Spezies beschriebene Sexualdimorphismus der Gl. extraorbitalis (WALKER[9]; BAQUICHE[10]; KITTEL[11]) könnte auch die unterschiedliche Reaktion des Drüsengewebes auf Kupfermangel geschlechtsspezifische Aufgaben der Extraorbitaldrüse andeuten, von der KITTEL[11] annimmt, dass sie beim Goldhamster bestimmte Duftstoffe, bei der Ratte Markierungsstoffe produziert.

In weiteren Untersuchungen muss geklärt werden, ob die beschriebenen Veränderungen des Drüsenbildes Folge einer fehlenden spezifischen Kupferwirkung auf die Gl. extraorbitalis sind oder ob sie infolge der verminderten Aktivität der Zytochromoxydase und anderer Zellhämine auftreten, deren Synthese an das Vorhandensein von Kupfer gebunden ist. Ausserdem müssten die Befunde SCHMIDT's[12] an den Extraorbitaldrüsen erwachsener Ratten nach Milchernährung ohne Zusatz von Spurenelementen und Vitaminen überprüft werden, da die von ihm beschriebenen Veränderungen möglicherweise nicht allein Folge der flüssigen Kostform sind, sondern eventuell Kupfermangelschäden darstellen[13].

Summary. Copper deficiency of the rat leads to a reduction of the histochemically demonstrable cytochrome oxidase activity of the glandula extraorbitalis, and to atrophy and fatty degeneration of the glandular cells. The histological alterations are more pronounced in the male than in the female.

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Carboxypeptidase A, Ionic Strength, and the Enhancement of Guinea-Pig Complement

Carboxypeptidase A enhances the formation of EAC $\overline{1,4,2}$ by guinea-pig complement (gp C) and sensitized sheep cells[1]. It has been reported that low ionic strength buffers in immune hemolysis prevent the dissociation of C1 from $SA\overline{C1}$, promoting the eventual conversion of $SA\overline{C1},4 \rightarrow SA\overline{C1,4,2}$[2]. Since carboxypeptidase A and low ionic strength exert similar effects on the immune hemolytic reaction, it was of interest to titrate gp C, by the method of MAYER[3], at varying ionic strengths in the presence and absence of the enzyme. Kinetic studies were also performed to determine the effect of time of introduction of low ionic strength and carboxypeptidase A on the immune hemolytic reaction.

Veronal buffers for the titrations were prepared according to the method of IBE and WARDLAW[4]. Low ionic strength buffers for the kinetic experiments were made by combining equal volumes of buffers ($\mu = 0.15$ and $\mu = 0.004$) to give a final ionic strength of $\mu = 0.077$. The sera from freshly drawn blood of healthy guinea-pigs were pooled, stored at $-52\,^{\circ}C$, and used as a source of gp C. Hemolysin was obtained by i.v. injection of rabbits with boiled stromata of sheep erythrocytes. Preparation of suspensions of sensitized erythrocytes is that described by MAYER[3].

Guinea-pig complement (2 ml) was diluted with 8.0 ml of buffer ($\mu = 0.15$). An additional aliquot of gp C (2 ml) was diluted with 8.0 ml of the same buffer containing carboxypeptidase A (Worthington Biochemical Corp., Freehold, New Jersey, Lot No. COA-DFP-6129) at a concentration of 8–10 µg/ml. These 2 samples were preincubated for 10 min at 37 °C[5] and then tested for complement activity by titration in buffers of varying ionic strength (Table). Enhancement by carboxypeptidase A was demonstrated at all ionic strengths tested, but was minimal at the optimum ionic strength (0.085). This optimum ionic strength is a higher value than previously reported[6] and may be a result of inadequate predilution of gp C in veronal buffer ($\mu = 0.15$) or possibly to the sugar, dextrose (Mallinckrodt Chemical Works, St. Louis, Mo., USA), used to maintain isotonicity. RAPP and BORSOS[6] have noted that the nature of nonelectrolyte used to maintain isotonicity does affect the activity of gp C. The additional increase of $C'H_{50}$ by carboxypeptidase A at the optimum ionic strength suggests that the enzyme either exerts a greater effect on components enhanced by low ionic strength, or affects, in addition, other components of complement.

The effect of time of introduction of low ionic strength or of carboxypeptidase A into the immune hemolytic reaction was studied in the following manner. Basic reaction mixtures of 5.0 ml of sensitized cells (EA 5×10^9 cells/ml) and 1.0 ml of guinea-pig complement (diluted 1:57) were placed in a 37 °C bath at zero time. At timed intervals, aliquots (6.0 ml) of veronal buffer ($\mu = 0.15$), containing carboxypeptidase A (8–10 µg/ml), or low ionic strength buffer ($\mu = 0.004$) were added to the reaction mixtures. Incubation was continued to 100 min, and the

Effect of carboxypeptidase A on guinea-pig complement at varying ionic strengths

$C'H_{50}$ Ionic strength	No enzyme	With enzyme	Enhancement (%)
0.15	218	278	27.4
0.10	358	419	17.0
0.085	393	455	15.3
0.075	386	455	17.8
0.050	192	242	26.0

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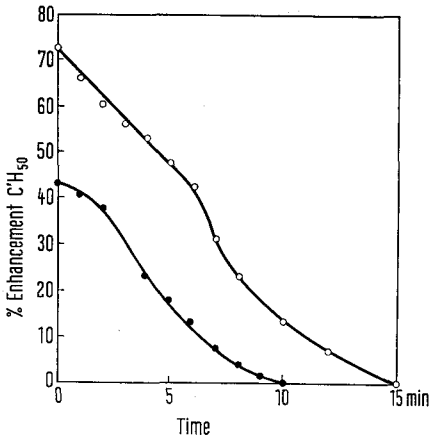
effect on the extent of hemolysis as compared to that of a control mixture is shown in the Figure. After 10 min, carboxypeptidase A no longer exerted any influence on the immune hemolytic reaction. However, enhancement of immune hemolysis by low ionic strength could be demonstrated up to 15 min after 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-

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