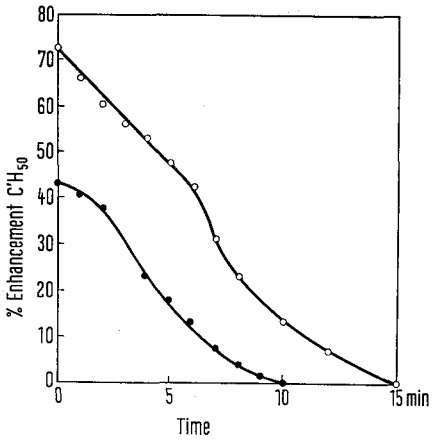
effect on the extent of hemolysis as compared to that of a control mixture is shown in the Figure. After 10 min, carboxypeptidase A no longer exerted any influence on the immune hemolytic reaction. However, enhancement of immune hemolysis by low ionic strength could be demonstrated up to 15 min after initiation of the reaction. Enhancement of gp C by low ionic strength beyond 10 min is evidence that one or more components of complement are affected by low ionic strength, which are unaffected by carboxypeptidase A.

It has been reported that C2, C4, and possibly C3 components of complement are affected by carboxypeptidase A[5]. Low ionic strength enhances the formation of SAC $\overline{1,4,2}$ by permitting firmer binding of C1 to SA and by preventing the macromolecular complex C1 from dissociating into nonactive forms[2]. Low ionic strength can exert both an enhancing and a detrimental effect on C3 or later acting components of complement[6]. The fact that low ionic strength affects the reaction for a longer period of time than carboxypeptidase A (Figure) suggests that it is enhancing a later step in the hemolytic reaction subsequent to the formation of EAC$\overline{1,4,2}$, which is unaffected by carboxypeptidase A. That carboxypeptidase A enhances gp C at the optimum ionic strength indicates that the enzyme exerts greater effect on mutual components or enhances component(s) C2 and/or C4, unaffected by low ionic strength[7].



Effect of time of introduction of low ionic strength and of carboxypeptidase A on immune hemolytic reaction. ●—●, carboxypeptidase A; ○—○, low ionic strength.

Zusammenfassung. Potenzierung von Meerschweinchenkomplement durch niedrige Ionenkonzentration und Carboxypeptidase A zeigt, dass das Enzym seinen stärkeren Einfluss während der Bildung von EAC$\overline{1,4,2}$ ausübt. Während der Immunhämolyse übte jedoch eine niedere Ionenkonzentration einen zusätzlichen fördernden Einfluss auf eine spätere Reaktion aus.

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Inhibitory Influence of Acid Citrate Dextrose on the Activity of Dehydrogenase in Erythrocytes

The inhibitory effect of some anticoagulants on the activity of enzymes[1-3] is not only of theoretical interest but has also some practical importance. We have therefore considered it important to publish our findings on the inhibitory influence of acid citrate dextrose (ACD), an anticoagulant solution often used in blood storage, on the activity of some dehydrogenases of red blood cell (RBC).

In the course of our studies with the erythrocytic enzyme, glucose-6-phosphate dehydrogenase (G-6-PD) we have registered a much slower reaction, catalysed by the above-mentioned enzyme, when blood samples treated with ACD (Figure, Table I). Ratio: ACD/blood 1:4 were compared to heparin samples. The enzymatic activity of G-6-PD in RBC was determined by the KORNBERG's and HORECKER's method[4] with some modifications[5]. By tracing this phenomenon in a greater number of blood donors, we have been able to prove by means of a doublet system method that the diminished erythrocyte activity of the enzyme G-6-PD in the samples of ACD has a statistical significance at 1% level, when H-test was used[6]. Differences of enzyme activity in both anticoagulant media in each blood sample examined are demonstrated in Table I. Therefore we tried to answer the question if the observed ACD-inhibitory effect is limited only to the enzyme G-6-PD or if it is of a more general importance. For this reason we decided to use another NADP dependent dehydrogenase glucono-6-phosphate dehydrogenase (6-PGD). The erythrocyte activity of this enzyme was determined by the method of KORNBERG and HORECKER[4] using identical conditions as described by G-6-PD. From 10 blood donors examined 9 showed the measured erythrocyte activity of 6-PGD to be higher when heparin was applied (Table I). However, differences in enzymatic activity were not of such a degree as to be statistically significant when tested with H-test. In another dehydrogenase, NADH dependent, lactic dehydrogenase (LDH), determined by the method of HORN and AMELUNG[7], we have been able to prove, when checking with heparin samples, statistically signif-

[1] P. M. EMERSON, J. H. WILKINSON and W. A. WITHYCOMBE, Nature *202*, 1337 (1964).
[2] H. D. HORN and F. H. BRUNS, Verh. dt. Ges. inn. Med. *65*, 407 (1959).
[3] G. FORNAINI, G. LEONCINI, P. SEGNI and A. FANTONI, Ital. J. Biochem. *6*, 366 (1964).
[4] A. KORNBERG and B. L. HORECKER, *Methods in Enzymology* (Academic Press, New York 1955), vol. 1, p. 323.
[5] J. PALEK, V. VOLEK, B. FRIEDMAN and V. BRABEC, Čas. Lék. čes. *105*, 775 (1966).
[6] W. H. KRUSKAL, Ann. math. Statist. *23*, 525 (1952).
[7] H. D. HORN and D. AMELUNG, Dt. med. Wschr. *82*, 619 (1957).

Table I. Enzymatic activities in blood samples collected in heparin and ACD

G-6-PD		6-PDG		LDH		PK	
HEP	ACD	HEP	ACD	HEP	ACD[a]	HEP	ACD
971	840	800	575	25.6	23.4	2.4	2.7
971	853	834	699	24.5	17.7	3.6	4.0
928	684	920	753	25.5	19.9	3.5	4.2
1189	759	1069	798	22.2	18.6	3.4	2.8
960	953	464	414	20.6	13.5	3.6	2.2
831	845	890	851	21.5	12.3	1.6	1.5
771	573	674	660	19.0	11.5		
1005	777	612	759	19.9	12.4		
773	562	635	496	18.6	12.5		
1105	817	760	557	14.6	9.9		
715	662						
738	681						
948	960						
1038	780						
875	763						
753	568						
851	648						
576	566						
815	581						
$\bar{x}$ 885	728	$\bar{x}$ 766	656	$\bar{x}$ 21.2	15.2	$\bar{x}$ 3.0	2.9
$P<0.01$		$P>0.05$		$P<0.01$		$P>0.05$	

Note: G-6-PD and 6-PGD activities are expressed in IU/1000 ml of RBC; LDH and PK activities are expressed in IU/ml of RBC. Heparin (HEP) was used in the identical final concentration as mentioned in Table II. [a] For LDH the ratio ACD/blood was 1:9.

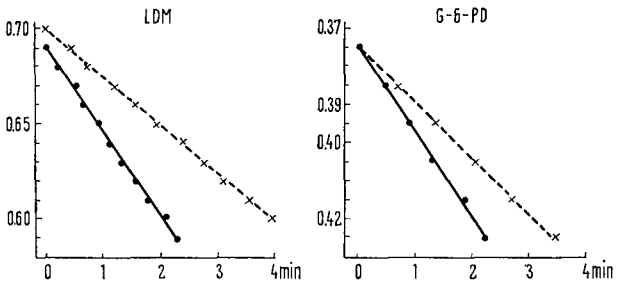
icant depression of the total erythrocyte activity of LDH in hemolysates of cells treated with ACD (Figure). This phenomenon has been observed even if blood samples were collected with ACD in the ratio ACD/blood 1:9 (Table I). LDH isoenzyme patterns determined in hemolysates, by agar-gel electrophoresis according to WIEME[8], have shown in ACD samples an evident restriction of all 4 bands which are usually obtained in matured RBC. The loss of total LDH activity is therefore divided in the same degree between all 4 erythrocyte LDH isoenzymes and the relationship between individual fractions remained unchanged when compared with heparin samples. In contrast to our above-mentioned findings in pyruvate kinase (PK), determined from RBC by the method of BÜCHER and PFLEIDERER[9], there are no differences in the erythrocyte activity of PK when compared heparin treated blood samples with ACD ones (Table I).

In addition to these studies we have performed some analyses with individual constituents of ACD solution (Table II). For this purpose we have chosen 2 erythrocyte enzymes, LDH and G-6-PD, according to the evident sensitivity of these enzymes on the inhibition effect of ACD. The constituents which have been tested are divided into 2 groups. The first group contains constituents of pH about 7 or higher (heparin, Na citrate, dextrose with Na citrate). The second group contains constituents with the same pH value (5.0) as was found in ACD solution. For this purpose Na citrate has been acidified to a fitting pH level by adding glacial acetic acid, whereas heparin has been acidified by adding of one drop of 1 N NCl. As shown in the Table II for interaction constituents of acid pH have been used, there is a significant loss of activity in both tested dehydrogenases (in LDH the loss of activity was about 50%, in G-6-PD the loss was about 25%). These results permit us to explain the partially irreversible loss of activity in tested enzymes by means of the lower pH of ACD.

Table II. A comparative analysis of the influence of individual components of ACD solution on the activity of LDH and G-6-PD in RBC: The demonstration of the inhibitory effect of acid pH

Examined constituents	pH	LDH activity %	G-6-PD activity %
Heparin[a]	6.6	100	100
Natrium citrate	8.94	95	95
Dextrose + Na citrate	8.94	94	100
ACD[b]	5.02	50	67
Acid citrate[c]	5.02	43	76
Dextrose + Acid citrate	5.02	42	71
Heparin acidified[c]	5.00	68	80
Dextrose + Heparin acid	5.00	–	69

[a] 0.04 ml of heparin or acidified heparin (5000 IU/ml) to 10 ml of blood was used (final concentration 20 IU/ml). In remaining cases the ratio of examined solution to blood was 4:16. [b] ACD solution: natrium citrate 1.1 g, acid citrate 0.4 g, dextrose 3.5 g, distilled water to 100 ml. [c] Acid citrate has been prepared from natrium citrate by acidifying with acetic acid (acidum aceticum glaciale). Acidified heparin has been prepared from heparin solution by acidifying with one drop of 1 N HCl.



A representative single probe with typical different course of enzymatic reaction in heparin and ACD samples. Extension 340. ○—○, heparin; ×---×, ACD.

Zusammenfassung. Der viel verwendete gerinnungshemmende Zusatz ACD (acid citric dextrose) vermindert die Aktivität der Glucose-6-Phosphat-Dehydrogenase und anderer Dehydrogenasen der Erythrozyten. Diese Wirkung kommt durch pH-Verschiebung zustande.

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First Medical Clinic, Charles University, Prague (Czechoslovakia), 1 June 1968.

[8] P. J. WIEME, *Studies on Agar Gel Electrophoresis* (Arscia Unitgaven, Brüssel 1959).

[9] T. BÜCHER and G. PFLEIDERER, *Methods in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 1, p. 435.