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ANALYSIS OF SERUM ZINC WITH THE AID OF FLAME-ABSORPTION PHOTOMETRY

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Abstract: The report indicates a simple and quick flame-photometric absorption method for the determination of zinc content of the serum. The author demonstrates that this method is sufficiently reliable and sufficiently accurate.

Introduction

Determination of the trace element zinc in serum has been made up to the present almost exclusively by means of chemical methods (15, 18, 20). Because of the difficulty of their execution and the time required, such methods of determination are not very suitable for routine clinical tests. Presently known methods for the determination of zinc in serum are polarography and the arc-emission method. The emission method utilizes the fact that a part of the zinc atoms raised to high temperature can be excited to emit according to the following relation

$$\frac{N_j}{N_o} = \frac{p_j}{p_o} \exp - \left(\frac{E_j}{kT}\right)$$

in which N_j = number of excited atoms, N_0 = number of atoms in the ground state, $p_{j,0}$ = statistical weight in excited and/or ground state, E_j = excitation energy, k = Boltzmann constant, T = absolute temperature.

In contrast to flame-emission (flame temperature 1,800-3,100° C and consequent low emission and low limits of demonstration), the conditions are more favorable for the higher temperature of the arc (about 4,500° C). Consequently, some analyses of serum zinc have been made with the aid of the arc (ref. 1, 2, 12). However, the arc method has the disadvantage that a well equipped spectrochemical laboratory and suitably trained personnel must be available. To our knowledge, no attempt has been made so far to

carry out analyses of serum zinc with the aid of the relatively new atom-absorption method (flame-absorption photometry). Such a method would have a greater chance, by reason of its simplicity, to be used more frequently in the clinical laboratory. It is the intention of the present communication to develop a method on this principle.

From the example of Alkemade and Wade (ref. 3, 19), the light of a spectral lamp, in our case a hollow-cathode lamp, is passed through a flame while measuring the absorption produced by the zinc atoms in the flame.

Figure 1 shows the trace of the beam in a diagram. Quantitatively, there is valid for the absorption of a spectral line:

$$\int K_\nu \, d\nu = \frac{\pi \cdot e^2}{m \cdot c} N_v \cdot f$$

in which k = absorption coefficient; e = electron charge; m = electron mass; c = velocity of light; N_v = number of atoms/cm^3 which are able to absorb radiation of frequency ν; f = oscillator intensity.

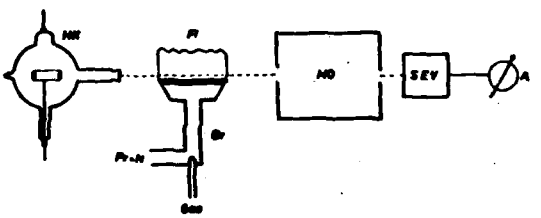

Figure 1. Diagram of beam path in the measuring arrangement utilised. HK = hollow-cathode lamp, F_l = flame, B_r = burner, P_r + N = compressed air + vapor, Mo = monochromator, FEV = secondary-electron multiplier, a = galvanometer.

The advantage of the absorption over the emission method is as follows:

the excitation energy E_j plays only a minor role in absorption (cf. below), as well as the temperature of the flame, because the last equation of absorption contains only the number of atoms (i.e., concentration in the flame) but not the flame temperature and the excitation energy

E_j. Upon closer examination, however, the excitation energy does play a role (even if this can be neglected) since the atoms absorb in the ground state but not in the excited state. The ratio of excited atoms to atoms in the ground state lies, according to Walch (ref. 19), for the zinc resonance line 213.9 nm and a flame temperature of 2,000° K at:

$$\frac{N_j}{N_o} = 7.29 \cdot 10^{-15}.$$

We see that, at the relatively low flame temperature, only a very small fraction of the atoms is in the excited state. Consequently, we can neglect excitation in the following considerations.

Moreover, we refer to the other advantages of flame-absorption photometry in contrast to the flame-emission photometry which are detailed in the pertinent literature (ref. 7, 19).

Measuring Arrangement

1. Spectral photometer

We had available for the experiments a Beckman spectral photometer, type DU, which we utilized with slit widths as low as 0.05 nm. In some cases, we utilized the accompanying recording instrument (Sera) whose output is connected with a Honeywell recorder (e.g., when photographing lamp spectra).

2. Light source

The source of background radiation was a hollow-cathode lamp of the Hilger Co. in London (Cu/Cn 227) which emitted among others the zinc resonance line at 213.856 nm employed by us (cf. Fig. 2).

We found the most favorable current intensity I of the hollow-cathode lamp for satisfactory lower limits of demonstration to be $I \geq 28$ mA. Since the useful life of the lamp decreases with increasing current intensity, we selected I = 28 mA for the further experiments.

In another case, an Osram zinc-spectrum lamp is employed. The spectrum of this lamp shows zinc lines at 307.6, 328.2, 334.6, 472.2 and 481.0 nm whereas the resonance line at 213.9 nm initially cannot be demonstrated. This is due to the fact that the external glass bulb of the lamp absorbs short-wave UV-radiation. Consequently, the glass jacket was removed in later experiments and the resonance line then shows at 213.9 nm in the spectrum. Under otherwise equal experimental conditions, the intensity of this line is greater than that of the hollow-cathode

lamp. However, its line width is evidently greater because, in later experiments (cf. section on limit of demonstration), it results that the limit of demonstration is not as good as when employing the hollow-cathode lamp.

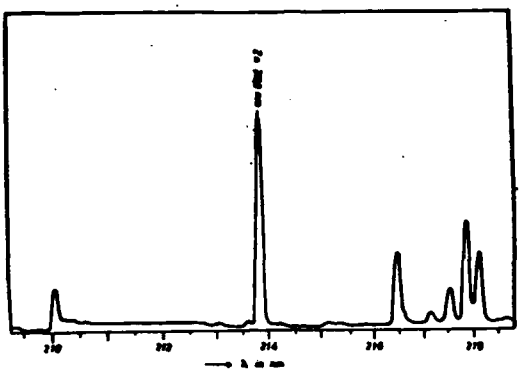

Figure 2. Spectrum of the copper/ zinc hollow-cathode lamp.

Let us mention in this connection that the line width of the lamp behaves inversely proportional to the limit of demonstration, provided that the line width of the flame is smaller than that of the lamp. This prerequisite is generally satisfied in practice, especially if we operate in the straightline section of the calibration curve (ref. 9).

3. Burner, gas, atomizing chamber, adjustment of flame height

For our first experiments, we utilized a water-cooled burner of our own design, similar to the Hilger type H1091, with a brass cap. With the idling flame, a perceptible absorption of zinc of more than 50% occurred. We ascribed this to evaporisation of burner material and therefore replaced the brass cap by a steel cap, as in the original Hilger burner, which eliminated the parasite absorption. For evaporizing and dissociating of the analyzed substance, we utilized municipal gas under pressure. A flame from propane under pressure furnishes almost the same extinctions. In some experiments carried out with a direct atomizer and an oxyhydrogen flame (Beckman burner 4020), the extinction values are definitely poor. A glass vessel with a capacity of 3 lit served as atomizer chamber. When using smaller chambers, the attainable extinctions are lower and, when using larger ones, only very little better. The larger

chambers have the disadvantage that we must wait a long time until the vapor concentration of the chamber reaches equilibrium (ref. 10). This would have resulted in a higher consumption of serum in our analyses. As shown by our own experiments, the adjustment of flame height is not critical, in contrast to the element magnesium, in making the zinc analysis because the extinctions remain the same in almost all suitable flame heights.

Lower Limit of Demonstration

By means of aqueous solutions of zinc as zinc chloride of decreasing concentration (diluted series), the lower limit of demonstration is determined by the following definition (ref. 11):

$$\underline{x} = \bar{x}_B + 3 \mid 2\, \sigma_B$$

in which $\underline{x}$ = lower limit of demonstration, $\bar{x}_B$ = mean blank value, σ_B = standard deviation of the blank solution.

When utilizing the hollow-cathode lamp (λ = 213.9 nm), we obtain 0.005 mg/100 ml as lower limit of demonstration. As comparison for this lamp, we also utilize an Osram zinc-spectrum lamp (cf. section on light source) for the same measurements of demonstration limit. The strongest of the lines transmitted by the original lamp (with glass jacket) lies around 481 nm. When using this line, we are unable to note any absorption of zinc. After removing the glass bulb, the zinc resonance line becomes easily demonstrable at 213.9 nm. In this case, we are able to reduce the slit to as low as 0.02 mm. In spite of this, the demonstration limit here is less by a factor of 10 than when utilizing a hollow-cathode lamp. Our further investigations were consequently carried out only with a hollow-cathode lamp. Since the mean zinc content of the serum (ref. 18) lies around 0.124 mg/100 ml, it results from this that a higher dilution of the sera prior to the actual flame-photometric analysis cannot be recommended. The extinctions would become too small and the errors too large. We must therefore improve the atomizing characteristics of the non-diluted serum which is done by deproteinization of the serum (cf. below).

We know from other flame-photometric investigations (ref. 4, 8, 13, 14) that the presence of protein can distort the measured values. For this reason and also in order to obtain better and more regular atomization of the natural serum, we recommend removing the protein prior to analysis. However, since the zinc is more or less firmly bonded to serum protein (ref. 17), the zinc must be leached out with acid before deproteinization or the entire serum must be incinerated. We selected the first method because it is simpler for routine analysis.

In order to dissolve the zinc bonded to the protein, we do not recommend the use of high concentrations of acid (e.g., 6 n-HCl) for flame-photometric investigations because this may cause corrosion in atomizers and burners and because the atomized acid may attack metal parts in and around the photometer. Dilution of the centrifugate cannot be considered because of the already low extinction (cf. above). We therefore utilized, in adaptation of the procedure in earlier flame-photometric copper-emission methods (ref. 6), the following process of serum preparation: we added 0.02 ml of concentrated HCl "p. A"[?]. Any coagulated protein is macerated with a glass rod. After leaving this to stand for 15 min, we add 0.2 ml of 20% trichloroacetic acid and stir again. After leaving this to stand again for 15 minutes, we centrifuge for 20 minutes at 600 g* ($g^* = 9.81\ m/sec^2$) and the centrifugate is measured without further preparation by the method referred to above.

Measurement and Calibration

In order to assign to the measured extinction E given concentrations of zinc C_{Zn}, we must know $E = E\ (C_{Zn})$. The determination of this function is made by recording a calibration curve. We utilized model sera with a varied zinc content but otherwise constant composition (cf. Table 1).

Table 1

Electrolyte Content of Model Sera for Recording of Calibration Curve (in mg/100 ml serum)

Nr.	K	Na	Ca	Mg	Cu	Zn
1	15	330	10	2	0,1	0,3
2	15	330	10	2	0,1	0,05
3	15	330	10	2	0,1	0,1
4	15	330	10	2	0,1	0,2
5	15	330	10	2	0,1	0,5
6	15	330	10	2	0,1	0,7

These solutions are prepared by dilution from stock solutions of higher concentration. A calibration curve is generally recorded by plotting the respectively measured extinctions over the concentration. This procedure would have the following disadvantages in our case: eventual changes in the conditions of atomization would change the extinctions of the calibration solutions and it would be scarcely possible to compensate this. In order to eliminate this disadvantage, we employ the quotient method (ref. 8), i.e., we form respectively the ratio of the extinctions E_x of the solution x to be measured to the extinction E_E (in practice usually averaged) of the main calibration solution (model serum

No. 1, cf. Table 1, whose zinc content should lie in the physiological range). In the preparation of this solution, we based ourselves on the since abandoned assumption that the mean zinc content of the serum, in accordance with the indications of ref. 16, lies around 0.3 mg/100 ml. The extinction of zinc for our main calibration solution (0.03 mg/100 ml) lies around about 0.2. The quotient method has one further advantage: we obtain, even with a change of concentration of the solution to be measured (caused e. g. by volume change, cf. below), the original concentration when the main calibration solution is subjected to the same change, provided that we are still on the straightline part of the calibration curve. The calibration curve recorded by us with this method is shown in Figure 3.

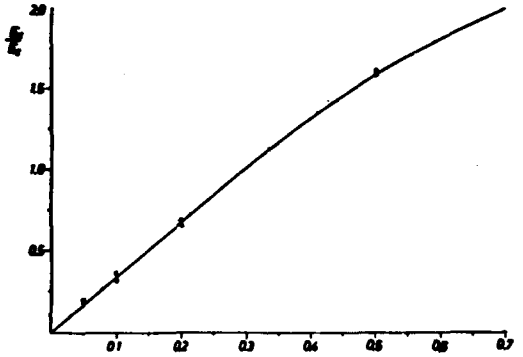

Figure 3. Calibration curve of zinc recorded with model sera.
x - x - x = measured against blank solution; ● - ● - ● = measured against double-distilled water.

We see that, in the physiological range from 0.1-0.2 mg/100 ml, the calibration curve has a straightline trace. The curvature of the calibration curve at higher concentration plays a role only in the additive method (cf. below). With the calibration curve showed above, analyses of serum zinc can be carried out in principle. The calibration curve shown in Figure 3 was measured against a blank solution (model serum without zinc). The question may now arise whether this expenditure of effort is necessary or whether the zero point (permeability = 100%) can be adjusted simply with double-distilled water. In order to examine this question, we carried out comparative measurements with the above blank solution as against double-distilled water. We tested moreover whether

the trichloroacetic acid added to the sera must also be added to the blank solution. It will be seen from Figure 3 that the points of the calibration curve measured for double-distilled water on the one hand and for the blank solution plus trichloroacetic acid agree within the accuracy of measurement. However, this result should not be generalized. We are never entirely certain whether the chemicals employed are sufficiently free of zinc. It is therefore recommended to use a blank solution plus trichloroacetic acid for the adjustment of zero point. In such a procedure, the influence of the zinc content on the measurement result are clearly defined, provided that the same chemicals are used for all solutions. This should always be the case.

Determination of Limits of Error

1. Reproducibility

In order to arrive at an indication of the accuracy of a measurement method, we determined the deviation $(\bar{M} - x)$ of the individual measured value x from the common mean value $\bar{M}$. This produces the dispersion σ from the following relation:

$$\sigma = \pm \sqrt{\frac{(\bar{M} - x)^2}{n - 1}} .$$

For the determination of σ, we carried out 25 measurements on mixed serum. This results as $\sigma = \pm$ 0.01 mg/100 ml of zinc. The σ obtained here lies more unfavorable than the σ of x in the determination of the lower limit of demonstration. The reason for this is that the measurements last named were carried out on aqueous solutions of zinc whereas the measurements made here were carried out on natural deproteinized serum. The sodium content as well as the content of the other substance in this serum resulted in irregular atomization due to the deposition of salt crystals at the atomiser jet, etc.

2. Errors due to preparation of the serum

The deproteinised serum obtained by the procedure indicated above possesses a different zinc concentration than the starting serum for reasons explained below.

Since the concentration c is defined as the quotient of the mass m of the respective substance to the respective volume V of the solution, i.e., $c = m/V$, it follows that the concentration will change with a change of volume or mass. Since the volume is decreased by the precipitation of the protein and increased by the addition of trichloroacetic acid and hydrochloric acid, the concentration consequently also changes repeatedly in different directions. Due to zinc not completely removed

before the precipitation of the protein and of the zinc-containing aqueous phase in the bottom sediment, m may also change. The following intends to investigate these errors and the possibilities for their elimination.

For the following calculation, we utilized the abbreviations listed below:

c = zinc concentration of serum

c_L = zinc concentration of supernatant

c_{WB} = zinc concentration of aqueous phase of bottom sediment

V_S = starting volume of serum

V_B = volume of bottom sediment

V_{WB} = volume of aqueous phase of bottom sediment

V_E = volume of precipitated protein

V_{Tri} = volume of trichloroacetic acid added

V_{HCl} = volume of hydrochloric acid added

m = total mass of zinc in serum

m_{WB} = mass of zinc in aqueous phase of bottom sediment

m_E = mass of zinc in precipitated protein

m_{Tri} = mass of zinc in trichloroacetic acid added

m_{HCl} = mass of zinc in hydrochloric acid added.

The significance of a part of the above symbols will be seen from Figure 4.

The "true" concentration c of the serum zinc to be determined is $c = m/V_S$. By precipitation of the protein, by centrifugation and by the addition of acids, the volume and the mass are changed (cf. above). Let V_x and/or m_x be the changes from V_S and/or m. We therefore measure a concentration c_L in the supernatant. There is valid:

$$(1) \qquad c_L = \frac{(m - m_x)}{(V_S - V_x)}$$

c results as:

$$(2) \qquad c = c_L \frac{(V_S - V_x)}{(m - m_x)} \frac{m}{V_S}$$

m can be eliminated through transformation:

$$(3) \qquad \frac{1}{c_L} = \frac{(V_S - V_x)}{(m - m_x)}$$

or

$$m = c_L(V_S - V_x) + m_x$$

If we enter equation 3 in equation 2, there follows:

$$(4) \qquad c = c_L \frac{(V_S - V_x)}{V_S} + \frac{m_x}{V_S}$$

V_x, the change of volume, is composed of the volume of the bottom sediment V_B and the volume of the added V_{Tri} and V_{CHl}, i.e.,

$$V_x = V_B - V_{Tri} - V_{HCl}.$$

V_B contains the volume of the aqueous phase of the bottom sediment V_{WB} and the volume of the precipitated protein V_E:

$$V_B = V_{WB} + V_E.$$

correspondingly, there is valid:

$$m_x = m_{WB} + m_E.$$

if no zinc is introduced by the acids added because we would otherwise have to take into account also the members m_{Tri} and m_{HCl}.

The unknown factors of equation 4 can be determined experimentally as follows:

V_B is read from graduated centrifuge tubes and varies between 1.8-2.0 ml for an initial volume of its serum V_S of 5 ml;

V_{WB} results from the following experiment: several centrifuge tubes with 2 ml residue each, remaining after the deproteinization of 5 ml each of serum, are dried for 48 hours under high vacuum ($p < 10^{-3}$ Torr). From the difference of the weights before (G_v) and after (G_n) drying, we calculate the volume of the aqueous phase V_{WB} (specific weight of H_2O is

assumed as 1.0) numerically as $G_y - G_n = V_{WB}$ or, in our case (V_S = 5 ml, V_B = 2 ml), as 1.8 ml;

V_E, the volume of precipitated protein, results from $V_B - V_{WB} = V_E$ and is around 0.2 ml for an initial volume of 5 ml (2 ml of residue);

V_{Tri} = volume of trichloroacetic acid added or, in our case, 1 ml (V_S = 5 ml);

V_{HCl} = volume of hydrochloric acid added or, in our case, 0.1 ml (V_S = 5 ml);

m_{WB}/V_{WB}; the residue of 5 ml serum is washed three times with 2 ml of double-distilled water and the zinc content of each wash is determined which furnishes the values shown in Table 2.

Table 2

n	0	1	2	3
c_A	0,125	0,063	0,032	0,019

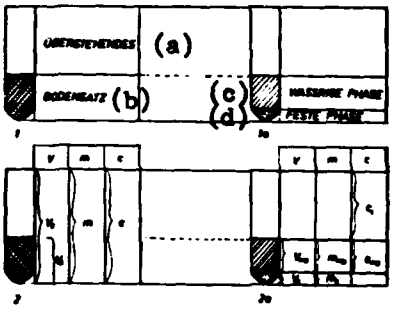

Figure 4. 1 and 2 correspond to actual conditions; 1a and 2a show the fictitious separation of the bottom sediment into two separate phases (cf. text).

KEY:

a - Supernatant

b - Bottom sediment

c - Aqueous phase

d - Solid phase

If we plot the concentration c_k against n (number of washes), we then see that c_k results from the relation (7) $c_k = c_0 a^{-n}$ with a = 2. c_0 results from extrapolation of the curve for the value n = 0. m_{WB}/V_{WB} results from summating of all concentrations c_k for n = 1 to n = ∞

$$(8) \qquad \frac{m_{WB}}{V_{WB}} = \sum_{n=1}^{\infty} c_k = c_0 \sum_{n=1}^{\infty} 2^{-n} = c_0$$

If we now compare the c_0 determined by extrapolation with c_L, we then find that both are equal, i.e., the concentration in the supernatant is equal to the concentration of the aqueous phase in the bottom sediment.

m_E/V_S; the residue from 5 ml of serum (2 ml) is incinerated with concentrated HNO_3 in a Kjeldahl retort after washing three times with double-distilled water. To the incineration residue are added 5 ml (= V_S) of double-distilled water and the zinc concentration is measured. From this concentration is deducted the zinc concentration of the aqueous phase of the bottom sediment which remains after three washes.

From the determination of m_{WB}/V_{WB} (cf. above), it results that $m_{WB}/V_{WB} = c_L$; this simplifies V_x and m_x to:

$$(9) \qquad V_x = V_E - V_{Tri} - V_{HCl} \qquad \left(\text{proof} - c_L \frac{V_{WB}}{V_S} + \frac{m_{WB}}{V_S} = 0 \right)$$

$$(10) \qquad m_x = m_E \qquad \left(\text{for} \quad \frac{m_{WB}}{V_{WB}} = c_L \right)$$

There consequently results for equation 4

$$(11) \qquad c = c_L \frac{(V_x - V_E + V_{Tri} + V_{HCl})}{V_x} + \frac{m_E}{V_x}$$

We see from this equation that two correction members occur. This first one depends only on the change of volume and the second only on the zinc remaining in the protein. If we add to the calibration solution the volume $V_x = V_{Tri} + V_{HCl} - V_E$, then the first member, i.e., $c = c_L + m_E/V_S$ (cf. quotient method above) is eliminated. The second member is added as an additive member to the measured result. It is here assumed that V_E/V_S and M_E/V_S are the same for each serum.

It results from our measurements that m_E/V_S amounts to 10% of

$$c_L \frac{V_x - V_E + V_{Tn} + V_{HCl}}{V_x}$$

i.e., 10% of its value must be added to the measurement result in order to obtain c if the volume correction is made by the addition of V_X.

Interference by Solution Partners

We know from other flame-photometric emission and absorption investigations that solution partners not directly of interest in the analysis can more or less distort the result of the latter (ref. 5, 8). In order to be certain that such interference does not occur here, we investigated the zinc absorption in model sera with constant zinc content but with contents of sodium, potassium, calcium, magnesium and copper varying within the physiological range. The model sera prepared for this purpose have the concentrations indicated in Table 3.

We find that zinc absorption within the limits of error of the method is independent of the respective concentrations of sodium, potassium, calcium, magnesium and copper. Consequently, we can neglect these parasite influences in routine tests.

Table 3

Concentrations of the Model Sera in the Investigation for Interference (in mg/100 ml)

Nr.	Na	K	Ca	Mg	Cu	Zn
7	*250*	15	10	2	0,1	0,3
8	*400*	15	10	2	0,1	0,3
9	330	*10*	10	2	0,1	0,3
10	330	*30*	10	2	0,1	0,3
11	330	15	*5*	2	0,1	0,3
12	330	15	*15*	2	0,1	0,3
13	330	15	10	*1*	0,1	0,3
14	330	15	10	*3*	0,1	0,3
15	330	15	10	2	*0,05*	0,3
16	330	15	10	2	*0,2*	0,3

Testing for Systematic Errors

A. Additive Method

Zinc solutions of increasing concentration are added to various mixed sera whose respective zinc content was previously determined flame-photometrically. After such addition, the zinc content is measured again. Theoretically, the zinc concentrations so measured must agree with those

calculated. Here we have to take into account the change of concentration through change of volume during addition. The result is shown in Table 4.

Table 4

Results of the Additive Method
(in mg/100 ml)

c_e = calculated concentration; c_g = measured concentration
S_i = i-th mixed serum; z_i = i-th zinc solution added.
a = error in percent

	c_e	c_g	(a) Fehler in %
S_1	—	0.193	—
$S_1 + Z_1$	0.293	0.270	— 8
$S_1 + Z_2$	0.418	0.440	+ 5
S_2	—	0.123	—
$S_2 + Z_1$	0.232	0.280	+ 21
$S_2 + Z_2$	0.357	0.43	+ 20
S_3	—	0.165	—
$S_3 + Z_1$	0.270	0.263	— 3
$S_3 + Z_2$	0.395	0.448	+ 13

In the discussion of the errors occurring in the last column, we must keep in mind that these are the inevitable errors of the method of determination (cf. section on reproducibility) and the inevitable pipetting errors which enter into this each twice. If we take this into account, the errors found lie within the limits which may be expected on the basis of the previous determination of σ. The result of the check by means of the additive method consequently shows that the physical method indicated by us is free of any large systematic errors. In spite of this, we carried out a further check of the method with the aid of comparative chemical determination.

B. Chemical Determination

For the chemical determination of serum zinc, we utilized the mixed color method (ref. 15) with the modification of preparing the solutions (buffer solution, leaching mixture) necessary for the extraction of the zinc with dithizone as indicated in ref. 20 and the preparation of the serum with the method indicated above (cf. section "preparation of serum"). The photometer utilized was a Zeiss spectrophotometer MLQII.

Measurement is made at λ_1 = 620 nm and λ_2 = 535 nm with the slit adjusted to 0.01 mm. This corresponds to a spectral band width of 0.7 nm (λ_1 = 620 nm) and/or 0.4 nm (λ_1 = 535 nm).

The zinc concentrations (cf. model sera above) utilized for calibration are plotted in the calibration curve against the extinctions corrected as follows:

$$E_{korr.} = E_{535} - \frac{E_{620}}{R}$$

in which R = extinction ratio E_{620}/E_{535} for dithizone in carbon tetrachloride, E_{535} = extinction at λ_1 = 535 nm, E_{620} = extinction at λ_2 = 620 nm).

R must be re-determined prior to each measurement series because the degree of oxidation of the dithizone changes and varied with us between 3.8 to 4.5. We divided different sera into two specimens each. One specimen is determined by means of the flame-photometric absorption method indicated by us above and the other by means of the chemical method referred to above. Table 5 compares the values obtained with the two methods:

Table 5

Comparison of Zinc Concentration in Different Specimens of Mixed Sera Determined by Chemical and Flame-photometrical Methods

Nr	(a) $C_{Zn\ chemisch}$	(b) $C_{Zn\ photometrisch}$	(c) Difference in %
1	0,10	0,12	+ 20
2	0,143	0,15	+ 5
3	0,185	0,153	— 17
4	0,08	0,097	+ 21
5	0,11	0,108	— 2
6	0,08	0,085	+ 6

KEY:

a - Chemically
b - Photometrically
c - Difference in percent

The comparison shows that the errors lie between 2 and 21%. We must here again remember that the errors of both methods enter simultaneously into the comparison. If we consider that we determine a σ of 0.01 mg/100 ml zinc (cf. section on reproducibility) and take into account the errors in the chemical analysis of zinc (ref. 15), we then see that the total error in the comparison is not any larger than may be expected on the basis of the circumstances. We find again that the flame-photometric absorption method is free of large systematic errors.

Discussion

By means of the flame-photometric absorption method, it is possible to obtain the results of analysis quickly and simply. The amount of time required for acidification, deproteinization and centrifuging each amounts to about 15 minutes where we should keep in mind that the actual working time is about 2-3 min for each. For the subsequent flame-photometric measurement about 1 min are required and for evaluation again 1 min. We consequently now have a possibility of quickly and simply carrying out series tests of zinc content in sera. The method is specific for zinc. Interferences by solution partners were not observed (cf. section on interference by solution partners). The flame-photometric absorption method has the following advantage over the chemical method: saving of time, simplification of work, saving of chemicals, no interference through other heavy metals.

Grateful acknowledgement is due to Dr. R. Hermann for his interest and suggestions and to Miss R. Karlewiecz for technical assistance.

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