Original Contributions

Continuous measurement of plasma protein content in the alert rat during and after 3 to 5 minutes of moderate activity

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Summary: A refractometric method was used for the continuous registration of plasma protein concentration in rats during and after 3 and 5 minutes of provoked activity. Simultaneous conductometric measurements of hematocrit (hct) showed that, although both invariably changing in the same direction, the relative change of protein concentration is always less than that of hct: plasma volume changes calculated from the former fall short of those calculated from hct by 34 ± 23% during the hemoconcentrative period during activity and by 52 ± 11% during the hemodilutary period after activity. The difference between these figures was significant, thus implying that fluid leaving the circulation during the filtration phase is less rich in protein than that entering it during the absorptive phase of microvascular adjustments. A kinetic analysis of the period after activity was made. The rate constants of fluid- and protein-flux were closely correlated. Both plasma volume and intravascular protein mass increased asymptotically to a new equilibrium 6% above control within 30 min after activity. It is suggested that the excess protein is mobilized from large parenchymatous organs, mainly the liver.

Key words: Plasma protein concentration, plasma protein mass, hematocrit, exercise, continuous recording

Introduction

Fluid exchange between blood and tissues during and after exercise or in postural changes in man have been the subject of many recent and less recent reports (4, 5, 7, 12, 13, 17, 18, 20, 24). The conflicting results of experiments in humans have been commented on in detail in a recent review (10). Both hematocrit and protein concentration have been used as indicators of blood fluid content. Lack of knowledge about the exact time course of events and insensitivity of analytical methods may have been the cause of some controversial views, indicating a demand for continuously monitoring and more sensitive measuring systems. Such systems have been developed, using various physical principles, such as blood conductometry (2), density measurements (14), photometry (19, 21) and refractometry (3). All have been applied successfully in work investigating fluid balance in the circulation. An evaluation of the efficiency of the several methods must take into account a number of factors such as specificity, time resolution and applicability in human physiology, besides sensitivity, and this would influence the decision on which system is chosen.

In this paper the newly developed refractometric method (3) was used to monitor protein concentration changes in the blood of unanaesthetized rats during and after a short spell of
motor activity provoked by an unstandardized sham-attack. All experiments were accompanied by simultaneous conductometric measurements of hematocrit (1, 2).

Methods and materials

Eight female albino rats of Wistar origin weighing 214 to 272 g were used in 10 experiments. They were provided with chronic nonobstructive catheters in two sites of the v. cava inferior and the portal vein. Three to six days after surgery, extracorporeal veno-venous shunts were established, in which a flowthrough conductivity cell, a roller pump and a plasma filter were interposed. The filtrate (flow rate 70–100 µl/min) was passed through a second conductivity cell and the half-cell of a recording differential refractometer (H. Knauer, Berlin) and returned to the animal via a caval catheter. The plasma filter was a Nuclepore(R) membrane, pore size 1.0 µm. The three parameters – whole blood conductivity (reciprocal hematocrit), filtrate electrolyte conductivity and filtrate refraction – were recorded continuously on potentiometric recorders.

When fairly stable recordings were maintained for 10–15 min, a rod of tightly wrapped gauze was intermittently introduced into and withdrawn from the experimental cage to provoke the animal to activity. This procedure was continued for 3 (5 experiments) or 5 minutes (5 experiments) with varying intensities of reaction. Instrumentation and experimental procedures have been described in detail (1, 2). The formula for the hematocrit-conductivity relationship in the present series of experiments was:

\[ -0.0649 \times \mu \text{Mho} + 88.46 \]

at 37 K and a cell constant of 8.134.

The performance of the protein-measuring rationale was examined in two series of experiments:

1. To see whether there was a difference between the protein concentration (Cprot) in the filtrate and that of blood passing through the filter capsule, appropriate simultaneous samples (0.1 ml) were taken at the beginning and end of experiments. Protein concentrations were determined by the Biuret method. Blood sample and filtrate had a Cprot of 54.7 ± 3.8 g/l (mean ± standard deviation) and 54.9 ± 3.7 g/l respectively (n = 17).

2. To see whether the refractometer was working satisfactorily in the course of experiments, samples of filtrate were taken. The Biuret value of the sample was compared with the calibrated value derived from the registration. Values were 55.3 ± 3.6 g/l (refractometer) and 52.5 ± 4.9 g/l (Biuret) in 9 measurements. The 5% difference was significant (p < 0.01) and may have been due to the different protein calibration standards used. Also, a time-lag between readings and sampling had to be taken into account. Blood volume was determined by injecting 0.5 ml homologous plasma and measuring the hematocrit change (2).

Statistical calculations were made, using Student's t-test for paired and unpaired values and computing linear regression coefficients and correlation coefficients.

Results

a) Qualitative description

Original tracings of reciprocal conductivity (hematocrit, hct), filtrate conductivity (essentially plasma electrolyte concentration, Cel) and filtrate refraction (plasma protein concentration, Cprot) are reproduced in Fig. 1. Hct and Cprot change in a parallel fashion throughout the registration, whereas “Cel” tends to take an antiparallel course. The inertia of the Cprot measuring system has only partly been compensated for by eliminating dead time, so that the peak changes in Cprot are somewhat blunted.

Nevertheless, corresponding values of Cprot and hct can be determined at appropriate locations where the changes are either slow or the slopes of the curves do not change. The result of the 5-minute provocation of the rat to activity is the steep rise of hct and Cprot in the beginning and the steep and then gradual fall to a new level below control after cessation of the stimulus. The small changes in “Cel” are partly due to changes of Cprot, a rise in this parameter causing conductivity to drop (16), so that actual Cel changes are negligible (2).