The activity of the enzyme increased with the increase in substrate concentration. The data regarding the effect of substrate concentration on esterase activity, when plotted in a Lineweaver-Burk plot for the Michaelis constant (K_m), gave a value of 2.1×10^{-3} M (figure 5).

1 The investigations are a part of the thesis presented to University of Lucknow, India.
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Estimation of pore passage time of red blood cells in normal subjects and patients with renal failure

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Summary. The average transit time of single red blood cells through a nuclepore membrane filter (pore diameter and length, 5 µm and 10 µm, respectively) was measured using an improved method and was shown to be an index of deformability. An increased passage time, indicating reduced deformability, was observed in renal failure.

Because of the importance of the deformability of red cells in relation to blood flow through the microcirculation, a number of methods to measure or quantify this property of red cells have been proposed and developed. In our previous study[2] a filtration method using a nuclepore membrane filter[3, 4] was applied to measure the deformability of red cells in fresh human blood exposed to hypercapnia. Although the method is open to ambiguity in the interpretation of the results, due to many factors affecting blood flow through the filter, its simplicity and speed made it possible to detect a rapid decrease of the deformability of red cells after blood sampling and its reduction by hypercapnia. In the present study, we tried to obtain a better index of red cell deformability than the originally proposed passage time of the whole blood[4], since the rheological property of blood in the filtration method is affected by the deformability of red cells and hematocrit as well. It seemed possible that a simple filtration model would permit an analysis of the hematocrit dependence of the filtration characteristics, and an evaluation of the average time required for a red cell to pass through a pore of the filter. Thus, a comparison of red cell deformability between normal subjects and patients suffering from renal failure accompanied with anemia could be made.

20 ml of venous blood from each healthy subject was drawn into a syringe containing 1 ml of heparin solution (1000 units). To obtain red cell suspensions with reduced hematocrits, the blood was divided into 5 parts, each of which was diluted by an appropriate amount of plasma from the fresh venous blood of the same subject. The time required for 0.5 ml of each sample to pass through a filter under a pressure difference of 10 cmH_2O was measured at 37 °C. The sample preparation and measurement were made within 30 min after blood sampling. Then the hematocrit of each sample was determined by a microhematocrit method. 2 ml of venous blood from patients was anticoagulated with

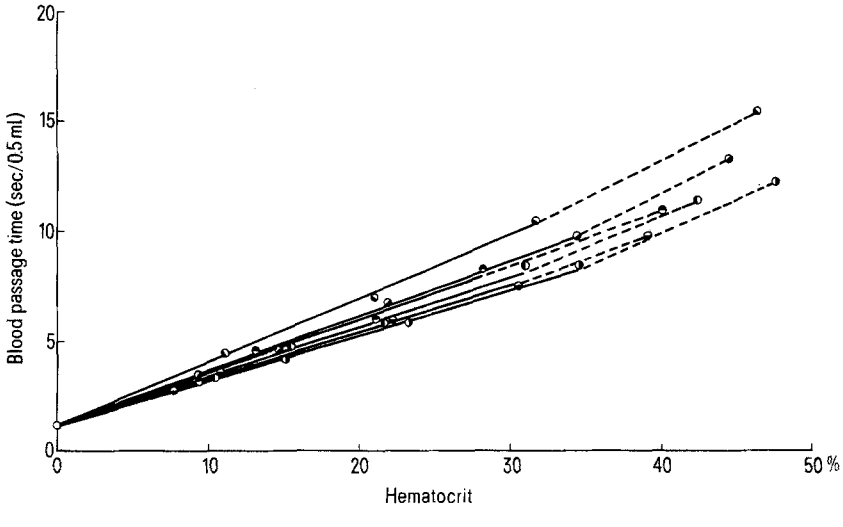

Fig. 1. Relation between blood passage time and hematocrit in 6 normal subjects.

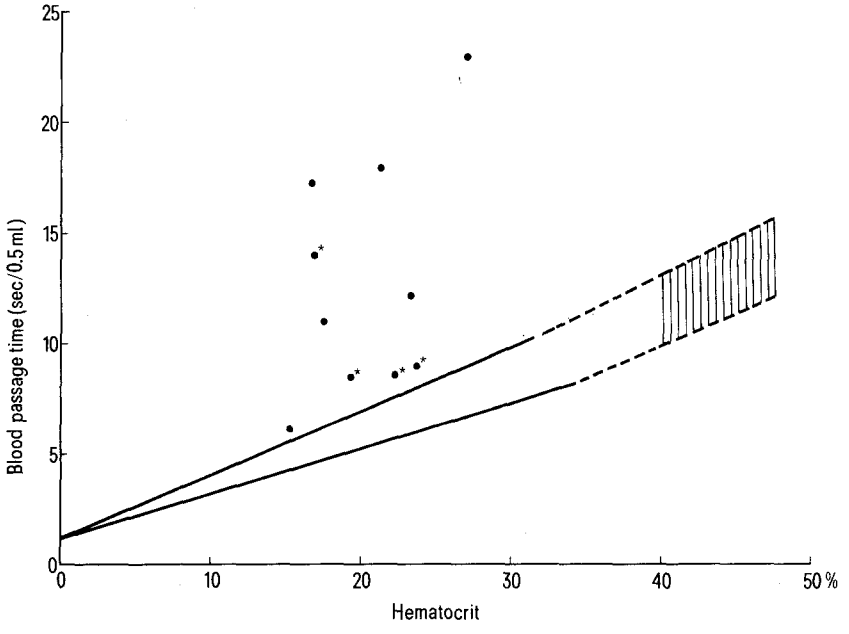

Fig. 2. Blood passage time of 10 renal failure patients with respect to hematocrit. The straight lines and dotted area indicate the region of diluted and undiluted normal blood shown in figure 1.

0.1 ml of heparin solution and used for measurement without dilution.

In figure 1 the passage time of samples from 6 normal subjects is plotted against the hematocrit. The passage time of plasma is essentially the same in all the 6 subjects, while those of the red cell suspensions increase linearly with a slightly different slope in each case when the hematocrit is increased from 0 to 33%. Therefore, individual serial measurements were connected with least square regression lines within the hematocrit range lower than 33% and combined with the undiluted values with dotted lines. The rate of increment seems to increase for hematocrit values above 33%.

In figure 2 the passage time of undiluted blood samples from 10 patients is plotted against the hematocrit. The straight lines and shaded area indicate the region of diluted and undiluted normal blood, respectively, shown in figure 1. The patients' blood had longer and highly variable passage times. The asterisk indicated blood samples of patients on hemodialysis.

The linear relation between the passage time and hematocrit in the range of 0–33% seems to be explained by the following consideraton. In figure 3 an average time for red cell to pass through a pore and the time required for each stage of the passage are schematically shown. The terms in the following speculation are used as follows:

- T time required for a unit volume (1 ml) of blood to pass through a filter,
- T_{in} time required for a red cell to enter into the pore at the upper opening,
- T_{out} time required for a red cell to flow out from the pore at the lower opening,
- T_{cp} $T_{in}+\frac{l-a}{V_c}+T_{out}$, time from the moment when a red cell blocks the opening of a pore to the moment when it leaves the lower opening completely,
- V_c velocity of a red cell passing through the pore,
- V_p velocity of a plasma column passing through the pore, i.e. when it is not slowed down by red cells passing through and blocking the pore,

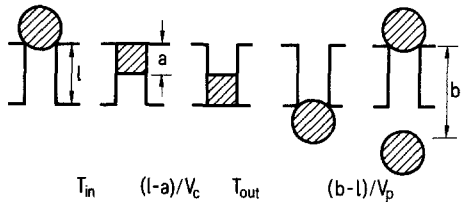

Fig. 3. A schematic time course of a red cell passing through a pore of the filter. The time required for each stage of the passage is represented by notations given below in the figure.

- a height of a red cell passing through the pore,
- b length of plasma column which flows between 2 cells passing succeedingly,
- l length of the pore, i.e. thickness of the membrane filter (10 μm),
- A effective area of the filter membrane (0.4 cm^2),
- d pore density ($4\times 10^5/cm^2$),
- S area of cross section of a pore ($19.6\times 10^{-8}\,cm^2$),
- H hematocrit value in percent,
- h $H/100$.

Since a red cell (volume 90–100 μm^3) occupies a half of a pore cylinder (volume 196 μm^3), a is equal to 5 μm. Since b is the length of the plasma column which flows between 2 cells flowing successively, b is related to H and a as

$$a/(a+b)=H/100=h. \quad (1)$$

When h is larger than $\frac{1}{3}$, b becomes less than l. Before a red cell flows out from a pore, the next red cell blocks the upper portion of the pore. No plasma space which can flow at the velocity V_p exists any more. T_{in}, T_{out} and V_c become hematocrit dependent. This situation is difficult to treat on the basis of simple assumptions. So we consider the case in which H is less than 33%. Then T is given as

$$T=(T_{cp}+\frac{b-l}{V_p})/[A\cdot d\cdot S\cdot(a+b)] \quad (2)$$

By putting (1) into (2), we obtain

$$T=[\frac{1}{V_p}+\frac{h}{a}\cdot(T_{cp}-\frac{a+1}{V_p})/(A\cdot d\cdot S) \quad (3)$$

This equation indicates the linear dependence of passage time on hematocrit. Since V_p can be determined from the measurement at $H=0$, T_{cp} for the blood from the normal subjects is estimated to be 0.85 ± 0.10 msec (mean $\pm$ SD). If we can assume that V_p for plasma of patients' blood is the same as the normal value (according to Dintenfass[5], plasma viscosity in renal failure does not differ from the normal value), T_{cp} for the patients' blood is estimated to range from 1.16 to 3.14 msec. In other words the increment of the passage time of the patients' blood can be attributed to a reduction of the red cell deformability in an agreement with Dintenfass[5] who deduced a reduced deformability of red cells from blood viscosity measurements in renal failure. The relation of the deformability with BUN-value is unclear, but as seen in figure 2, hemodialysis seems to ameliorate the deformability of red cells of the patients. The reduced red cell deformability will increase red cell trapping at the spleen, which may be one of the causes of the anemia in renal failure.

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An experimental study of thiamine metabolism in acute ethanol intoxication[1]

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Summary. A dose of 3.9 g/kg b.wt of ethanol was administered to rabbits, and ethanol and thiamine concentrations, transketolase activity and thiamine pyrophosphate (TPP) effect in blood samples were determined. It was established that ethanol ingestion produced a rapid decrease in blood thiamine levels and transketolase activity in erythrocytes and an increase in TPP effect in erythrocytes. These values reverted to normal within 2 or 3 days after the ingestion of alcohol.

Alcoholics are nearly always in a state of thiamine deficiency[2–4], and although this deficiency may be partially attributed to a low dietary intake of thiamine[5] other mechanisms are also probably involved[6,7]. A number of experimental studies on the relationship between thiamine metabolism and chronic ethanol administration have been done, but since there is little information on thiamine metabolism in cases of acute ethanol intoxication, we carried out studies in an attempt to clarify the relation.

Materials and methods. Male rabbits, *Oryctolagus cuniculus* var. *domesticus,* weighing about 2500–3000 g were used. A restricted diet (100 g/day) of commercial rabbit chow (Oriental Co., Japan, thiamine concentration: 0.8 mg/100 g) was ingested by every rabbit during the experimental period. A dose of 13 ml/kg b.wt of 30% ethanol (about $\frac{1}{3}$ of LD_{50}) was administered orally by a gastric tube, and blood samples were obtained from the vena praeauriculares. In the control rabbits, 14.2 ml/kg of 50% glucose was administered by a gastric tube to equalize the energy intake.

Ethanol concentration was determined by the method of Eriksson[8] with several modifications. A 0.2 ml aliquot of blood was placed in a 10 ml vial to which 0.2 ml of n-propanol solution (1 g/l n-propanol in isotonic saline) was added. The vial was sealed with a butyl rubber septum, then heated at 55 °C for 20 min. A 1.0 ml volume of the head-space gas was injected into a gas chromatograph, and chromatography was performed with an instrument equipped with a flame ionization detector (Shimadzu Co., Japan, model GC-4BMPFE). A glass column (2m) packed with 15% polyethylene glycol 1500 on chromosorb (60–80 mesh) was used. Detector temperature was maintained at 150 °C, the injection temperature at 150 °C, the oven temperature at 80 °C and the nitrogen flow rate at 70 ml/min. Glucose was determined by the method of Hultman[9]. Thiamine was determined by the thiochrome method of Fujiwara and Matsui[10]. Transketolase (EC 2.7.1.1) activity was assayed by the method described by Itokawa[11]. The thiamine pyrophosphate (TPP) effect is the percentage stimulation of enzyme activity above the origi-

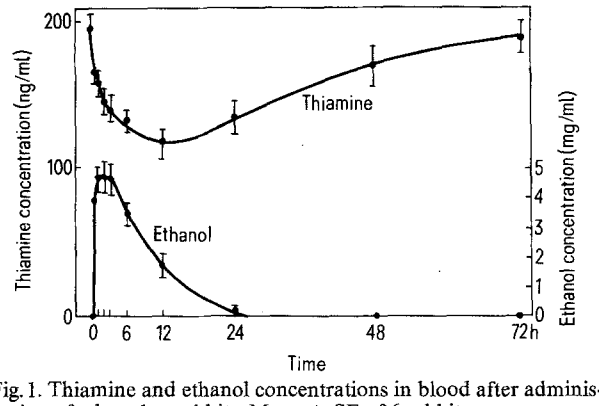

Fig. 1. Thiamine and ethanol concentrations in blood after administration of ethanol to rabbits. Mean ± SE of 6 rabbits.

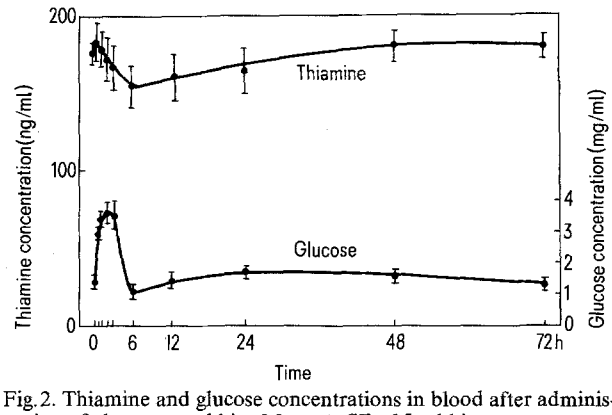

Fig. 2. Thiamine and glucose concentrations in blood after administration of glucose to rabbits. Mean ± SE of 5 rabbits.