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The use of regulation characteristics of erythropoiesis for analysing data on computer-assisted erythrokinetics [2]

Abstract

In a new method of analysing erythrokinetic data the erythrocyte iron turnover was calculated from ^{51}Cr-ery-vita and the erythrocyte maturation time by analysing the utilisation curve. Four regulation characteristics of erythropoiesis are defined: Normal anaemia characteristic, inverse anaemia characteristic of self-compensation, normal polyglobulia characteristic and inverse polyglobulia characteristic of self-compensation. The application of these characteristics is demonstrated analysing clinical data of patients with renal anaemia, liver cirrhosis, polyglobulia and polycythemia.

Method

Since the introduction of erythrokinetic investigation, the quantitative calculation of erythropoiesis has been based on the analysis of the plasma iron activity disappearance curve. The use of multi-compartment models extends the analysis of the first rapid slope of the disappearance curve to the further slower part of the curve. The result is a more exact determination of the erythrocyte iron turnover and the possibility of estimating certain metabolic pools. Independently of the discussion about the various kinetic models [1-6] we have modified the method of erythrokinetic investigation [7, 8].

We omit the slow part of the disappearance curve. Instead, we determined the rate constant of this slope by analysis of the utilisation curve (cf. fig. 1). The erythrocyte iron turnover was calculated using total body haemoglobin and erythrocyte life of the ^{51}Cr-ery-vita.

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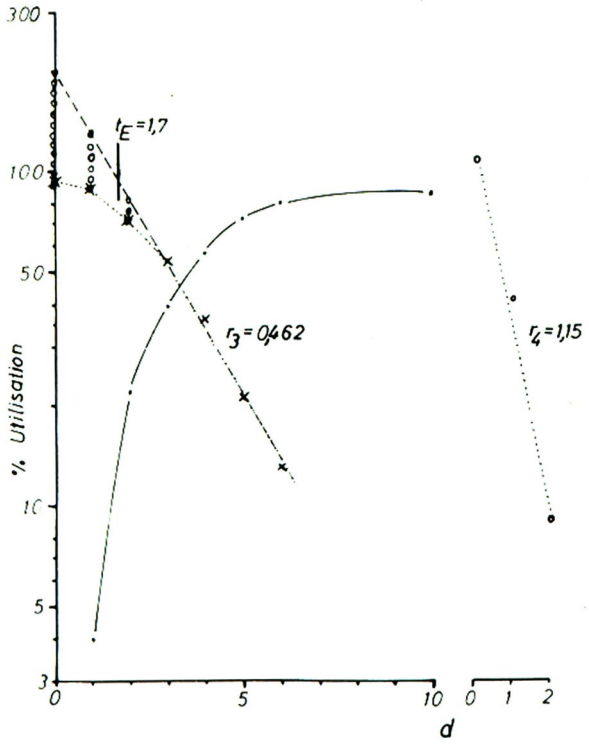

Fig. 1: Analysis of the utilisation curve (— • —). The computer program imitates the demonstrated graphic analysis. The linear part of the complementary curve (– · · · –) produces r_3. The interception of the extrapolated linear part (– – –) and the 100% Value lead to the "erythrocyte maturation time" (t_E). The difference between the extrapolated linear part and the real complementary curve (o o o) is plotted to obtain r_4 (· · · ·).

This method was introduced for three reasons:

1. The activity in the plasma is so low that it is very difficult to obtain good results even when using detectors with high sensibility, long counting times and high doses. Furthermore, in each sample possible hemolysis must be excluded.

2. To get enough plasma, samples of more than 10 ml each must be obtained. Together with other laboratory investigations the total amount of blood soon exceeds 100 ml, an amount which markedly influences the erythropoiesis which is to be measured without disturbance.

3. The common methods of analysing iron kinetics using the plasma disappearance curve are based on an single determination of plasma iron. It must be recognized that the day-to-day variation of plasma iron introduced an error into the extrapolation from a moment value to an extended process such as erythrocyte life span and erythrocyte iron turnover. This error is at least of the magnitude of the error resulting from estimation of the erythrocyte life span from the half life of ^{51}Cr-ery-vita.

Lastly the omission of the analysis of the slow plasma disappearance curve has the disadvantage that some details of subcompartments and the possible extent of intramedullary hemolysis cannot be determined quantitatively. But other typical signs indicate such relevant pathologic deviations qualitatively and pathognomically. But the quantitative calculation of these metabolic values is, in any case, connected with a large error.

This modified method of analysis is independent of the metabolic model. The rapid disappearance rate of plasma iron, the slow rate obtained by the utilisation curve, and the erythrocyte iron turnover can be used to calculate the pool sites. The "erythrocyte maturation time" derived from analysis of the utilisation curve in the classic manner is influenced by both the effective maturation and the slow rate constant. Therefore we simplified the iron in the erythroblasts to one pool and calculate from the utilisation curve the rate constant generated by this catenary pool (cf. fig. 1). This rate constant (r_4) expresses erythropoiesis per unit of erythroblast iron and is called "erythropoetic effectiveness".

To obtain enough values for good analysis of the utilisation curve we collect two samples per day in the first 4 days. All the samples were stored until the end of the investigations and then measured using an automatic sample changer with a two-channel-γ-spectrometry unit. To ensure sufficient statistics each channel counts with a separate count preset. After reaching preset counts in each channel an AND-logic starts the change of the sample. The sample number and the elapsed time in each channel are punched down. The punch strip is completed with clinical and standard data. This procedure is done using a format controlled puncher system. Then the data are transmitted to the computer centre using the internal telex network of the university. There the data are processed with a TR 4 by a program written in algol.

Besides the saving of time, the main advantage of data processing is the possibility of determining the half life of ^{51}Cr-ery-vita by linear regression of the logarithm of the net ^{51}Cr-activity of the blood samples as a function of time. It has proved necessary to repeat this calculation one to two times after monitoring outlayers. Experience shows that in graphic evaluation of the regression line through scattering points there is a tendency of overestimate the regression coefficient and to underestimate the half life. Thus we observed an underestimation of the half life of 51Cr-ery-vita in an mean of 2,89 $\pm$ 3.8 days). But, in some cases of systematic error, e. g. partial clotting of some of the subsequent samples before pipetting, it may happen that the program falsely eliminates the good values and calculates a wrong regression. In other cases, e. g. in the existence of two erythrocyte populations or random destruction, the prerequisite of linear regression is not fulfilled, and therefore the result also is wrong. To detect such situations a printout of the picture of the regression with signing of the eliminated values is important (cf. fig. 2).

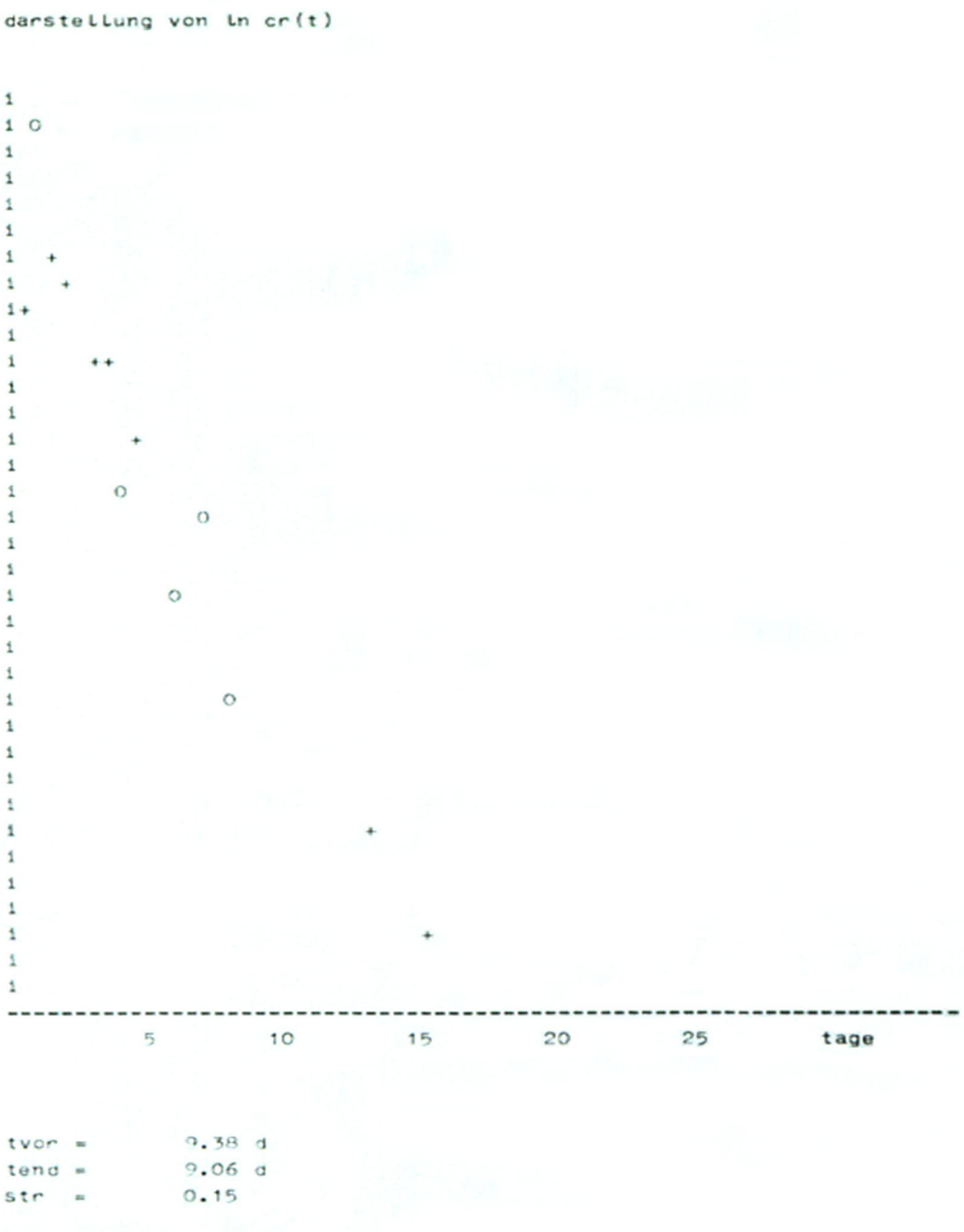

Fig. 2: Printout of the logarithm of the net ^{51}Cr activity in the blood samples (ln cr(t)) versus time in days (tage). tvor: half life before correction, tend: half life after omitting the values with excessive scattering (0).

Results

Of the multiple aspects of judging erythrokinetic data one will emphasized here. The common method is the isolated judging of each result. The correlative examination of the relationship between the two findings may give a better look to the mechanism of some pathological deviations. In the relationship between erythropoiesis and haemoglobin level two kinds of interdependence must be recognized:

1. The true erythropoietic regulation. It can be accepted that in the physiological regulation of the erythropoiesis the haemoglobin concentration will be the main regulatory factor. A diminution of the haemoglobin concentration will be answered by an increase of erythropoiesis. The diminution of the haemoglobin concentration may be the result of blood loss, mild reduction of erythrocyte life span, etc. In a two-dimensional diagram with the haemoglobin level on the abscissa and the erythrocyte iron turnover on the ordinate we called the line connecting all cases with true erythropoiesis regulation after a decrease of the haemoglobin concentration the **normal anaemia characteristic** (cf. fig. 3).

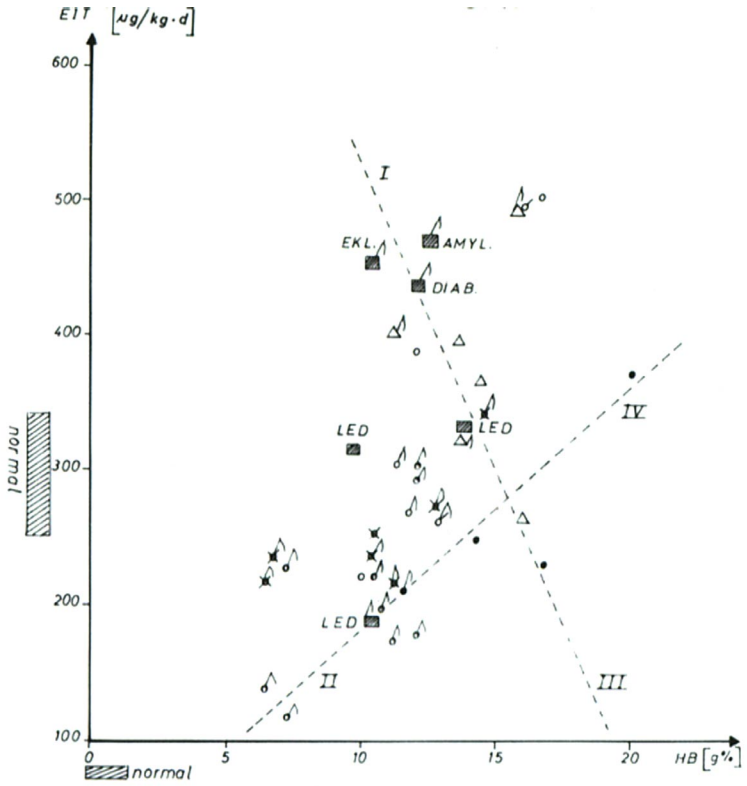

Fig. 3: Correlation between erythrocyte iron turnover (EIT ordinate) and haemoglobin concentration (HB abscissa). Normal anaemia characteristic (I), inverse anaemia characteristic of self-compensation (II), normal polyglobulia characteristic (III) and inverse polyglobulia characteristic of self-compensation (IV).
Chronic pyelonephritis, chronic glomerulonephritis, patients with retention of creatinine, hypertension, nephrotic syndrome.

2. The second kind of interdependence in the relationship between erythropoiesis and the haemoglobin concentration is due to a simple self-compensation mechanism. In each case of impaired erythropoiesis daily haemoglobin synthesis is lower than daily haemoglobin destruction. The result is a diminishing of the haemoglobin concentration. But, the reduction of the haemoglobin concentration leads to a reduced haemoglobin mass and so to a reduced daily haemoglobin destruction. This process continues until the haemoglobin concentration has sunk so low that the daily haemoglobin destruction can be compensated by the impaired daily synthesis. In the correlative examination we called this regulation characteristic **the inverse anaemia characteristic of self-compensation**. This is the common finding in marrow insufficiency. A good example is the special marrow insufficiency of secondary renal anaemia due to chronic inflammatory renal diseases. With increasing renal insufficiency, patients with chronic glomerulonephritis and pyelonephritis and LED show a reduction of haemoglobin synthesis, while patients with non inflammatory renal insufficiency are able to increase erythropoiesis according to the decreased haemoglobin concentration, due to the reduction of erythrocyte life span (cf. fig. 4).

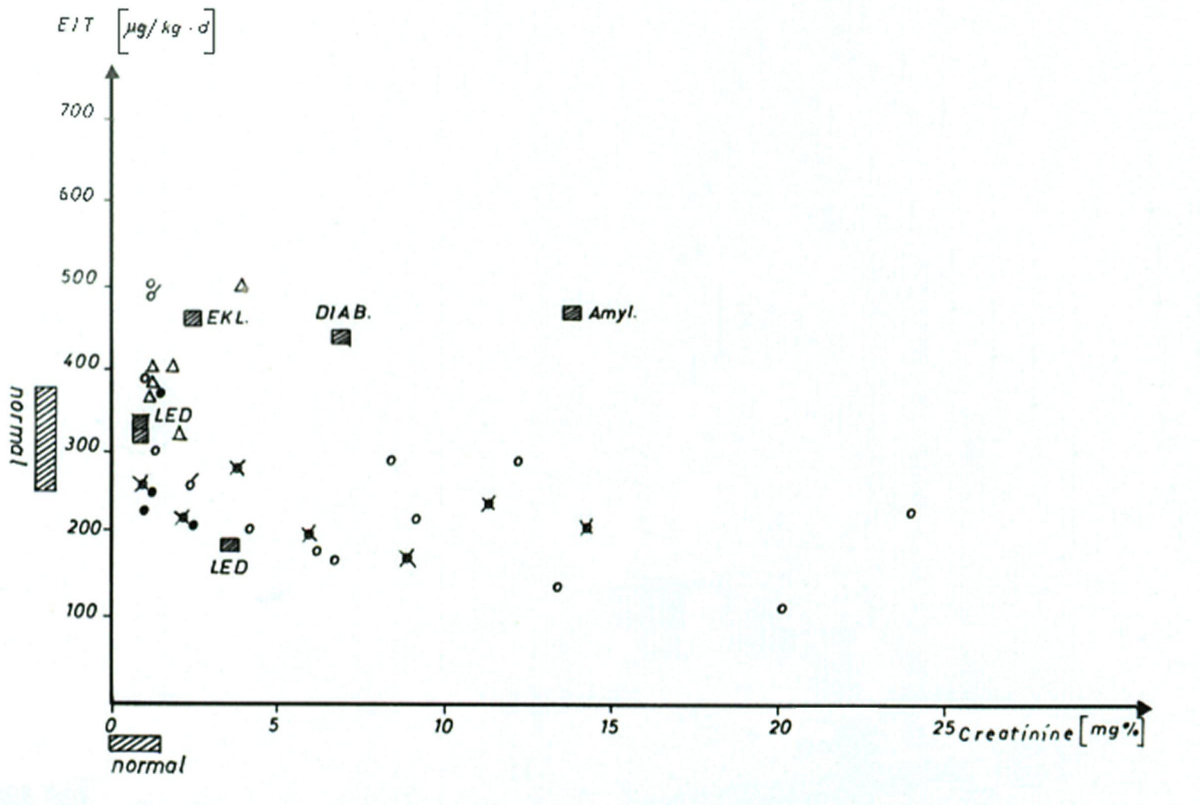

Fig. 4: Erythrocyte rin turnover (EIT ordinate) in relation to creatinine retention. Symbols cf. fig. 3 (p. 5)

It is very interesting that both the groups, inflammatory and non inflammatory insufficiency, show an increasing reduction of "erythropoietic effectiveness" (r_4) with elevated serum creatinine (cf. fig. 5, p. 7). Thus, the erythropoietic effectiveness does not depend upon which of the two regulation characteristics the renal-anaemia patient has [9]. Renal insufficiency is regularly connected with a reduced erythrocyte life span, especially in patients with chronic pyelonephritis; the more increased the observed retention of creatinine in the serum the shorter the life span is. The additional reduction of erythrocyte life span produces in the diagram a shift to the left. Without reduction of erythrocyte life span we assumed a diminution of the haemoglobin concentration to half the normal value, due to a reduction of haemoglobin synthesis to half the normal value.

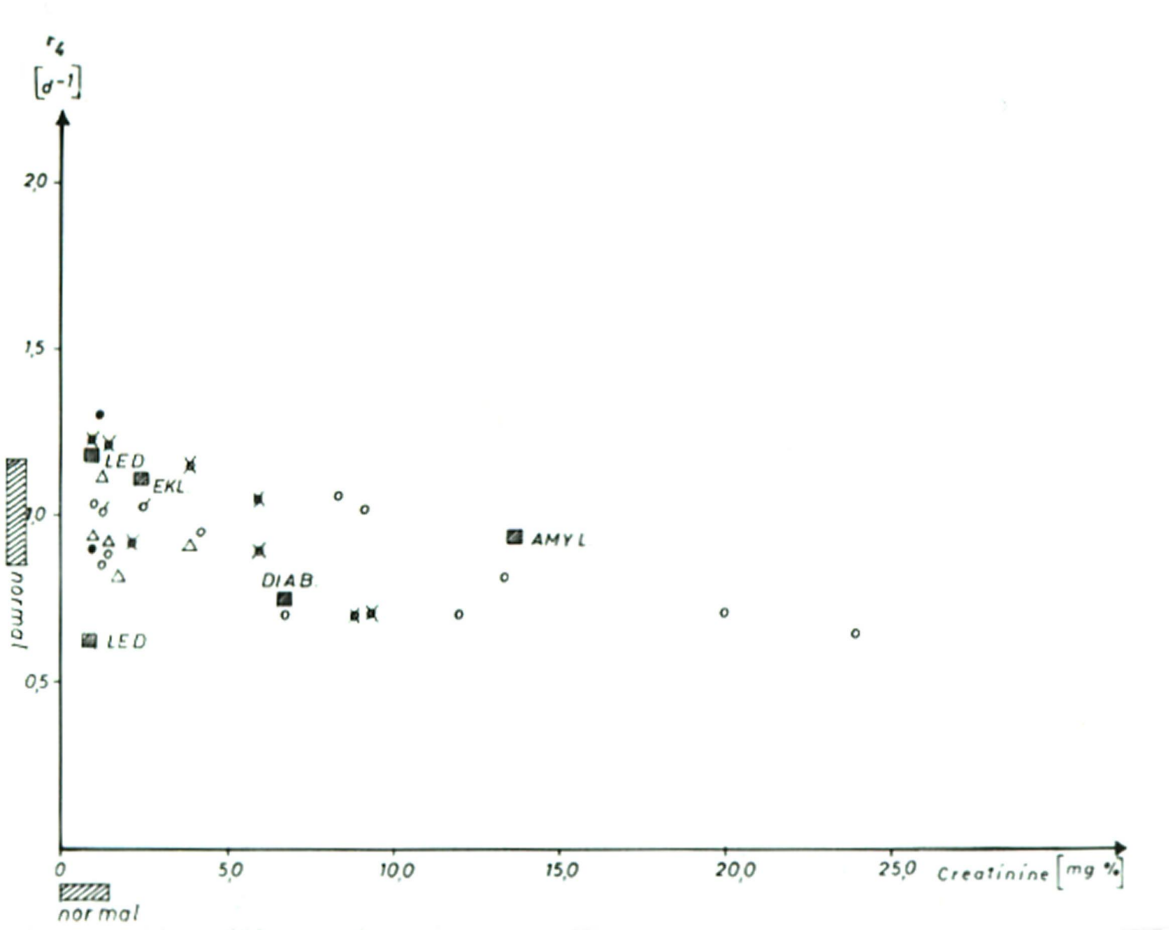

Fig. 5: "Erythropoietic efficiency" (r_4 ordinate) in relation to creatinine retention. Symbols cf. fig. 3 (p. 5)

The normal reaction of erythropoiesis to an elevated haemoglobin concentration is a decrease in erythrocyte iron turnover. This condition can be established in overtransfusion experiments and is seen very seldom spontaneously, e. g. in a case of pseudopolyglobulia accompanying duodenal ulcer. This regulation characteristic we called the **normal polyglobulic characteristic**.

Instead, another finding is very common in polyglobulic and polycythaemic patients. The increased daily haemoglobin synthesis leads to an increased haemoglobin mass and thus to an increasing daily haemoglobin destruction. The haemoglobin concentration rises until the increased haemoglobin mass compensates the stimulated synthesis via the increased daily destruction. This we call the **inverse polyglobulic characteristic of self-compensation**. This is observed in both polyglobulias and polycythaemias. As in the inverse anaemia characteristic (cf. fig. 6) a reduction of erythrocyte life span shifts the patients in the diagram to the left.

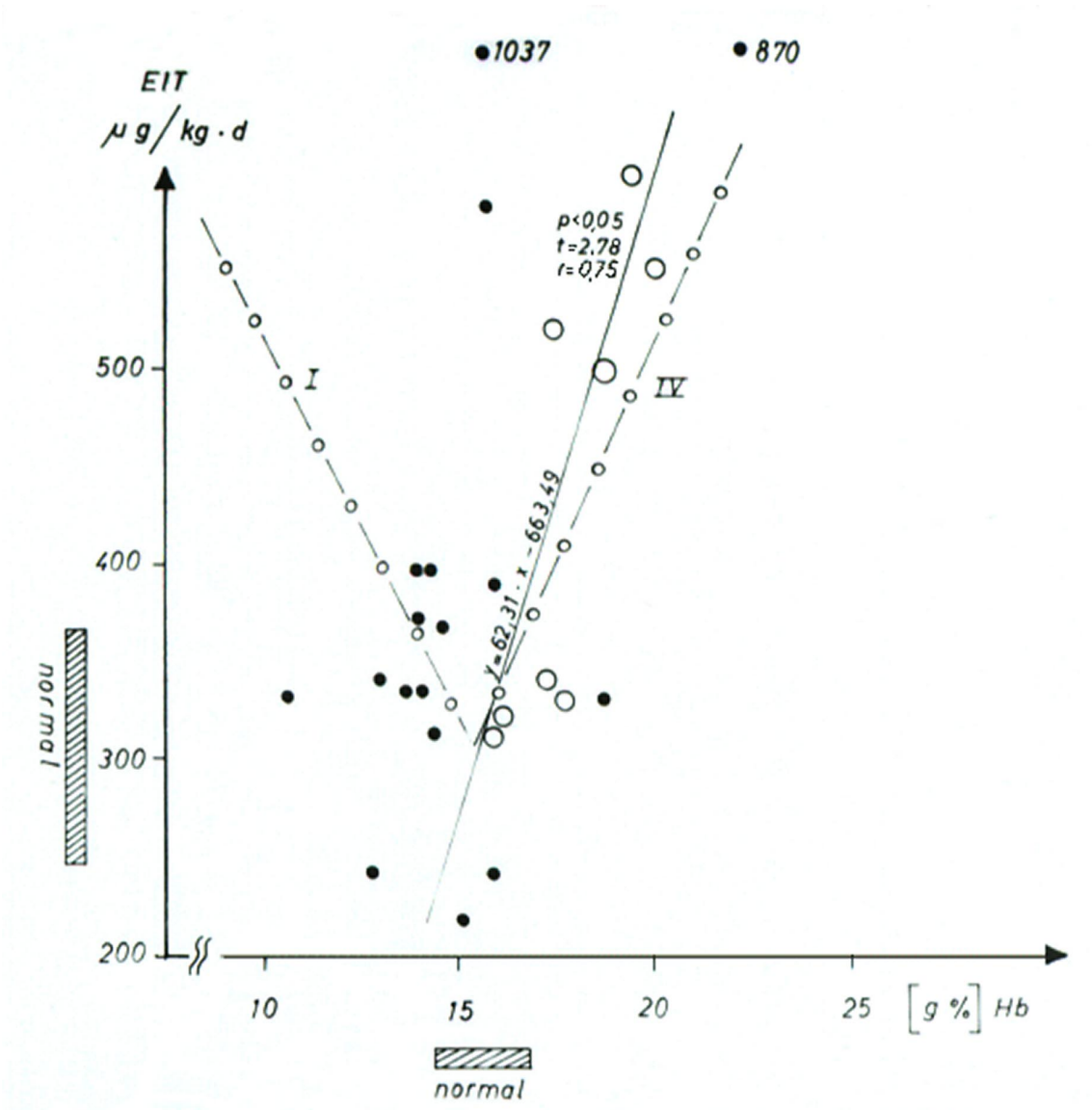

Fig. 6: Regulation characteristics as in fig. 3 (p. 5). Patients with polycythemia ○ patient with $r_4 \geq 1.4$. ● Patients with $r_4 < 1.4$. The two patients with EIT 500 µg/kg * d have a reduced ^{51}Cr-ery-vita. The full line marks the linear regression of the patients with $r_4 \geq 1.4$.

We assume a doubling of haemoglobin mass due to a doubling of haemoglobin synthesis. But in accordance with Huber et al. [12] we assumed an elevation in the haemoglobin concentration with increasing haemoglobin mass following the equation: haemoglobin concentration elevation in percent of normal value is equal to 0.73 times haemoglobin mass increasing in percent of normal value. It is noteworthy that the most of the patients belonging to this regulation characteristic group show an "erythrocyte effectiveness" (r_4) of more than 1.4 (normal range 0.85-1.15).

In this area of the diagram we have also observed patients with an increased haemoglobin synthesis instead of a normal haemoglobin concentration. We observed this behaviour in many non anaemic patients with compensated cirrhosis of the liver (cf. fig. 7).

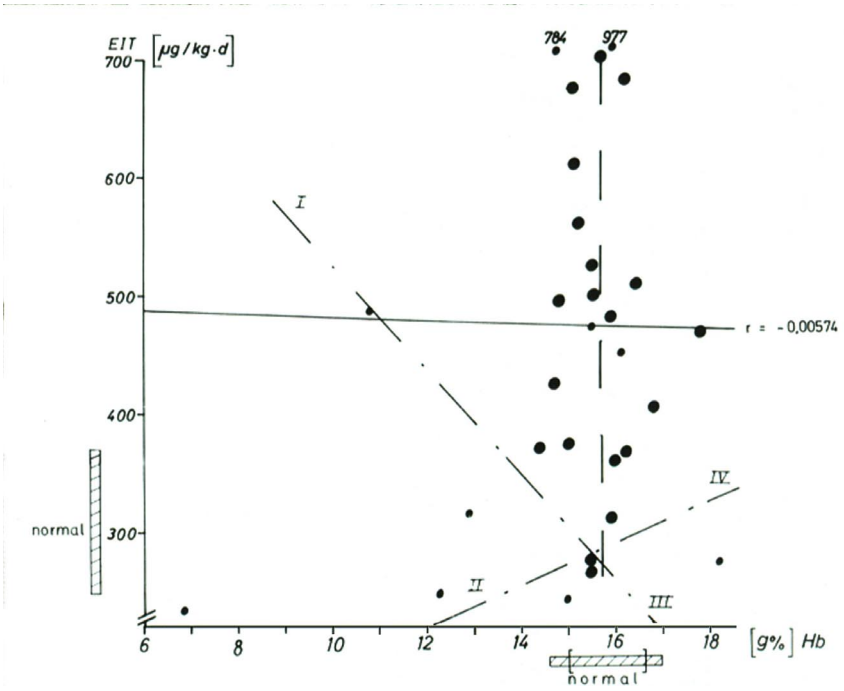

Fig. 7: "Independent characteristic" obtained by the linear regression of 20 compensated liver cirrhoses without anaemia (full dots).

In this group we found a significant correlation between the increase of haemoglobin synthesis and bromsulfthalein retention (cf. fig. 8, p. 10) [10, 11]. One of the causes is that the mean corpuscular volume (MCV) and the mean haemoglobin content of the erythrocyte (MCH) were elevated to the extent of the observed increase in bromsulfthalein retention (cf. fig. 9, p. 10). The daily production of erythrocytes corresponded approximately to the normal anaemia characteristic (cf. fig. 10, p. 11). The increased haemoglobin content of the cells leads to an elevated haemoglobin output and increase in haemoglobin mass. This kind of "independent characteristic" can be interpreted in part as a pseudo-decompensation resulting from the quantitative dissociation between red cell production and haemoglobin synthesis.

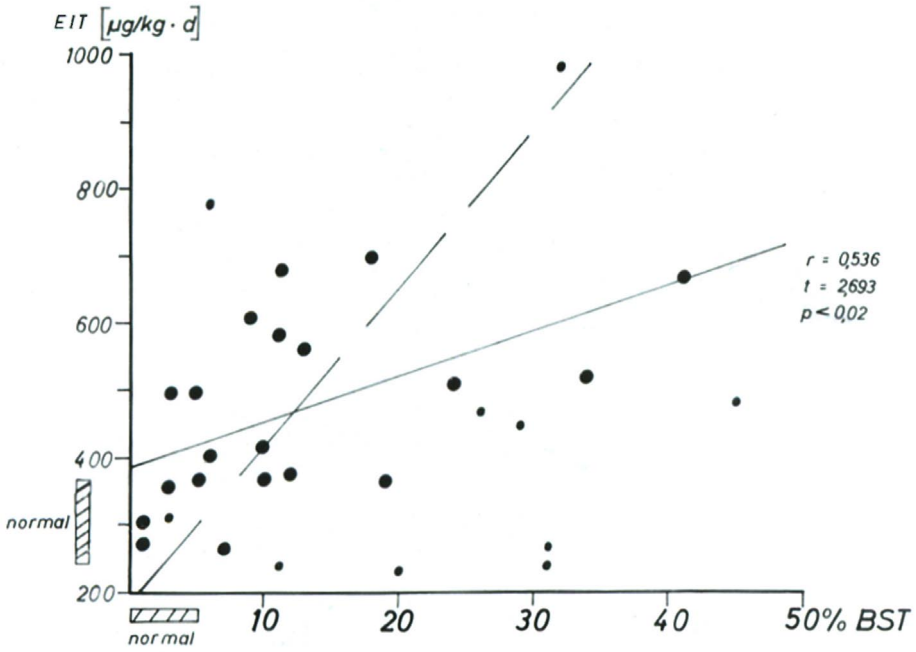

Fig. 8: Erythrocyte iron turnover (EIT ordinate) in relation to bromsulfthalein retention (BST abscissa) in the same cases as fig. 7 (p. 9).

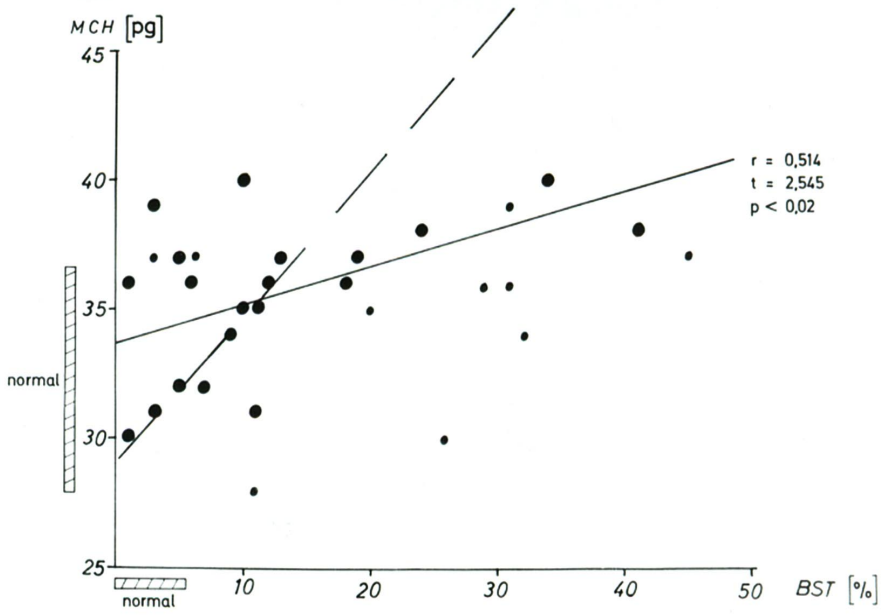

Fig. 9: Mean corpuscular haemoglobin (MCH ordinate) in relation to bromsulfthalein retention (BST abscissa) in the same cases as fig. 7 (p. 9).

The examples demonstrated show that correlative examination is very useful in interpreting the qualitative and quantitative influence of organ function on erythrokinetic data. Vice versa, based on this there is the possibility of detailed analysis of complicated haematological pictures using correlative examination. Furthermore the recognition of relative marrow insufficiency is very simple. The judgement of the position of a certain case in the diagram of correlation shows very clearly whether an increase of haemoglobin synthesis is adequate to the extent of the diminishing of the haemoglobin concentration.

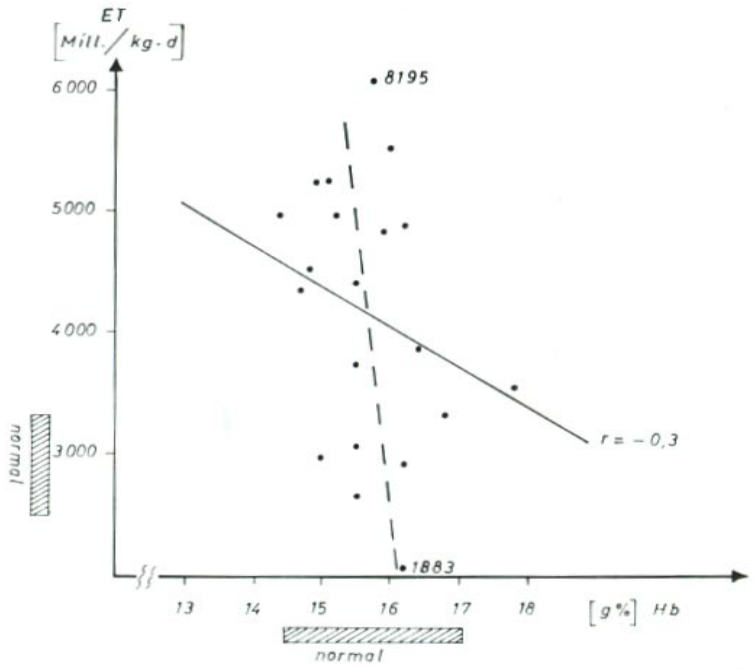

Fig. 10: Erythrocyte turnover (ET ordinate) in relation to haemoglobin concentration (abscissa) in the same cases as fig. 7 (p: 9)

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