Hemodynamic studies in acute venous stasis edema in rats

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Summary. An increase in venous pressure in the rat tail is known to result in acute edema. Acute venous stasis edema of the rat tail was induced by applying a force-controlled banding of standard tension (200 g) proximally for a period of 6–12 h. The hemodynamic changes of acute venous stasis edema were evaluated using non-invasive plethysmography, fluorescence angiography, computer thermography and invasive radioactive microsphere techniques. It is shown here that reduction of tail circulation to 40% of the control value is followed by prolonged vascular disorder characterized by genesis of reversible edema, increased total blood flow to the tail and decreased local cutaneous blood flow, without affecting the general hemodynamics. The cutaneous circulation (decreased blood flow) seems to be principally involved in the edemogenic response, whereas the deeper vessels (hyperemia) may or may not play a determinant role in acute experimental venous stasis edema in rats.

Rat tail has been used as a model for study of acute edemogenic response to various pharmacological[2,3] and mechanical stimuli[3]. We have made several attempts to induce acute venous stasis by progressively increasing the venous tensions in the tail of the rat during simultaneous recording of distal coccygeal arterial pressure. These attempts have enabled us to choose a standard tension of 200 g (ligature) as a stimulus for selectively occluding the cutaneous venous compartment of the tail[4].

Acute venous stasis edema of the rat tail was induced by applying a ligature of a standard tension of 200 g proximally for a period varying between 6 and 12 h. The hemodynamic changes like edemogenic response, mean blood pressure, heart rate, cardiac output, stroke volume, total blood flow to the tail and the cutaneous blood flow in the tail region were evaluated using non-invasive and invasive means. The non-invasive techniques included plethysmography[5,6], fluorescence angiography[6,7] and computer thermography[5]. The invasive technique[8] involved catheterization of the heart and femoral arteries and use of radioactive microspheres.

Materials and methods. 61 male rats of Tif RAI (f) strain, weighing between 200 and 230 g were used. They were acclimatized to our standard laboratory conditions for 8 days before the experiment. The whole study was carried out in 4 series of experiments as specified in the text from A to D. The ligature (adhesive PVC tape, Coroplast®, 12 × 0.25 × 300 mm) was applied with a tension of 200 g. The ligature was applied for periods ranging between 6 and 12 h[3–6].

A) Plethysmographic evaluation of the edemogenic response: Plesthysmography was performed in 15 rats before and at 1, 4, 6, 8, 10 and 12 h after putting the ligature on the tail. Another measurement was made at 24 h after release of the ligature. The details of the plethysmographic technique, which involves volume displacement by an immersion method, were essentially the same as described previously[5,6]. The volume of the displaced fluid (90% ethanol) up to a reference mark on the tail was weighed using an electronic balance (Mettler, Greifensee, Switzerland, ref. PL 1200) directly connected to a computer (Hewlett Packard HP 9815, USA). This allowed an immediate calculation of the volume increment as a percentage of the control value for each animal.

B) Fluorescein angiographic evaluation for cutaneous irrigation: The details of the method were essentially the same as described previously[6,7]. 20 rats were used; they were divided into 2 groups each representing sham-ligated (n = 10) and ligated (n = 10) rats. 8 h after sham-ligature/ligature, they were put under light ether anesthesia. To obtain a hyperemic reaction, a pressure cuff was placed on the tail proximal to the site of ligature and inflated to 200 mm Hg so that the arterial blood supply to the tail was totally blocked. During the following min, 1 ml Na^+ fluorescein 10% (Fluka, Switzerland) was injected i.v. through the penile vein, the ligature was removed and the animal was placed on an adequate support under UV light (Reprostar®, λ = 366 µm, Camag, Switzerland). Exactly 1 min after placing the cuff, the pressure was removed and sequential photographs were taken every 30 sec up to 10 min (Nikon F2, motor MD 11, objective micronikor 55/35 – Nikon, Japan; flash Mecablitz 212 – Metz, Germany – with 2 mm cobalt filter).

C) Computer thermography for evaluation of locally emitted heat: 6 rats were used in this experiment. Thermography was done on individual rats using an IR camera, before ligature and 8 h after putting on the ligature (Inframetrics, model 525 – USA). The line scan of the camera was positioned on the median longitudinal axis of the tail and the thermal profile of the brightened line was displayed and registered on a video tape recorder (Philips, VHS system). The playback of the tape was analyzed frame by frame and the integration of the thermal profile was calculated by a gravimetric method using 3M (ref. 686) transparency film.

D) Radioactive microsphere technique for evaluation of different hemodynamic parameters: 20 rats were divided into 4

Table 1. Hemodynamic changes in acute venous stasis edema of the rat tail evaluated by non-invasive techniques

A) Edemogenic response: Plethysmography: Percentage change in volume of the tail during the ligature period of 12 h and 24 h after ligature release

Time after release	1 h	4 h	6 h	8 h	10 h	12 h	24 h
n = 15	6.58 ± 1.01	17.98 ± 3.41	26.09 ± 4.02	36.92 ± 4.02	42.08 ± 4.28	47.46 ± 4.39	28.00 ± 6.1

B) Cutaneous irrigation: Fluorescence angiography: Photodensity (arbitrary units) of the tail immediately after the release of an 8-h ligature

Time after release	0 min	1 min	2 min	3 min	4 min	5 min
Control n = 10	181.2 ± 15.7	189.3 ± 7.0	186.5 ± 15.6	186.7 ± 10.5	191.7 ± 2.8	192.7 ± 2.0
Ligated n = 10	78.5 ± 30.7**	97.0 ± 30.1**	107.5 ± 21.8**	113.6 ± 19.8**	116.0 ± 16.7**	118.4 ± 21.0**

Time	6 min	7 min	8 min	9 min	10 min
Control n = 10	194.4 ± 4.2	192.7 ± 4.3	191.9 ± 4.9	193.0 ± 4.4	192.0 ± 1.4
Ligated n = 10	121.4 ± 17.9**	122.1 ± 17.7**	124.1 ± 19.2**	125.4 ± 19.1**	134.8 ± 13.5**

C) Local heat emission: Computer thermography; integrated longitudinal heat emission profile (g) of the tail before, during and after the release of an 8-h ligature

Time	0 h (without ligature)	8 h (with ligature)	0 h (after release)	3 min (after release)	3 h after release
n = 6	2.61 ± 0.75	0.91 ± 0.20**	0.95 ± 0.26**	1.10 ± 0.38**	1.77 ± 0.42*

* $p < 0.05$; ** $p < 0.001$.

groups containing 5 rats in each group. These groups represented: a) control, b) experimental with 7-h ligature, c) experimental, immediately after release of 7-h; ligature; d) experimental, 3 h after release of 7-h ligature.

The general hemodynamic parameters (mean blood pressure, heart rate and cardiac output) and coccygeal blood flows were determined using the radioactive microsphere technique as described previously[8]. The animals were anesthetized (Na^+ pentobarbital 50 mg/kg i.p.). A tracheotomy was performed for free ventilation and the left ventricle of the heart was catheterized through the right carotid artery. The 2 femoral arteries were catheterized, one for continuous recording of the mean blood pressure by connecting the proximal end to a pressure transducer (HP-1280 C), which in turn was connected to a Hewlett Packard multichannel recorder. The other femoral artery was catheterized for collecting the timed reference blood sample at a constant speed of 0.477 ml/min. Heart rate was claculated from the triggered pulse tracing recorded at specified time intervals. 0.2 ml of the suspension containing approximately 6×10^5 radioactive microspheres (^{51}Cr) were injected into the left ventricle of the heart. The total radioactivity injected into the system was determined, calculating the difference between the radioactivity taken in the syringe at the start and that retained in the syringe needle and catheter after injection of the microspheres. Microspheres were injected over a 30-second period, while the reference sample was being withdrawn from one of the femoral arteries using a pre-calibrated withdrawal pump (B. Braun, Melsungen AG, Germany). At the end of the experiment, the animals were sacrificed using a large dose (500 mg/kg) of pentobarbitone. The tail was isolated and weighed. The radioactivity of the reference sample and that of the tail was measured in a MR 252 λ-counter for 5 min.

The flow to the tail was determined from the equation:

$$\text{Blood flow} = \frac{\text{Radioactivity in the tail}}{\text{Radioactivity in the reference blood sample}} \times \text{reference blood sample flow rate (0.477 ml/min)}$$

The cardiac output was determined by the equation:

$$\text{Cardiac output} = \frac{\text{Total radioactivity injected}}{\text{Radioactivity in the reference blood sample}} \times \text{reference blood sample flow rate (0.477 ml/min)}$$

The percentage distribution of the cardiac output to the tail was determined by the equation:

$$\text{Percentage distribution of cardiac output} = \frac{\text{Radioactivity in the tail}}{\text{Total radioactivity injected}} \times 100$$

Results. Plethysmography. The percentage increase in the volume of the tail during a ligature of 12 h was 47.16 ± 4.39 which decreased to 28 ± 6.1 at 24 h after the release of the ligature (table 1, A).

Fluorescence angiography. Fluorescence angiography performed immediately (1–10 min) after the release of an 8-h ligature demonstrated that venous stasis edema was accompanied by a decrease in the cutaneous blood flow (1.5-fold) (table 1, B).

Computer thermography. The levels of heat emitted were significantly decreased during and immediately after (0 and 3 min) release of the ligature (table 1, C).

Radioactive microsphere technique. The results of general and local hemodynamic aspects are presented in table 2. Mean blood pressure, heart rate and cardiac output remained unchanged throughout the study period (table 2, A, B, C), except that an immediate hypotensive response was evident on release of the ligature (table 2, A). The tail blood flow decreased during the ligature period accompanied by a similar decrease in percentage distribution of cardiac ouput to the tail. A sustained hyperemic response was observed after release of the ligature (table 2, D, E).

Discussion. To define venous stasis precisely, we have reported earlier[4] that, when the mean arterial pressure of the tail after ligatures of different tensions was measured, the coccygeal arterial pressure was unaffected by ligatures up to a tension of 200 g. Thereafter, the pulse wave started to diminish in amplitude and at 300 g tension the pulse was no longer discernible; at 400 g tension the mean arterial pressure of the tail dropped from initial values of 70 ± 5 mm Hg to 10 mm Hg. We were unable to demonstrate a consistent edemogenic response with tension below 200 g. The tension of 200 g was therefore considered as an ideal stimulus to induce edema restricted to cutaneous venous stasis.

We cannot ignore the fact that the deeper veins and arteriovenous anastomoses escaped the effect of ligature, and as a result there was only moderate swelling, of the ordeer of 47% within 12 h, which was reduced to 28%, 24 h after release of the ligature. The 'reopening phenomena' due to increased capillary pressure may also participate in generating a moderate edemogenic response.

From the present findings we can safely conclude that the cutaneous vessels are principally involved in the edemogenic response, whereas the deeper vessels may or may not play a determinant role in acute experimental venous stasis edema in the rat.

The hemodynamic changes during and after release of a 7-h ligature were also evaluated by the radioactive microsphere technique in anesthetized rats. The general hemodynamic parameters (mean blood pressure, heart rate and cardiac output) remained unchanged when determined at the end of a 7-h ligature period (table 2, A, B, C); however, an immediate hypotensive effect was evident on release of the ligature which might be due to sudden pooling of blood to the tail (hyperemic reaction). At this stage the blood flow increased 5-fold, doubling the normal tail blood flow (table 2, D). The tail blood flow was decreased during the ligature period, thus supporting our earlier data obtained by non-invasive techniques. Release of the ligature produced an immediate hyperemic response (marked increase in the organ blood flow) which was of the sustained type despite recovery of the general hemodynamic response (mean blood pressure returned to initial levels). It has to be emphasized that, in situations where cardiac output does not change, the percentage change in distribution of the cardiac ouput to a particular organ reflects the local regulation, contributing to a change in blood flow to that particular organ. In the present study, the observed changes in tail blood flow during the experiment were associated with similar changes in the percentage distribution of cardiac output to the tail, thus emphasizing the importance of local blood flow regulation. This sustained hyperemic response might be due to increased sensitivity of the tail blood vessels to locally released vasodilator mediators like kinins and prostaglandins.

The sensitivity of the tail blood vessels to chemical[2] and thermal[10] stimuli has been well documented. Gemmel and Hales[11] have provided histological evidence for the existence of arteriovenous anastomoses in the rat tail which could explain the disparity of results obtained by fluorescence angiography and

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