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Training induced effects on blood volume, erythrocyte turnover and haemoglobin oxygen binding properties*

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Summary. The effect of three weeks ergometer training (Tr) 5 times a week for 45 min at 70% $\dot{V}_{O_2max}$ by 6 subjects on erythrocyte turnover and haemoglobin O_2 affinity has been studied. Increased reticulocytosis could be observed from the second day after beginning Tr until a few days after its end, probably caused by increased erythropoietin release by the kidney. Erythrocyte destruction was most pronounced in the first and markedly reduced in the third week of Tr. Elevated glutamate oxalacetate transaminase activity and creatine as well as lowered mean corpuscular haemoglobin indicate a younger erythrocyte population in the first week of recovery. Total blood volume increased during the course of Tr by 700 ml, mainly caused by a raised plasma volume (74%). Red cell volume increased later with maximal values one week after Tr (+280 ml). In this week the standard oxygen dissociation curve was most shifted to the right (P_{50} increased from 3.77 ± 0.05 kPa to 3.99 ± 0.07 kPa) and the Bohr coefficients had their lowest values. Both effects are completely explainable by the haemoglobin O_2 binding properties of young erythrocytes.

After training, all parameters of physical performance ($\dot{V}_{O_2max}$, maximal workload, heart rate during rest and exercise) were markedly improved, indicating fast adaptation mechanisms. The increased erythrocyte turnover, including higher erythropoiesis, seems to be one important part of these effects.

Key words: Reticulocytes — Red cell age — Erythropoietin — Bohr effect — Plasma volume

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Introduction

The endurance performance of working men depends primarily on the oxygen supply to the active muscle tissue. Well known adaptations leading to the ability for high oxygen consumption in endurance trained athletes are an increased total blood volume (e.g. Brotherhood et al. 1975), higher heart stroke volume (Ekblom and Hermansen 1968), improved conditions of oxygen diffusion from the blood to the working tissue, caused by an effectively developed close-meshed capillary system (Hudlicka 1982) and favourably changed haemoglobin oxygen dissociation properties. Characteristic of O_2 binding by haemoglobin in the blood of endurance trained athletes, when compared with normal untrained men, is a right shifted and steepened haemoglobin-oxygen dissociation curve (Braumann et al. 1979) especially under acidotic conditions (high Bohr effect), leading to improved oxygen delivery conditions from blood to tissue at rest and during heavy muscular work (Böning et al. 1975; Böning et al. 1982; Braumann et al. 1982).

During physical training, destruction of erythrocytes occurs. This so called "sports anaemia" is indicated by a decreased haemoglobin concentration in blood and a lowered haematocrit value (e.g. Dressendorfer et al. 1980; Eichner 1985; Falsetti et al. 1983; Poortmans and Haralambie 1979; Yoshimura et al. 1980). The reason for the destruction is not yet fully clarified. One cause is mechanical damage to red cells in the foot capillaries which occurs during running, but other reasons might exist because of the appearance of sports anaemia in other kinds of sport (e.g. cycling).

On the other hand, the well known increased total blood volume, including red cell mass, in

highly endurance trained athletes (Brotherhood et al. 1975) suggests increased training dependent erythrocyte formation. This fact, as well as the exercise destruction mainly of old erythrocytes (Reinhart et al. 1983), which are characterized by decreased mechanical and osmotic resistence (La Celle et al. 1973), should reduce the mean age of the erythrocytes in the blood of athletes (Mairbäurl et al. 1983).

Young erythrocytes are characterized by a right shifted and steeper oxygen dissociation curve than that of old cells or of the whole population (Edwards et al. 1967, Schmidt et al. 1987). Therefore the training-dependent increase in destruction and formation rate of erythrocytes might be the cause for the improved haemoglobin oxygen (Hb-O_2) binding properties (Braumann et al. 1982).

The aim of this study was to investigate various parameters of the training dependent erythrocyte turnover and its correlation to variations in Hb-O_2 binding properties. Since generally all training effects are most pronounced at the beginning of a training period, we observed untrained subjects before, during and after a three week training programme.

Methods

9 young, healthy, nonsmoking untrained male volunteers — 6 test subjects (age 23.6 ± 2.4 years, weight 76.2 ± 3.4 kg without change during the observation time) and three controls (age 23.7 ± 4.7 years, weight 74.6 ± 2.4 kg) were investigated during seven weeks, which consisted of one week prephase, three weeks training and three weeks postphase.

Since two subjects in the training group fell ill (common cold) after two weeks of training, their training programme was interrupted for one week and their values could not be used for time-linked comparisons of various results.

Exercise lasting 45 min after 5 min warming up at 100 W was performed on five consecutive days of the week by use of bicycle ergometers (Monark, Wilken and Ergotest, Jaeger, FRG) at heart rates between 165—175 beats · min^{-1} in the last 30 min of exercise corresponding initially to about 70% $\dot{V}_{O_{2max}}$. Because of the improvement in each subject's performance during the training programme, the mean daily work load increased from 193 ± 40 W to 235 ± 43 W from the first to the last day of training. The mean lactic acid concentration during work (samples were taken after two weeks of training) increased from 1.5 ± 0.3 mmol · l^{-1} at rest to 5.0 ± 1.6 mmol · l^{-1} after 5 min exercise and 7.9 ± 2.3 mmol · l^{-1} in the last minute of working.

Maximal work load (W), maximal oxygen uptake ($\dot{V}_{O_{2max}}$), (closed system, Meditron Magna Test 710) and the performance at 4 mmol · l^{-1} lactic acid in earlobe blood (according to Mader et al. 1976; test kit No 124842, Boehringer, FRG) were determined immediately before and one day after the exercise period by a performance test on a bicycle ergometer (Meditron Type 799; vita maxima, beginning with 100 W, workload increased stepwise every 3 min by 50 W until exhaustion).

Every morning 200 µl blood were sampled from the ear lobe of each subject for the determination of reticulocytes (expressed as per thousand of 2000 counted red cells from a bloodsmear dyed with Brilliant-Cresyl-Blue).

To determine the time-course of reticulocyte appearance in the peripheral blood after a single bout of exercise we performed an additional experiment. In six subjects (not those of the main study) the reticulocyte count was measured immediately before and for three days after a $\dot{V}_{O_{2max}}$-test as described above.

Each week blood samples were taken in the morning between 8 and 9 o'clock from the cubital veins of both arms. After 25 min in the supine position 5 ml were collected from the left vein, then 3 ml Evans Blue solution (0.3% T 1824 in 0.9% NaCl, for the determination of plasma volume) were injected into the same vein. The syringe was rinsed three times with the blood of the subject. Exactly 10 min later, 45 ml of blood were taken from the other arm, from which we determined the following parameters in a whole blood sample: haemoglobin (Hb) concentration (test kit, Merck, FRG), 2,3-diphosphoglycerate (DPG) concentration (test kit, Sigma, FRG), and haematocrit value (Hct, microhaematocrit centrifugation at 20900 g).

In plasma we determined plasma volume (PV) from the absorbance of both samples at 640 nm (according to von Porat 1951) and total plasma protein (Biuret method, test kit, Merck, FRG). In the erythrocytes, creatine concentration (according to Griffiths and Fitzpatrick 1967) and glutamate oxalacetate transaminase (GOT) activity (test kit, Boehringer, FRG) were measured as indicators of mean cell age.

Using the following equations (1—3) mean corpuscular haemoglobin concentration (MCHC), total blood volume (TBV) and red cell volume (CV) were calculated:

$$MCHC = (Hb \times 100) \cdot Hct^{-1} \quad (1)$$

$$TBV = (PV \times 100) \cdot (100 - Hct \times F_t \times F_c)^{-1} \quad (2)$$

($F_t = 0.96$, factor for trapped plasma according to Convertino et al. 1980; $F_c = 0.91$, cell factor for body haematocrit according to Fricke 1965).

$$CV = TBV - PV \quad (3)$$

In the weekly collected blood samples we also measured the Hb-O_2 binding properties under various conditions (A—D); A: Equilibration gas consisting of 5% CO_2 in air, 37° C; B: 10% CO_2, 37° C; C: 5% CO_2, 10 mmol · l^{-1} lactic acid, 37° C; D: 5% CO_2, 41° C.

Measurements of oxygen partial pressure (P_{O_2}) and of pH were made with the blood micro system BMS 3 MK 2 and pHM 73 and of oxygen saturation (S_{O_2}) with the haemoximeter OSM 2 (all Radiometer, Copenhagen, Denmark) in order to determine the oxygen dissociation curves (ODC) (using the mixing technique of Braumann et al. 1982). In additional experiments comparing blood samples treated with and without T-1824 we could not detect any effect of T-1824 on S_{O_2}. At every mixing step the pH of the intracellular milieu (pH_i) was also measured (freeze — thaw method).

The Bohr coefficients for CO_2 (BC_{CO_2}) and lactic acid (BC_{Lac}) as well as the temperature coefficient (TC) were calculated at constant S_{O_2} with the following formulae:

$$BC = \Delta \log P_{O_2} \times \Delta pH^{-1} \quad (4)$$

$$TC = \Delta \log P_{O_2} \times \Delta T^{-1} \quad (5)$$

Table 1. Haematological data and haemoglobin-oxygen binding parameters of the control subjects during the observations

Observation week	1	4	7
[Hb] ($g \cdot 100\ ml^{-1}$)	15.4 ±0.5	15.3 ±0.8	14.8 ±0.5
Hct (%)	43.7 ±0.3	43.4 ±1.9	42.8 ±1.0
Plasma prot. ($g \cdot 100\ ml^{-1}$)	7.46 ±0.22	7.35 ±0.21	7.55 ±0.14
PV ($ml \cdot kg^{-1}$)	38.3 ±0.5	40.6 ±3.1	41.0 ±3.7
MCHC ($g \cdot 100ml^{-1}$)	35.2 ±1.1	35.3 ±0.4	34.6 ±1.7
GOT ($U \cdot gHb^{-1}$)	3.13 ±0.16	3.25 ±0.08	3.43 ±0.11
[Creatine] ($\mu mol \cdot gHb^{-1}$)	1.10 ±0.05	1.15 ±0.06	1.11 ±0.13
[DPG] ($mol \cdot molHb^{-1}$)	0.79 ±0.06	0.81 ±0.05	0.77 ±0.08
P_{50} (kPa)	3.72 ±0.20	3.72 ±0.07	3.76 ±0.11
Hill "n" (at 50% S_{O_2})	2.76 ±0.11	2.72 ±0.25	2.73 ±0.22

Values are means ±SD; $n=3$. Abbreviations see text

The oxygen dissociation curve was then calculated to standard conditions (pH 7.4, 37° C) with the individual BC_{Lac}.

Since the position of the ODC and the Bohr coefficients could be influenced by variations in intraerythrocytic properties during the training period, the Bohr coefficients were calculated for intraerythrocytic conditions according to formula 6:

$$BC_i \times BC_e^{-1} = \Delta pH_e \times \Delta pH_i^{-1} \quad (6)$$

(indices e and i indicate extra- and intracellular space, respectively).

For statistical calculations we used the Student t test (for two means), as well as analysis of variance (Anova, for multiple means) and of regression, all according to Winer (1971).

Results

All measured quantities of the control group, which should indicate the possible influence of the weekly blood loss on haematological parameters, did not change during the observation time (Table 1). Therefore their values are not considered in the following text.

As shown in Table 2 the parameters of physical performance, tested immediately before and after the training programme, indicate significant improvements after training.

Haematological data from the 4 subjects investigated during 7 weeks without interruption are given in Table 3. [Hb] and Hct showed the expected fall during the training period. In contrast, the total haemoglobin mass calculated by use of the red cell volume (see below) increased; this is especially clear, if all 6 subjects are considered (+39 g or 5.0% from the initial value to the first week after training, $2p < 0.01$).

Plasma protein concentration temporarily rose during the second week of training (Table 3). PV and TBV increased continuously until the end of the training programme and decreased thereafter. In contrast, CV increased later and had a maximum in the first week of recovery. All changes in relative volume ($ml \cdot kg^{-1}$ body weight, were significant (Anova: $p < 0.025$). For all 6 subjects also absolute volume increases could be statistically confirmed (changes in PV and TBV from the initial values to the end of training: PV from 3469 ± 572 ml to 3982 ± 640 ml; TBV from

Table 2. Spiroergometric data of the test subjects before and after the training period

	HR_{rest} ($beats \cdot min^{-1}$)	HR_{work}	$\dot{V}_{O_2max}$ ($ml \cdot kg^{-1}$)	An Thr (Watt)	Lac max ($mmol \cdot l^{-1}$)	Work max (Watt)
Before Training	75 ± 7	158 ±19	46.1 ±6.6	211 ±27	12.3 ±1.2	286 ±37
After Training	66** ±10	148* ±16	51.8*** ±5.0	248*** ±26	13.6 ±1.6	329*** ±34

Values are means ±SD, $n=6$. HR_{rest} = heart rate immediately before the test; HR_{work} = heart rate during the test at 200 W (workload, which could be performed for three minutes by all test subjects during both tests); $\dot{V}_{O_2max}$ = oxygen uptake in the last minute before exhaustion; An Thr = anaerobic threshold according to Mader et al. 1976; Lac max lactic acid concentration three minutes after the test; Work max = maximal workload in the last minute before exhaustion. Degree of significance in t-test (paired samples):
* $2p < 0.05$; ** $2p < 0.01$; *** $2p < 0.001$

Table 3. Haematological data and parameters of mean erythrocyte age in the test subjects

Observation week	1 Before	2	3 Training	4	5	6 After	7	Anova
[Hb] ($g \cdot 100\ ml^{-1}$)	15.8 ±0.5	14.7* ±0.4	14.4* ±0.6	14.5* ±0.8	15.1 ±0.4	15.0 ±0.5	14.7 ±0.4	$p<0.01$
Hct (%)	44.0 ±1.2	41.9* ±1.8	41.4* ±1.9	41.8 ±2.5	43.8 ±0.8	43.7 ±1.8	42.5 ±0.8	$p<0.05$
[Plasma prot.] ($g \cdot 100\ ml^{-1}$)	7.46 ±0.29	7.58 ±0.31	7.85* ±0.51	7.49 ±0.26	7.09 ±0.53	7.56 ±0.41	7.58 ±0.33	
TBV ($ml \cdot kg^{-1}$)	72.3 ±9.1	72.8 ±8.0	75.9 ±6.2	79.7* ±8.1	77.8* ±9.2	— —	72.1 ±9.7	$p<0.025$
PV ($ml \cdot kg^{-1}$)	44.6 ±6.1	46.3 ±5.5	48.5* ±3.8	50.6** ±5.9	48.0 ±5.3	— —	45.1 ±5.2	$p<0.025$
CV ($ml \cdot kg^{-1}$)	27.7 ±3.1	26.5 ±2.9	27.4 ±2.8	29.1 ±2.8	29.8* ±3.8	— —	26.7 ±4.0	$p<0.025$
MCHC ($g \cdot 100\ ml^{-1}$)	35.9 ±1.3	35.0 ±0.6	34.7 ±0.8	34.8 ±0.5	34.5* ±0.4	34.5* ±0.7	34.7 ±0.8	
GOT ($U \cdot gHb^{-1}$)	2.92 ±0.13	3.19 ±0.15	3.50*** ±0.08	3.69** ±0.24	3.77*** ±0.13	3.70* ±0.24	3.59* ±0.39	$p<0.01$
[Creatine] ($\mu mol \cdot gHb^{-1}$)	1.11 ±0.16	1.11 ±0.21	1.26 ±0.20	1.19* ±0.20	1.23* ±0.22	1.11 ±0.21	1.15 ±0.27	

Values are means ±SD; $n=4$. Abbreviations, see text. Anova = significance of changes during the observation time. *t*-tests (paired samples) were performed between the initial and all following values
* $2p<0.05$; ** $2p<0.01$; *** $2p<0.001$

5585±872 ml to 6280±725 ml; changes in CV from the initial value to the first week of recovery: 2116±307 ml to 2298±306 ml; in all cases $2p<0.01$).

The indicators of mean red cell age (GOT activity, MCHC, [creatine]) clearly demonstrate a younger erythrocyte population after the training programme (Table 3). Most pronounced was the increase in GOT activity during training (from 3.01±0.18 $U \cdot gHb^{-1}$ to 3.82±0.16 $U \cdot gHb^{-1}$; $n=6$, $2p<0.001$), which remained at a high level in the following three weeks.

During and some days after the training programme there was a highly significant elevation of the reticulocyte count in the peripheral blood (Fig. 1). But it must be noted that because of the increase in CV during the training period the absolute number of produced red cells was higher at the end of the training programme than indicated by the relative reticulocyte count. In the last week of observation the reticulocyte number fell below the average values observed during the week before training ($p<0.01$).

The behaviour of reticulocyte release from the bone marrow could be demonstrated in the additional experiments after a single bout of exercise. As shown in Fig. 2 the appearance of reticulo-

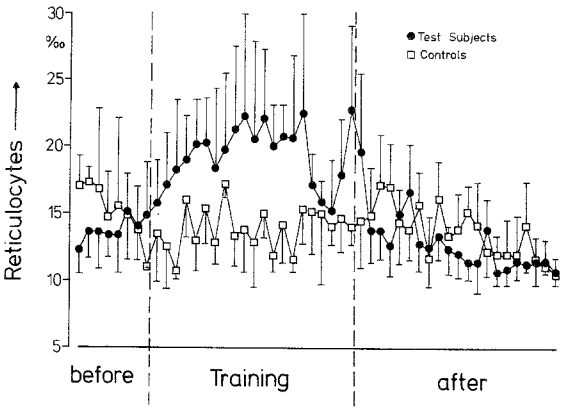

Fig. 1. Reticulocytes in the peripheral blood of test subjects ($n=4$) and controls ($n=3$) during the seven week observation time. Each symbol represents one day. Significance of the time courses *(Anova)*: test subjects $p<0.001$, controls ns, difference between both groups $p<0.01$

Table 4. Haemoglobin-oxygen binding characteristics in the blood of the test subjects

Observation week	1 Before	2	3 Training	4	5	6 After	7	Anova
[DPG] (mol · molHb^{-1})	0.73 ±0.05	0.87* ±0.10	0.85** ±0.06	0.84* ±0.07	0.80** ±0.05	0.79** ±0.05	0.81 ±0.04	$p<0.01$
P_{50} (kPa)	3.77(*) ±0.05	3.68(*) ±0.08	3.80(*) ±0.07	3.85 ±0.05	3.99* ±0.07	3.80 ±0.08	3.87 ±0.08	$p<0.01$
Hill "n" (at 50% S_{O_2})	2.85 ±0.11	2.88(*) ±0.07	2.96 ±0.09	3.06 ±0.10	3.09 ±0.17	2.88 ±0.07	2.91 ±0.16	
BC_{CO_2e} (at 50% S_{O_2})	0.51 ±0.05	0.49 ±0.04	0.48 ±0.06	0.58 ±0.19	0.40 ±0.09	0.42 ±0.13	0.51 ±0.05	
$BC_{lac,e}$ (at 50% S_{O_2})	0.43 ±0.05	0.49(**) ±0.07	0.50(**) ±0.02	0.39 ±0.13	0.34 ±0.06	0.38 ±0.13	0.38 ±0.18	
BC_{CO_2i} (at 50% S_{O_2})	0.64 ±0.05	0.61(*) ±0.07	0.70(*) ±0.08	0.95 ±0.44	0.51 ±0.09	0.54 ±0.21	0.62 ±0.08	
$BC_{lac,i}$ (at 50% S_{O_2})	0.56(*) ±0.03	0.63 ±0.13	0.68(**) ±0.12	0.63 ±0.24	0.43* ±0.07	0.44 ±0.19	0.46 ±0.15	$p<0.05$
TC (at 50% S_{O_2})	0.020 ±0.002	0.025 ±0.004	0.025* ±0.002	0.019 ±0.004	0.015 ±0.004	0.022 ±0.005	0.025 ±0.003	$p<0.001$

Values are means ±SD; $n=4$. Abbreviations see text. *t*-tests were performed as decribed in Table 2, as well as for the Bohr coefficients, P_{50}, and Hill's "n" between the values of the fifth week (youngest state of the erythrocyte population) and all other values. In these cases: (*) $2p<0.05$; (**) $2p<0.01$

cytes in the peripheral blood was most pronounced two days after heavy exercise.

Data concerning the haemoglobin-oxygen binding characteristics are shown in Table 4. [DPG] increased in the first week of training, staying at a higher level during the following time. The position of the oxygen dissociation curve (ODC) expressed as half saturation pressure (P_{50}) does not exactly correlate to [DPG]. P_{50} was significantly increased only in the first post-training week. The apparent systematic increase of Hill's "n" up to the first week of recovery could not be fully confirmed by Anova ($p<0.1$). The Bohr coefficients had their highest values during the training period and lowest values in the first recovery week; the differences between these extreme values are partly significant (BC_{CO_2i}, $BC_{Lac,e}$, $BC_{Lac,i}$). The temperature coefficient (TC) showed a time course similar to BC, with a significant increase during the training period (Table 4).

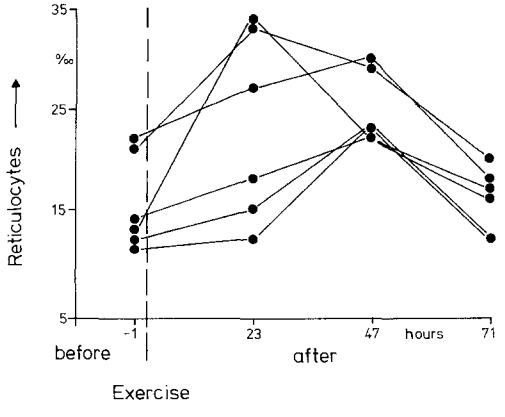

Fig. 2. Reticulocyte number in the peripheral blood immediately before and up to three days after a single bout of exercise until exhaustion in 6 subjects. (Significance of the difference from the initial value $2p<0.001$ two days after exercise)

Discussion

Haematological status

In the course of the training programme TBV increased by about 700 ml ($n=6$) or 570 ml ($n=4$), which was caused by an elevated PV (74% or 81.5%) and CV (26% or 18.5%). The rise in PV is in the same range as that reported by Convertino et al. (1980). The exact reason is not yet clear. Some fast effects on PV might be the elevation of renin and aldosterone as well as ADH concentrations after a single endurance training bout, which

leads to increased PV for up to 48 hours after prolonged exercise (Costill et al. 1976).

For long term adaptation we suggest that the following sequence of steps during training is responsible for the increase in plasma volume: 1. Production of plasma protein increases; each 1 g binds 14—15 ml of water (Convertino et al. 1980). A rise in plasma protein concentration has been observed as early as a few hours after exhausting exercise (Böning et al. 1979). In our study protein concentration (Table 3) and protein mass (+37 g) increased until the second week of training. 2. In the following week PV continued to increase, whereas protein mass remained stable and protein concentration even tended to decrease. This must result from additional water preservation. Indeed, the usual reaction to increased filling of the venous system — namely diuresis resulting from high central venous pressure during immersion (Henry-Gauer reflex) — is reduced in athletes (Böning and Skipka 1979). The underlying mechanisms might be an enhanced aldosterone effect after training (Skipka et al. 1979) or changes in the long postulated and now established atrial natriuretic factor (e.g. Weidmann et al. 1986).

In contrast to PV, the increase in CV during training is delayed, obviouly resulting from a strong initial haemolysis. The relative share of daily red cell formation and destruction can be estimated from our data as follows: we assume an average lifespan of 120 days for the erythrocytes in the prephase. Under this condition the daily turnover amounts to 0.83% of the total red cell population. By means of the reticulocyte number and the changes in CV, the daily formation (EF) and destruction (ED) of red cells (%, Fig. 3, or ml)

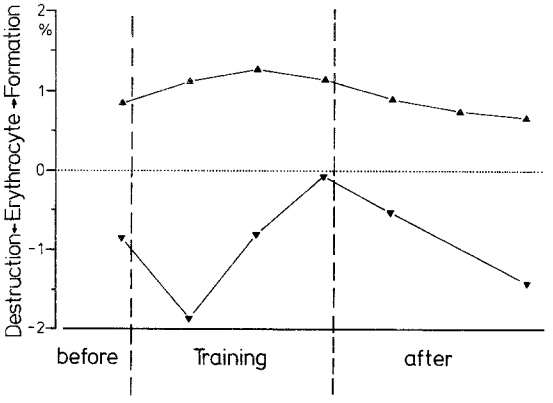

Fig. 3. Daily erythrocyte turnover in the blood of the test subjects before, during and after the training period (*each symbol* represents the mean of seven days). For conditions of calculation see text

can be calculated by formulae 7 and 8 (%) or 7a and 8a (ml), respectively:

$$EF\ (\%) = R \times f \tag{7}$$

$$EF\ (ml) = R \times f \times (CV \times 100^{-1}) \times F_1 \tag{7a}$$

R = reticulocyte count (‰, mean of seven days); f = factor to calculate the daily red cell formation: normal daily reproduction = 0.83% of the cell volume, reticulocyte count in the prephase = 13.5‰ corresponding to the daily production of 0.83% of the total red cell mass, $f = 0.83 \times 13.5^{-1} = 0.061$; CV = red cell volume (weekly estimated), F_1 = cell age dependent volume factor (1.114 for young cells, if the whole population is set at 1.000, calculated according to Piomelli et al. 1967).

$$ED\ (\%) = EF\ (\%) + (CV_1 - CV_2) \times (7 \times F_2)^{-1} \times (100 \times CV_1^{-1}) \tag{8}$$

$$ED\ (ml) = EF\ (ml) + (CV_1 - CV_2) \times 7^{-1} \tag{8a}$$

$(CV_1 - CV_2)$ is the weekly difference in red cell volume, which must be divided by 7 in order to get the daily variation. If one assumes that mainly old erythrocytes are destroyed, the factor $F_2 = 0.886$ for old erythrocytes is valid (Piomelli et al. 1967).

In the first week of training the daily cell destruction amounts to about 2% of the whole population, exceeding the production of reticulocytes (1.1%), the latter being greater in the following three weeks (Fig. 3).

The high destruction rate at the beginning of the training period may be caused especially by the elimination of old erythrocytes (Eichler 1985; Reinhart et al. 1983). These cells are characterized by reduced osmotic and mechanical resistance (La Celle 1973); they should especially be damaged by the training induced mechanical and biochemical stress and subsequently destroyed. Another reason may be the early appearance of plasma factors released from the spleen impairing the mechanical properties of the erythrocyte membrane, as was demonstrated by Yoshimura et al. (1980). If in fact particularly old erythrocytes are haemolysed during exercise the progressive reduction of cell age during training must decrease the destruction rate. This is demonstrated in Fig. 3.

The mechanisms regulating the exercise induced erythropoesis have not been sufficiently investigated. As demonstrated by Wichmann et al. (1983), a negative correlation between Hct and the erythropoietin titre in the blood exists. Thus the

good correlation between Hct and reticulocytes in this study ($r = -0.71$, Fig. 4) suggests the release of erythropoietin into the blood. One may hypothesize that the reduced renal blood flow during exercise (Poortmans 1984) causes a decreased venous P_{O_2} in the kidney leading to the formation and liberation of erythropoetin (Jelkmann 1986). The beginning of the reticulocytosis two days after the first training bout or, as demonstrated in the additional experiments, after a single short exercise until exhaustion, correlates well with the time linked effect of erythropoetin (Papayannopoulou and Finch 1972). Indeed it could be demonstrated by Lindemann et al. (1978) that the erythropoetin titre in the blood of young soldiers was slightly increased after a four days training programme. But because of the relatively unspecific methods for determination of erythropoetin (Jelkmann 1986) at least nine years ago, one must confirm their results with newly developed methods (radioimmunoassay, Cohen et al. 1985).

Additionally to erythropoetin release of the kidney, other hormones released during exercise such as cortisol, growth-hormone, adrenaline and thyroxine, which are known as erythropoetic factors, may contribute to the training dependent erythropoiesis.

The high destruction rate especially of old erythrocytes (Reinhart et al. 1983) and the reticulocytosis during and immediately after the training period lead to a shift to a younger erythrocyte population. This is confirmed by the marked increase in GOT activity, which is known to be one of the most sensitive indicators of red cell age (Fisher and Walter 1971). Additionally, the increase in red cell [creatine] and the decrease in MCHC observed in this study and also reported by Eichner (1985) and Reinhart et al. (1983) after long distance races are further proofs of a younger mean red cell population after training.

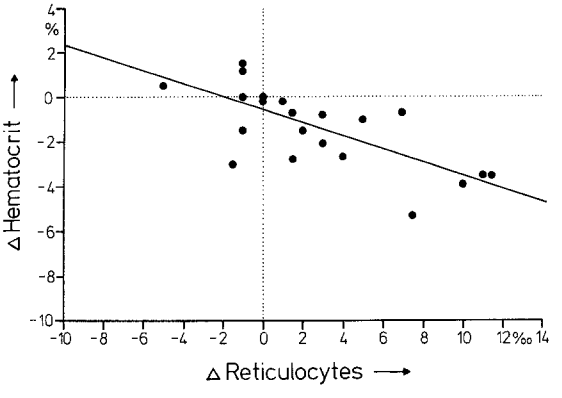

Fig. 4. Correlation between weekly changes in haematocrit and reticulocyte number (mean values from seven days) compared to the initial values in the blood of the test subjects during the observation time. $n = 24$; $Y = -0.29 \times -0.56$; $r = -0.71$; $p < 0.001$

Thus the nearly constant low values of [Hb] and Hct during the training period are caused by totally different mechanisms: namely the increased haemolyis in the first week, and the relative greater increase in PV than in CV in the second and third weeks.

Haemoglobin-oxygen-binding properties

The different time course of the Hb-O_2 binding parameters (P_{50}, "n", BC) demonstrates that more than one cause is responsible for the observed changes. One of these causes is the training dependent increase in [DPG], which again is due to different mechanisms. Firstly, [DPG] is negatively correlated to haemoglobin concentration in whole blood, the cause of which is not fully elucidated. Secondly, there are many changes in blood during and after exercise (e.g. desaturation of haemoglobin, acid base changes, elevation of inorganic phosphate and pyruvate, secretion of hormones), which might influence DPG turnover. Thirdly, young erythrocytes possess high DPG concentrations resulting from higher enzyme activities (e.g. Schmidt et al. 1987). This effect should be most important at the end of the training period and at the beginning of the postperiod.

In spite of increased [DPG], P_{50} shows no significant variations during the whole training period, also observed in similar experiments by Shappell et al. (1971). One week after the training programme, however, when the erythrocyte population is at its youngest mean age but the exercise stimulus is no longer present, Hb-O_2 affinity is mostly reduced. In this week Hill's "n" seems to have the highest value. Young erythrocytes are characterized by a right shifted and steepened ODC (Schmidt et al. 1987). Therefore, the appearance of a young cell population could explain the variations in these Hb-O_2 binding properties after the training period. Its effect is an improved oxygen unloading from the blood to the tissues, when compared to the initial values before training.

A further property of young red cells is decreased Bohr coefficients (Schmidt et al. 1987). Therefore one should expect a continuous decrease in the BCs during the training period. However, low values are only observed when the training stimulus is withdrawn (first recovery week). During the training period, part of the BCs

were significantly higher than in the first recovery week, which is consistent with our earlier observations of large BCs in highly trained active athletes (Böning et al. 1982; Braumann et al. 1982). Therefore, a younger cell population is not responsible for all changes in Hb-O_2 binding parameters in endurance trained athletes, as was suggested by Braumann et al. (1982). Probably a special training factor increasing the BC is partly antagonized by the decreasing cell age.

The dependency of TC on training has never been investigated. Since the relatively small differences in [DPG], as found in this study, do not yet influence TC (Benesch et al. 1974) and since apparently no variations of other known TC influencing factors exist (e.g. P_{CO_2}), the observed changes in TC cannot yet be explained.

Physical performance

It must be assumed that, besides the well known adaptation factors (e.g. higher enzyme activity in the working muscle and a more economic capillary blood flow), activation of the erythropoetic system is one cause for the observed improvement in endurance capacity of the test subjects (Table 2). One week after training the increase in total haemoglobin mass by 5% improves the O_2 transport capacity of the body. The right shift in the ODC, especially during heavy work (Bohr effect), compensates for the fall in haemoglobin concentration. Furthermore, because of the elimination of the oldest cells, which possess more useless haemoglobin molecules for oxygen transport (e.g. methHb, Hb_{AIc}), the O_2 transport capacity of a given blood volume should be augmented after training.

Finally it should be noted that, in spite of increased red cell volume, the rheological properties of blood might be improved because of lower haematocrit values and a younger mean erythrocyte population, both characterized as factors which lower blood viscosity (Tannert et al. 1977).

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