Table 2. Hemodynamic changes in acute venous stasis edema of the rat tail evaluated by invasive techniques before, during and after the release of a 7-h ligature in anesthetized rats (n = 5/group)

Time	0 h	7 h (with ligature)	0 h (after release)	3 h (after release)
A) Arterial pressure (mm · Hg)	99.4 ± 4.6	97.8 ± 2.3	81.0 ± 7.7	98.6 ± 6.4
B) Heart rate (beats/min)	442 ± 21	426 ± 14	378 ± 19	398 ± 15
C) Cardiac output (ml/min/kg)	296 ± 20	257 ± 29	235 ± 30	260 ± 5
D) Blood flow in the tail (µl/min/g)	82.6 ± 11.6	30.4 ± 10.0*	165.0 ± 34.0*$^{+}$	149.0 ± 8.0*$^{+}$
E) % of cardiac output in the tail	0.128 ± 0.028	0.051 ± 0.013*	0.320 ± 0.077*$^{++}$	0.270 ± 0.011**$^{++}$

* $p < 0.05$, ** $p < 0.01$: values compared to time 0 h; $^{+}$ $p < 0.01$, $^{++}$ $p < 0.001$: values compared to time 7 h with ligature.

computer thermography (markers of cutaneous blood flow) from those obtained with the radioactive microsphere technique (marker of organ blood flow) in the acute and sustained hyperemic situations. The sensitivity of the blood vessels to norepinephrine is directly proportional to the internal diameters of the blood vessels in a micro-vascular bed, which could be due to the higher density of innervation in the bigger dissociation of blood flows in the skin and the whole organ (tail) in the hyperemic situation after release of the ligature.

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Systemic oxygen transport and erythropoiesis in the mouse[1]

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Summary. Removal of 15% of blood volume int he mouse increases erythropoiesis by a factor of 2.2 when measured 12 h after bleeding. Exposure of normal mice to 40% reduced barometric pressure for the same period of time increases erythropoiesis only by a factor of 1.6. The response to hypoxia takes place in the presence of a 40% reduction of oxygen consumption and tissue-venous P_{O_2}, changes which are concomitant with a 5-fold increase in plasma erythropoietin activity. The larger response in anemic animals on the other hand occurs without any detectable change in these parameters. These results cast serious doubts about the interpretation of the quantitative homeostatic control of erythropoiesis based solely on the action of erythropoietin.

The presence of erythropoietin (Epo) is essential in the course of erythroid development to induce differentiation of erythroid comitted stem cells (ESC) into erythroblasts, as this compartment is not self-maintaining[2,3]. Administration of the hormone causes an increase in erythropoiesis whereas injection of a specific anti-Epo serum almost completely eradicates erythroid cells from the spleen and bone marrow in the mouse[4]. These, among other actions of the hormone, have led to the idea that Epo is the sole quantitative regulator of erythropoiesis. According to this interpretation variations in oxygen exchange O_2Ex) would be expected to produce changes in the bio-genesis and in the plasma concentration of Epo in exact proportion to the variations, and in the same direction[3].

There are, however, major objections to this unitarian theory[5–7]. Among the most important is the notion that even minute changes in the hemoglobin mass (Hb-M) should necessarily result in strictly commensurate variations in O_2Ex. If this were to be the case, then no other physiological mechanism in the systemic oxygen transport (SOT) would be operative or even exist for the compensation of small blood losses.

Here we report data that indicate that erythropoiesis in the mouse can be increased significantly in the absence of changes in the O_2Ex. The model used was the increase in erythropoiesis that followed a mild experimental anemia (MEA), which was compared with the changes in erythropoiesis caused by exposure of mice to hypoxic hypoxia (HH).

Material and methods. We used F_1 C3H/FWD female mice 7–9 weeks of age, MEA was produced by draining 15% of the blood volume from the retrorbital venous sinus of the eye. After centrifugation, the plasma was reinjected i.v. Groups of normal mice were placed in a chamber at low barometric pressure with pressure adjusted to 450 mm Hg. The chamber was opened daily for cleaning and for food and water renewal. Temperature was kept at 22 °C, humidity near saturation point and air flow at a rate of 20 l. Oxygen consumption ($\dot{V}_{O_2}$) was measured with the method described by Grad[8]. ($\dot{V}_{O_2}$) in mice exposed to HH was

Experimental condition	$\dot{V}_{O_2}$ (cc/100 g b.wt)	$P_{v\text{-}tO_2}$ (mm Hg)	$P_{v\text{-}tO_2}$ (mm Hg)	P 50% (mm Hg)	Plasma Epo activity*	Bicarbonate concentration**	Ht
Controls	10.5 ± 1.1****	40.1 ± 1.6	41.9 ± 1.3	39.5 ± 1.3	4.2 ± 1.0	24.3 ± 1.8	43.3 ± 1.2
After 8 h							
Hypoxic (9)***	*6.8 ± 0.9*	*25.0 ± 1.7*	*32.1 ± 1.9*		*19.9 ± 3.0*		44.0 ± 1.5
Anemic (10)	10.7 ± 1.1	39.9 ± 2.0	42.2 ± 2.3		4.7 ± 2.0		*37.0 ± 2.2*
After 24 h							
Hypoxic (9)	*6.3 ± 1.4*	*26.0 ± 1.9*	*30.0 ± 2.2*	*52.1 ± 2.3*	*12.0 ± 1.5*	*14.7 ± 1.9*	44.2 ± 2.0
Anemic (10)	10.7 ± 2.0	40.0 ± 2.2	42.9 ± 3.0	*53.1 ± 3.0*	5.0 ± 0.9	21.1 ± 1.9	*38.0 ± 2.1*
After 48 h							
Hypoxic (9)	*6.9 ± 2.0*	*28.0 ± 2.2*	*33.3 ± 3.0*		7.0 ± 0.8		44.0 ± 2.0
Anemic (10)	10.6 ± 1.6	40.6 ± 3.3	41.2 ± 2.8		4.6 ± 0.3		41.0 ± 1.9

* Measured by the Hodgsons's Index. ** Bicarbonate concentration (real mEq/l). *** Number of mice in the group. **** Mean ± SE. Values set in italic type differ from controls by p of 0.05 or less.

measured after thoroughly washing the respirometer-chamber with a mixture of air and nitrogen to yield an atmosphere of 12.5 ± 0.5% of oxygen. Venous-tissue P_{O_2} ($P_{v\text{-}tO_2}$) and venous-tissue P_{CO_2} were determined using the gas-pocket technique as described by Rahn[9]. Analyses of the gases in the pocket were performed after 8 h of equilibration using the Scholander 0.5-ml apparatus[10]. Values for oxygen affinity (P 50%) were obtained as described elsewhere[11]. Acid-base status was evaluated 6 h after insertion of the dialyzing bag, using the Astrup equilibration technique and a Radiometer gas-analyzer as previously described[12]. Plasma erythropoietin activity was measured at various times by a bio-assay using as recipients mild polycythemic transfused mice as follows: On day zero recipients were given 0.8 ml of washed isologous erythrocytes i.p. On days 2 and 3, 0.5 ml of the plasma under study was injected i.p. On day 4 0.25 µCi of ^{59}Fe were injected i.v. and 3 h later the percentage of the injected radioiron taken up by the spleen, both femurs and circulating reticulocytes was calculated. For calculation of RBC ^{59}Fe uptake blood volume was assumed to be 7% of b.wt. Total bone marrow was estimated as 7 times the fraction contained in both femurs. Controls received normal plasma in equivalent volume. Using these values the amount of iron 59 going to the erythroid tissues per h was calculated according to Hodgson's formulae[13]. This assay proved effective to detect activities as low as 0.02 U (IRP). 72 h after hypoxia exposure or bleeding some animals in each group were injected with 0.25 µCi of ^{59}Fe and the topographic distribution of the radiodose and the Hodgson's Index were calculated as above.

Results. Erythropoiesis as measured by the Hodgson's Index in normal unmanipulated mice was 27.7 ± 2.8 (SE). This value rose to 43.5 ± 3.1 in the HH group and to 58.9 ± 3.3 in the MEA when measured 72 h after either hypoxia exposure or bleeding. The table shows the values of $\dot{V}_{O_2}$, $P_{v\text{-}tO_2}$, P 50%, plasma Epo activity, acid-base balance and hematocrit in normal controls and in MEA and HH mice at various times after onset of hypoxia or anemia. $\dot{V}_{O_2}$ and $P_{v\text{-}tO_2}$ in HH mice were consistently reduced at all times covered by these observations. On the other hand, and in spite of the reduction of the hematocrit from 43 to 37, MEA mice showed normal $\dot{V}_{O_2}$. $P_{v\text{-}tO_2}$ in this group was slightly reduced in a few animals although the change was not statistically significant when compared with normal controls. P 50% was similarly increased in both experimental groups. Plasma Epo activity was increased significantly at 8 and 24 h in the HH animals whereas no detectable change was found in MEA mice at any time. Ventilatory rate as estimated by the acid-base balance reflected a highly significant increase in HH whereas it remained normal in MEA animals.

Discussion. The interpretation of the increase of erythropoiesis that follows MEA or HH, in accordance to the unitarian theory, implies that in both cases the first step in the response is a reduction in the O_2Ex. namely functional hypoxia. Such an interpretation is entirely consistent with the results found in the HH group which shows that a marked decrease in $\dot{V}_{O_2}$ is associated to a similar reduction in $P_{v\text{-}tO_2}$. These changes that take place in spite of the increase in ventilatory rate are also concomitant with a clear increase in plasma Epo activity and are followed 72 h later by a 1.6-fold increment in erythropoiesis.

The results in the MEA mice, on the other hand, differ considerably from those in the HH group. The most conspicuous difference was an even larger increase in erythropoiesis, which occurs, paradoxically, in the absence of changes in O_2Ex. A 15% reduction in the hematocrit is not reflected by any modification of the $\dot{V}_{O_2}$, which remains essentially normal. In this regard it should be pointed out that this parameter in rats subjected to the same anemic experimental condition was also found to be unchanged after reduction of the hematocrit from 44 to 34[14]. The fact that no detectable plasma Epo activity was found at any time in MEA mice also stands against any substantial reduction in O_2Ex.

The normality of these oxygen-dependent parameters in the face of marked reductions in the Hb-M therefore provides evidence that under these circumstances SOT contains mechanisms for compensation in the oxygen provision to the body. Decreases in the hematocrit initiate, without a threshold, a circulatory response directly related to a lowering in blood viscosity and thereby in the systemic vascular resistance, which results in a more efficient venous return[14,15]. To these adjustments should be added the possibility of an increase in arterio-venous oxygen extraction made possible by a shift of ODC to the right as indicated by the increase in P 50%. A Bohr effect is compatible with slight increase in $P_{v\text{-}tCO_2}$. Also, a more efficient oxygen unloading would be facilitated by an increase in the erythrocytic 2,3 DPG content[16].

Although erythropoiesis was comparable in both experimental conditions, at the onset of either anemia or hypoxic stimuli. The intensity of the resulting hypoxemia was not. Even though a small and undetectable degree of functional hypoxia might have been present in the MEA animals, its magnitude must have been drastically smaller than in HH.

Consequently, the larger erythropoietic response in the MEA does not appear to depend solely on the action of EPO, that is, on a strictly commensurated relation between O_2Ex, Epo and erythropoiesis. This lends support to the theory that accessory mechanisms exist in the quantitative control of erythropoiesis. Admitting that in the steady state,

plasma Epo concentration stays at a constant and low level, feeding new erythroblasts into the erythroid compartment at a fairly constant rate, a locally acting agent(s) may be responsible for the fine quantitative regulation of erythrocytic production. The so-called hematopoietic-inductive-microenvironment (HIM)[17], possibly involving cell to cell interactions as well as intercellular communications mediated by short-distance-acting metabolites, may well be part of the local regulating factors proposed here.

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Effect of pectin and cellulose on formation and regression of gallstones in hamsters[1]

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Summary. Male Syrian hamsters were fed a lithogenic diet containing 7% cellulose or 4.2% pectin. After 50 days, pectin was 76% and cellulose 64% less lithogenic than the control diet. Hamsters fed the control diet for 50 days were maintained on that diet for another 50 days or fed diets containing cellulose or pectin. There was a 52% increase in gallstone incidence in hamsters continued on the control diet and a 9% increase in those on cellulose. Pectin promoted regression of gallstones (by 52%).

Bile is a finely balanced solution of cholesterol, bile salts and phospholipids. Oversecretion of cholesterol or undersecretion of bile salts will result in bile which is supersaturated with cholesterol and gallstones will result. Epidemiologically, gallstones are considered to be a disease of highly developed societies[3,4]. One dietary difference between developed, gallstone-prone societies and underdeveloped, gallstone-free societies lies in the fact that the former ingest a diet higher in refined carbohydrate and poorer in fiber than the latter. It has also been shown[5,6] that wheat bran will increase the size of bile salt pools in man, a condition which would inhibit gallstone formation. No direct studies on the effects of dietary fiber on gallstone dissolution in man have been carried out.

Dam and Christensen[7] demonstrated in 1952 that gallstones could be produced in hamsters by feeding a diet containing 74.3% sucrose, 20% casein, 5% salt mix, 0.5% vitamin mix and 0.2% choline. Although many variations of this diet have been suggested[8,9], it remains the standard means of inducing gallstones in hamsters. Heaton[3] has summarized the dietary means of causing gallstones in experimental animals and all of them involve feeding large amounts of refined carbohydrate and no fiber. Gallstone formation can be inhibited by replacing the sucrose by whole wheat, rolled oats or sorghum[10] and by replacing or diluting the casein with soy protein[11,12]. Bergman and van der Linden[13] and Rotstein et al.[14] have shown that gallstone formation can be partially inhibited by addition of 5% pectin to the diet and almost completely inhibited by adding 5% lignin. We thought it of interest to compare the effects of pectin and cellulose on gallstone formation, liver cholesterol levels and biliary lipids. Pectin has been shown to reduce cholesterol levels in rats[15] and might be expected to affect the level of biliary cholesterol. We also studied the effects of pectin and cellulose on pre-established gallstones on the premise that readjustment of bile composition might lead to dissolution of existing gallstones.

Materials and methods. Male Syrian hamsters weighing 60±5 g were maintained 5 to a cage in an air-conditioned (21 °C) animal room and subjected to a 12-h light/dark cycle. Control hamsters were fed the lithogenic diet of Dam and Christensen[7]: 74.3% sucrose, 20% casein, 5% salt mix, 0.5% vitamin mix and 0.2% choline chloride. The composition of the salt and vitamin mixes has been detailed elsewhere[11].

Hamsters on the cellulose-containing diet received 67.3% sucrose and 7% cellulose, the other components of the diet being unchanged. The pectin group was fed 7% of a pectin formulation[16] containing 60% apple pectin (NF XV, methoxyl content 9.5%), 37% sucrose and 3% excipient. Thus they received 69.9% sucrose and 4.2% pectin. A group of 100 hamsters was fed the control lithogenic diet for 50 days and groups of 25 hamsters each were fed the cellulose and pectin diets for the same period. At 50 days all the hamsters on the test diets and one-quarter of those on the control diet were killed and their gallbladders examined for stones. The remaining hamsters were either maintained on the control diet or fed one of the fiber-containing diets for 50 days more. Livers were removed and aliquots assayed for total and free cholesterol content[17]. Bile was aspirated