determined in duplicate by the technique described by BRINKMAN and JONXIS[13]. The measurements of DPG were performed using the enzymatic method of TOWNE et al[14].

For the analysis of the results, it was assumed that the O_2 affinity of whole blood depends on the level of DPG, on the relative proportions of HbA and HbF and on the relative effect of DPG on the O_2 affinity of HbA and HbF. The relation among these variables can then be expressed by the following equation:

$$P50 = m \times [(\alpha \times DPG \times HbA) + (\beta \times DPG \times HbF)] + c \quad (1)$$

where α and β represent the relative effect of DPG on the P50 of HBA and HbF, respectively; P50 is expressed in mm Hg, DPG in nmoles/ml RBC and HbA and HbF as percent of total Hb; m and c are arbitrary factors representing the slope and the intercept of the regression line.

Equation No. 1 can also be written as follows:

$$P50 = m \times \alpha \times DPG\,[HbA + \beta/\alpha\, HbF] + c \quad (2)$$

where the the ratio β/α represents the effect of DPG on the P50 of HbF in relation to the effect of DPG on the P50 of HbA.

Regression equations were calculated for different values of β/α (0.1 to 1.0), for each group of experiments. The equations with the best fit, as judged by the correlation coefficients, were used to express the results and are shown in Figures 1 and 2.

The results in the two groups of experiments were very similar. There were no significant differences between the slopes or the intercepts of the two equations and in both cases the best correlation was obtained with a value of $\beta/\alpha = 0.4$. This suggests that in whole blood, both in vitro as well as in vivo, the effect of DPG on the P50 of HbF is 40% of that of HbA. This value is in excellent agreement with those found by previous investigators using hemoglobin solutions[4,5,15].

Riassunto. Experimenti condotti sul sangue intero di individui neonati e adulti hanno dimostrato che l'affinità del sangue per l'ossigeno (P50) dipende dalla concentrazione intraeritrocitaria di 2,3-difosfoglicerato (DPG) e dalle proporzioni relative di emoglobina adulta (HbA) e fetale (HbF). I risultati ottenuti indicano che nel sangue in toto l'effetto del DPG sulla P50 della HbF è circa il 40% di quello sulla P50 della HbA.

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Intracellular pH of Red Cells Stored in Acid Citrate Dextrose Medium

The importance of hydrogen ion cencentration during the storage of red cells has been noted and actually the controlled lowering of pH by citrate buffer was the major improvement in blood preservation. More recently, the levels of ATP and 2,3-diphosphoglycerate, which play an important role in the cells, have been shown to be greatly affected by the pH of the storage medium[1,2]. Although there have been several reports on the pH change during storage, the measurements were done at 37°C[2] and the data obtained were extracellular pH (pH_e). It seems more interesting to know the intracellular pH (pH_i) of red cells if we aim to improve storage conditions, especially so in relation to the stability of intracellular enzymes or metabolic intermediates during storage. Moreover, the pH at the temperature of storage, i.e. at 4°C, may have more meaning for this end than the pH at body temperature. The present investigation is on the changes of the pH_e and the pH_i at 4°C of ACD blood during storage.

Methods. The pH_i was measured by using 5,5-dimethyl oxazolidine-2,4-dione (DMO) essentially according to the method of CALVEY[3]. To 100 ml of acid citrate dextrose (ACD) blood in a storage bottle, about 4 µCi of 2-C^{14} DMO (New England Nucl. Corp., spec. act. 10.1 mCi/m*M*) was added with a syringe and the blood was preserved at 4°C. Every 2 or 3 days, an aliquot of the sample (about 5 5 ml) was taken out by a syringe and used for the assay. The pH of the suspension (pH_e) was measured by a Hitachi-Horiba expanded scale pH-meter F-5 at 4°C. The samples were taken out before and after centrifugation (5,000 rpm for 10 min in a refrigerated centrifuge), using a precalibrated micropipette (99.8 µl). DMO was extracted from each sample and measured by a liquid scintillation spectrometer. The water content of the suspension was measured by drying the sample in a hot air oven at 110 ± 10°C for 24 h. The content of extracellular water in packed cells was measured by C^{14}-inulin for another batch of ACD blood and found to be 2 to 4% of packed cell volume, which showed no appreciable change during storage and contributed to the calculation of pH_i to a negligible extent. The pH_i was calculated according to the equation derived by IRVINE et al.[4], except that the measured value at 4°C of $pK' = 6.52$ was used for DMO.

Results and discussion. Typical data of ACD blood stored for 1 month are shown in the Table. The pH_i decreased as the pH_e of the blood decreased during the storage. The pH_i was always higher than the pH_e and the decrease of the pH_i during the storage was slower than that of the pH_e. The pH_e of ACD blood shown here is appreciably higher than that reported by other workers[2] because of the measurement at 4°C. The higher value of the pH at 4°C can be explained by big negative temperature coefficient ($\Delta pH/\Delta t$) of protein solution as a buffer system. For example, the pH of 2-day-old ACD blood was 7.37 at 4°C and 6.92 at 37°C. The pH of ACD plasma was affected by temperature to lesser extent than that of the suspension, e.g. 7.35 at 4°C and 7.08 at 37°C, indicating that the pH_i is affected by temperature change more than the pH_e.

It has been known that the pH_i of red cells is lower than the pH_e of fresh blood or the cells suspended in

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pH_e and pH_i at 4 °C of red cells in ACD medium

Days of storage	Hematocrit value	Total water content (%)	DMO in suspension/ DMO in suspernatant	pH_e	pH_i
1	46.4	80.0	0.85	7.50	7.61
2	46.3	79.6	0.85	7.46	7.55
4	46.4	80.0	0.83	7.42	7.47
6	45.6	80.0	0.85	7.35	7.44
8	46.3	79.8	0.85	7.34	7.39
10	45.8	80.0	0.86	7.30	7.40
12	45.8	80.0	0.86	7.28	7.39
14	46.1	80.0	0.88	7.19	7.33
17	46.7	79.9	0.90	7.12	7.28
19	46.5	79.9	0.91	6.99	7.18
22	46.0	79.9	0.92	6.98	7.20
25	45.5	79.9	0.91	6.80	7.06
28	45.0	79.7	0.94	6.84	7.12

buffered saline. However, as shown in the present study, the pH_i was higher than the pH_e in ACD blood, which was further confirmed by freezing and thawing of ACD-stored packed red cells covered with liquid paraffin. The pH at 4°C of the red cells increased from 7.29 to 7.39 by hemolysis. The observation that the pH_i of ACD blood is higher than the pH_e can be explained by Gibbs-Donnan equilibrium based on the impermeability of citrate ion to the cell membrane[5]. The increase of the difference between the pH_i and the pH_e observed during the storage can be explained by the acidification of the suspension and is not due to the aging of the cells. The similar increase was observed when fresh ACD blood was acidified with lactic acid.

The characteristics of the glycolytic reaction in ACD blood are determined by at least 2 factors: pH_i and low temperature. Although data are available about the effect of the pH_e on the glycolysis at 37°C[6], no data are available about the effect of the pH_i. On the other hand, as the pH_i is extremely susceptible to temperature change, the data obtained at different temperature[7,8] need to be reconsidered in relation to the shift of the pH_i during the temperature change. Improvement of blood preservation method may be attained by examination of the pH_i of red cells in different storage medium and the effect of the pH_i on the glycolysis.

Zusammenfassung. Nachweis, dass in ACD-Blut das intrazelluläre pH höher ist als das extrazelluläre pH und das es während der Lagerung bei 4°C langsamer sinkt.

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Effect of Non-Narcotic Analgesics on Anticoagulant-Induced Hypoprothrombinemia in Rats

It has been suggested by several writers[1,2] that salicylates potentiate the action of oral anticoagulants. High doses of acetylsalicylic acid (ASA) have been shown to augment hypoprothrombinemia in humans[3]. Similar effects have heretofor not been demonstrated in other species. An earlier report[4] from our laboratory showed that a single oral dose of 100 mg/kg of ASA decreased the hypoprothrombinemia induced in male rats by *bis*hydroxycoumarin (BHC). The results of further studies on the pharmacologic interaction of analgesics and oral anticoagulants in rats are described in the present communication.

Following the procedures described earlier[4], the effect of oral administration of ASA, 100 mg/kg, daily for various periods up to 35 days on the prothrombin time of blood was investigated in male and female adult Wistar rats treated with BHC by the oral and intraperitoneal routes. The results are summarized in Figure 1. ASA decreased the hypoprothrombinemic effect of BHC in both sexes and there is no indication that chronic administration increased the magnitude of the anti-BHC action of the analgesic. The time course of this effect is illustrated in Figure 2. It becomes significant 18 h after ingestion of ASA; after 24 h the prothrombin time of the BHC-treated groups is still elevated while that of the animals administered both drugs has returned to normal levels.

Treatment	Day 1	Day 2	Day 3
BHC	20 mg/kg i.p.	15 mg/kg i.p.	15 mg/kg i.p.
BHC+	20 mg/kg i.p.	15 mg/kg i.p.	15 mg/kg i.p.+
ASA	—	—	100 mg/kg orally
ASA	—	—	100 mg/kg orally

Tail blood was taken on Day 3 at time intervals, commencing 2 h after the drugs were administered. Each point represents the mean ± standard error of determinations on blood from 6 animals. * $P < 0.05$.