the method of protein precipitation used. Even if all the iron added to serum is recovered, this does not prove that the iron originally present is being extracted completely.

(3) Iron present as haemoglobin may be released during the analysis and give false high values, particularly in methods which employ wet digestion or ashing to remove protein.

The object of the present investigation was to devise a reagent which would be as sensitive as 4 : 7 diphenyl 1 : 10 phenanthroline, but which would give a water-soluble colour with iron.

Experimental

The substance 4 : 7 diphenyl 1 : 10 phenanthroline is a very stable solid, insoluble in water, and should be capable of sulphonation without destroying its structure. Attempts at sulphonation were made, first with sulphuric acid and then with fuming sulphuric acid. Both attempts were partially successful, but the resulting derivatives, though giving an intense red with ferrous iron, were

themselves too highly coloured to give satisfactory results. Finally it was found that chlorosulphonic acid was an ideal sulphonating agent. The resulting sulphonic acid was lightly coloured and gave a water-soluble red colour with ferrous iron, of about the same intensity as that given by the unsulphonated compound.

Having prepared a satisfactory colour reagent, methods of extraction were examined. Of these, that of Peterson (1953) appeared to be the most promising. Peterson extracts the iron by precipitating the protein with hot trichloroacetic acid-thioglycollic acid ; after centrifuging and decanting the supernatant fluid, the protein precipitate is washed with a further portion of trichloroacetic acid. Peterson states that the percentage recovery of added iron decreases as increasing amounts of serum are precipitated from the same final volume of solution. This apparent decrease in recovery is entirely due to inadequate washing of the protein precipitate, and if allowance is made for the amount of fluid trapped in the precipitate the recoveries by Peterson's method are quantitative no matter what quantity of serum is taken for analysis. Peterson's method of protein precipitation was therefore adopted, but thioglycollic acid was not added to the trichloroacetic acid, as it does not affect the recovery of iron and it causes some breakdown of haem iron during the analysis.

Method

Reagents and Apparatus.—The apparatus used should be rendered iron-free by any of the accepted methods. The reagents should be prepared from chemicals of the highest available purity and the trichloroacetic acid must be redistilled from an all-glass apparatus. In this laboratory distilled water of the ordinary quality obtained from a Manesty still was found to be entirely satisfactory, and the total blank on the reagents was 0.2 μg./determination, half of which was due to the initial colour of the iron reagent and the other half to iron in the reagents.

Reagents.—All reagents are made up in aqueous solution and should be stored in pyrex or plastic bottles.

Sodium Acetate, $C_2H_3O_2Na.3H_2O$, 40 g./100 ml.

Trichloroacetic Acid (T.C.A.), 20 g./100 ml.

Sulphuric Acid 1 : 1.—Concentrated sulphuric acid, 50 ml., is cautiously added to 50 ml. of water.

Standard Iron (2.8 μg. Fe/ml.).—Ferrous ammonium sulphate, 1 ml. 0.01N, in 0.1N sulphuric acid, is diluted to 200 ml. with water.

Iron Reagent.—One hundred milligrams 4 : 7 diphenyl 1 : 10 phenanthroline (Light) are weighed into a boiling tube and 0.5 ml. of chlorosulphonic acid added. The mixture is boiled for 30 seconds over the pilot flame of a Bunsen burner. After cooling, 10 ml. of water is cautiously added and the tube is heated in a glass water-bath at 100° C. until the precipitate has dissolved completely (after five to 15 minutes). After adding sufficient water to bring the volume to 100 ml., 1 ml. of thioglycollic acid is added.

All the reagents keep indefinitely.

Procedure

Serum, 2 ml., is transferred to a 15 ml. cylindrical centrifuge tube and 2.5 ml. of water and 1.5 ml. of 20% T.C.A. added with mixing by lateral shaking. The tube is covered with an aluminium cap and heated for 10 minutes in a glass water-bath maintained at 90–95° C. The contents of the tube are mixed by lateral shaking after five minutes and again just before removal from the water-bath. After cooling and centrifuging for a few seconds to dislodge droplets of condensed water, the tube is shaken to mix the contents ; it is then centrifuged (still covered with the aluminium cap), at 4,000 r.p.m., radius 6 in., for 15 minutes. Clear supernatant fluid, 4 ml., is transferred to a test tube. A blank is prepared by heating a mixture of 3 ml. of water and 1 ml. of 20% T.C.A. at 90 to 95° C. in a test tube covered with an aluminium cap. After heating for 10 minutes the mixture is cooled. To each tube 0.2 ml. of iron reagent, 0.6 ml. of 40% sodium acetate, and 0.4 ml. of 1 : 1 sulphuric acid is added in that order ; the contents of the tubes are mixed after each addition. A photoelectric absorptiometer is set with the blank, and the optical density of the unknown is read using the Ilford 624 green filter and cells of the maximum possible light path. If a spectrophotometer is used a wavelength setting of 535 mμ is suitable. The red does not alter in optical density for at least one hour. The iron content of the serum is read from a calibration graph prepared by treating 3 ml. quantities of water containing 0.7 to 5.6 μg. iron with 1 ml. of 20% T.C.A., etc., in the same manner as the blank and plotting the optical densities of the resulting colours in the usual way. The colour deviates only slightly from Beer's law (Table I). The quantities of iron used in the standards correspond to serum iron values of 52.5 to 420 μg./100 ml., and, if 20 mm. cells are used to obtain the readings, the corresponding optical densities are 0.1 to 0.8, the range in which the relative error is reasonably low. Many photoelectric instruments can be adapted to take small "tintometer" cells of 20 mm. light path, but some instruments will only take cells of 10 mm. light path. If the latter type of instrument is the only one available, the usefulness of the proposed method is somewhat reduced, but even so the optical densities of the colours obtained will be two to three times greater than those obtained using conventional methods.

TABLE I

DATA FOR CALIBRATION GRAPH

Iron (μg./5·2 ml.)	Serum Iron (μg./100 ml.)	Optical Density
0·7	52·5	0·100
1·4	105	0·198
2·8	210	0·395
4·2	315	0·585
5·6	420	0·770

Final volume of solution 5·2 ml. Filter Ilford 624. Cells 20 mm. light path.

Results

Recovery experiments were performed by adding known amounts of ferric iron to pooled serum. The resulting solutions were analysed by the proposed method, and the results shown in Table II indicate that

the recovery was quantitative. The addition of 120 μg. of haem iron/100 ml. of serum was found to have no observable effect on the results obtained by the proposed method. This amount of haem iron is only introduced when there is marked haemolysis.

TABLE II

RECOVERY OF IRON ADDED TO SERUM

Ferric Iron Added	Iron Found	Iron Recovered	Percentage Recovery
0	98	—	—
140	237	139	99·5
210	312	214	102
280	383	285	102

* All results expressed as μg. 100 ml. of serum.

Discussion

The iron reagent is reasonably specific for ferrous iron. Only copper of the metals commonly present in blood gives any colour with the reagent. The slight yellow due to copper is destroyed by mineral acid, whereas the red ferrous complex is unaffected by such treatment.

The recovery figures were regarded as satisfactory, although there is no proof that all the iron originally present in the serum was being extracted. Ramsay (1953, 1954) introduced methods which in his hands gave serum iron values 30–60 μg. 100 ml. higher than the levels given by other methods. Ramsay suggested that the results obtained by his methods were the true values. Ten consecutive serum samples analysed by Ramsay's (1954) technique and by the proposed method gave average results which were almost identical. Care was taken to ensure that the coloured supernatant solutions obtained using Ramsay's method were optically clear, as the colours are so pale that the slightest turbidity invalidates the results. It was sometimes necessary to centrifuge the coloured solutions for over one hour to achieve the required clarity.

One further point worthy of note is the possibility of inaccuracy due to loss of water during heating in the water bath at 90–95° C. Experiments showed that the volume loss during the whole process of heating and subsequent centrifuging was less than 0.015 ml., and this loss will scarcely affect colorimeter readings, as it only amounts to 0.25% of the total volume. As the blank and standards are similarly heated, the loss of water may be taken to be negligible if the tubes are covered with aluminium caps during the heating and subsequent centrifuging.

Summary

An improved method for the determination of iron in serum is presented.

The iron is extracted with trichloroacetic acid at 90–95° C.

Sulphonated 4 : 7 diphenyl 1 : 10 phenanthroline reacts with the iron in the presence of thioglycollic acid to produce a water-soluble red two to three times as intense as that produced by conventional reagents.

Moderate haemolysis does not affect the results.

The recovery of added ferric iron is quantitative.

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