Discussion. It was observed that glomerulopressin increased the rate of passage of T-1824. That this effect was due to glomerulopressin itself was suggested by the fact that after inactivation with β-glucuronidase the rate of passage was similar to that in the control animals (figure).

That the only route for the passage of T-1824 was through the lymphatic hearts was suggested by the observation that when they were destroyed the dye did not appear in the blood (table).

Recent reports have documented that tranylcypromine reduced the activity of prostacycline synthetase[9].

Tranylcypromine did not alter the effect of glomerulopressin, therefore it can be deduced that glomerulopressin did not stimulate prostacycline synthesis.

Indomethacin is an inhibitor of prostaglandin synthesis[10]. In other studies it has been shown that glomerulopressin acts through the synthesis of prostaglandins, for example in isolated strips of stomach fundus, duodenum and bladder[11], in ovarian blood flow[12] and mesenteric blood flow (in preparation).

In this study it was observed that the treatment with indomethacin completely inhibited the effect of glomerulopressin (figure) suggesting that the effect of glomerulopressin on the toad's lymphatic heart was also due to a stimulation of prostaglandin synthesis.

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Expansibility of erythrocytes during the course of hypotonic hemolysis

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Summary. A quick method for measuring the expansibility of erythrocytes during the course of hypotonic hemolysis has been developed, using the MCV continuous analyzer. By this method not only the volume changes during hypotonic hemolysis, but also the critical volume, at which hemolysis of the cells occurs, could be measured in a few minutes. Furthermore, data are presented demonstrating the following; when the MCV or the MCH was larger, the critical volume increased, but the expansion ratio (critical volume/initial volume) was almost constant, about 1.90, for most erythrocytes.

Erythrocytes increase their volume under hypotonic conditions and hemolysis occurs at the critical volume of the cells[1,2]. The main determinants of in vitro hemolysis are the critical volume and the total number of intracellular osmotically active constituents. The critical volume is a useful parameters; it is dependent on quantitative and qualitative factors associated with the membrane lipid and protein[3]. The determination of the critical volume is a good way to analyze the mechanism of hemolysis. Usually, the microhematocrit method is used to measure the volume. But the method is not convenient and not very accurate; the procedure is very time-consuming, and Guest and Wing's correction has to be used to calculate the critical volume[4]. Therefore, we developed a new direct method to measure volume changes (MCV) during hemolysis, and the critical volume, as reported in this paper.

To measure cell volume, the MCV continuous analyzer (TOA Medical Electronics Co., Kobe-Los Angeles-Hamburg), which can measure mean cell volume and record it continuously at time intervals of 1–10 sec, was employed. The principle for measuring cell size is the same as that of the Coulter counter[5]. But the machine has 2 kinds of special circuits; a conductivity-error compensation circuit and a temperature-error compensation circuit. The machine can determine cell-volume accurately (determination range; 1–1000 fl/cell) in hyper- or hypo-tonic media or at low or high temperatures (4–40 °C)[6,7]. We installed a water pump in the machine to dilute the cell suspensions. Before every experiment we checked the compensation functions by using standard resin particles.

2 µl of heparinized blood were suspended in 40 ml of Celluent (TOA Medical Electronics Co., cell diluent for counting blood cell number, 265 mOsM). After checking the stability of the machine for 15 sec, distilled water was added continuously at a flow rate of 0.5 ml/sec into the cell suspension. Changes in mean cell volume were measured at 5 sec intervals and recorded on a chart.

By the above procedure hemolysis curves like that shown in figure 1 could be obtained. On the chart V_0 means the initial volume (MCV; mean corpuscular volume), water ↓ means the starting point of water addition, p indicates the peak point of the curve, and V_1 means the maximal expansion volume. The curve was much the same as Seeman had proposed previously[1]. The maximal expansion volume, V_1, corresponded to the critical point of Seeman, at which hemolysis had occurred. The time from the starting point of water addition (0 sec) to point p (t sec) could be converted into osmolarity (h) of suspending medium by the following formula; h (mOsM) $= 265 + 1.656 \times t$. Mean osmolarity of the hemolysis point in erythrocytes obtained from 239 subjects was 135.8 ± 22.3 mOsM. The value is very close to the 50% hemolysis value obtained by Dacie's method[8].

The degree of expansibility during hypotonic treatment was determined by the height of p (V_1) and time to p (t). The ascending foot of the curve in figure 1 could be expressed

by the simplified formula; $V_1 = V_0 + v \times t$ (V_1; maximal expansion volume or cirtical volume, V_0; initial volume or MCV, $v = dV/dt$ or rate of volume increase, t; hemolysis time). From this equation t could be calculated by $(V_1 - V_0)/v$. The v means the rate of volume increase which was induced by increasing internal pressure caused by the osmotic effect of intracellular hemoglobin. As the rate of water addition, and therefore the rate of change of osmolarity in the cell suspending media, was strictly controlled in our experiments, v should be regulated by the hemoglobin content of the cell. As a matter of fact, a high correlation could be observed between the value of v and MCH (mean corpuscular hemoglobin), as shown in figure 2. The data indicated that intracellular hemoglobin concentration was one of the major determinants of water influx rate.

In each specimen, V_1 was the most stable value. When Celluent was changed to saline; when EDTA or ACD was chosen as an anticoagulant, instead of heparin, when the temperatures of the Celluent and of the added water were changed (4–40 °C), or when the speed of addition of water was changed (0.066–0.66 ml/sec), the value of V_1 was not influenced. The results suggest that V_1 does reflect the expansibility of the cells; an inherent property reflecting the stable configuration of the membrane constituents.

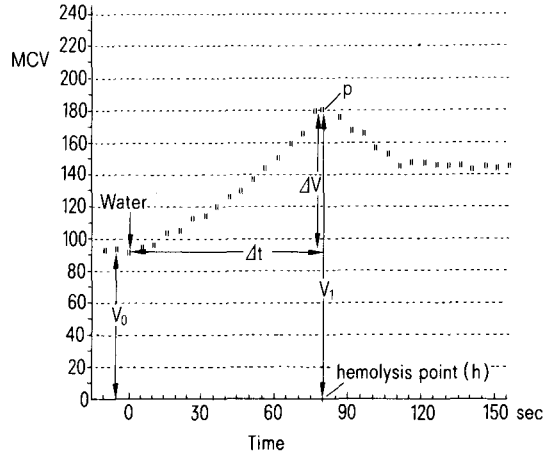

Fig. 1. Hemolysis curve obtained by volumetry on the course of hypotonic hemolysis. (Mean ± SD, n = 239.) V_0; initial volume (88.0 ± 11.8 fl/cell); V_1; maximal expansion volume (163.3 ± 25.3 fl/cell); h; osmolarity at mean hemolysis point (135.8 ± 22.3 mOsM); t, hemolysis time (78.0 ± 13.5 sec); p, peak point. Arrow indicates the start of water addition.

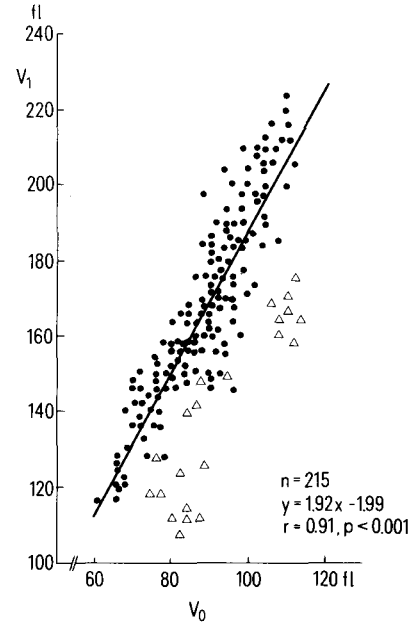

Fig. 3. Correlation between initial volume and maximal expansion volume. X axis, initial volume, V_0; Y axis, maximal expansion volume, V_1. Open triangles; specimen obtained from patient with hereditary spherocytosis (16 cases), chronic liver diseases combined with increased hemolysis (6 cases) or autoimmune hemolytic anemia (2 cases). Closed circles; specimen which did not show any unusual increased of red cell fragility.

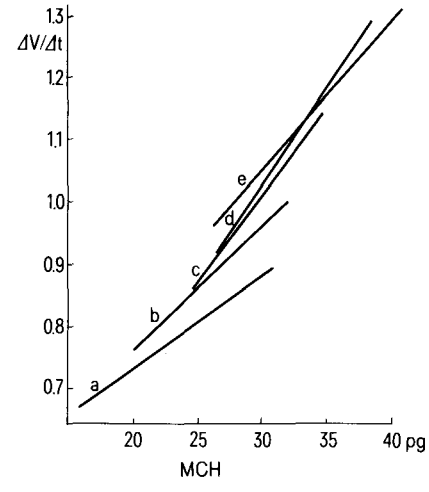

Fig. 2. Correlation between volume increasing rate and MCH. X axis, MCH; Y axis, volume increasing rate, $\Delta V/\Delta t$.
a) MCV ≦ 70, $y = 0.42 + 0.015 \cdot x$, n = 19, $r = 0.81$.
b) 70 < MCV ≦ 80, $y = 0.32 + 0.022 \cdot x$, n = 39, $r = 0.82$.
c) 80 < MCV ≦ 90, $y = 0.11 + 0.030 \cdot x$, n = 69, $r = 0.84$.
d) 90 < MCV ≦ 100, $y = 0.09 + 0.031 \cdot x$, n = 51, $r = 0.73$.
e) 100 < MCV, $y = 0.48 + 0.020 \cdot x$, n = 37, $r = 0.63$.

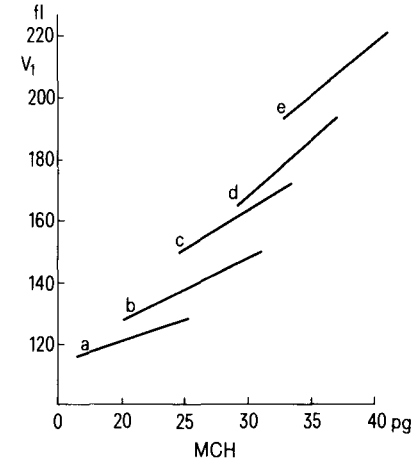

Fig. 4. Correlation between V_1 and MCH. a) MCV ≦ 70, $y = 97.5 + 1.2 \cdot x$, n = 11, $r = 0.91$. b) 70 < MCV ≦ 80, $y = 87.4 + 2.0 \cdot x$, n = 35, $r = 0.82$. c) 80 < MCV ≦ 90, $y = 86.2 + 2.6 \cdot x$, n = 45, $r = 0.86$. d) 90 < MCV ≦ 100, $y = 68.0 + 3.4 \cdot x$, n = 42, $r = 0.87$. e) 100 < MCV, $y = 85.2 + 3.3 \cdot x$, n = 26, $r = 0.72$. Specimens were limited within mean of expansion ratio ± 2 SD.

Between V_1 and V_0 a close correlation could be observed, as shown in figure 3, except with the hereditary spherocytosis cells (HS cells). Weed et al.[3] reported that HS cells showed a reduced critical volume. The hemolysis curve could show the reduction of cell volume. All our cases of HS did show the reduced critical volume (figure 3).

MCH showed high correlation with V_1, as shown in figure 4. That is, microcytic hypochromic cells exhibited small expansibility and macrocytic hyperchromic cells exhibited large expansibility. The maximal expansion volume, the critical volume, was determined not only by MCV, but also by MCH.

On the other hand, the V_1/V_0 value (maximal expansion ratio) was 1.90 ± 0.11, and its coefficient of variation was 5.8%, which was lower than that of V_0 (13.4%) or V_1 (15.6%). This meant that all the ratio values were distributed within a limited narrow range, close to 1.90. But V_1/V_0 in HS cells was 1.47 ± 0.17. It could be considered from the results of our hemolysis curve that HS cells showed an extremely small expansibility, compared with other erythrocytes.

In conclusion, the method of drawing hemolysis curves by measuring the change of cell volume during hypotonic hemolysis has the following advantages: data concerning not only the volume change, but also the critical volume, can be obtained directly and accurately, 2. the test requires only a small volume of blood and the whole process is completed in a few minutes, and 3. interference by plasma components or preexisting hemolysis can be eliminated, since the sample blood is diluted 2000 times at the starting step. Thus, the method might be useful as a tool in diagnostic and experimental medicine.

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