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THE DECREASE OF EFFICIENCY OF POVIDONE-IODINE PREPARATIONS  
BY BLOOD: MODEL EXPERIMENTS ON THE REACTION OF IODINE  
CONTAINING DESINFECTANTS WITH PROTEIN CONSTITUENTS

Waldemar Gottardi and Waltraud Koller

ABSTRACT

The reaction of blood with povidone-iodine containing preparations results in a decrease of titrable iodine ( $C_{ox}$ ) and a significant raise of the iodide concentration. As a consequence the concentration of free iodine ( $[I_2]$ ), decreases more than  $C_{ox}$ , because the equilibrium of the triiodide formation,  $I_2 + I^- \rightleftharpoons I_3^-$ , is shifted to the right. The extent of the decrease of  $C_{ox}$  and  $[I_2]$  is not proportional to the amount of blood but decreases with the latter. Thus the reaction of a 10% povidone-iodine solution with 20% blood gave rise to a decrease of 27-40%  $C_{ox}$  and 35-55%  $[I_2]$ , while in the case of 100% blood the decrease was 80-88 resp. >90%.

Since  $[I_2]$  is an important parameter, which largely correlates with the rate of killing microorganisms, the action of blood (mainly at concentrations >20%) on povidone-preparations can cause a significant loss of disinfecting power.

The reaction with blood takes place immediately and its extent - as well as the fast reaction rate - depends, as can be shown by experiments with defined protein constituents, mainly on the content of protein bound sulfur, in particular of the amino acids cysteine and methionine. Histidine, thyrosine, cytosine, albumine and uracil react also reducing iodine, but much slower than do cysteine and methionine. Alanine, glycine, arginine, asparagine, lysine, guanine, adenine and cystine however did not react with iodine under the selected conditions (aqueous solution, pH 7, 25°C).

## INTRODUCTION

At the application of a disinfecting or antiseptic agent it should be considered that besides the desired reaction with living germs - which causes their death - also reactions with not living material (dead micro-organisms, skin surfaces, corpuscular and dissolved proteins) occur, which are both not desired and give rise to a decrease of the degerming activity.

In the case of disinfectants on halogene basis the latter are due above all to the substitution of covalent bounded hydrogen by halogene which results in compounds, whose oxidation power is reduced (e.g. N-halogene compounds) or which are not at all oxidizing (e.g. C-halogene compounds). It can be expected that according to the generally decreasing reactivity of the halogenes with growing atomic weight the halogene demand will be the smallest in the case of iodine. This could be verified in vitro at the reaction with peptone (Fig. 1), although bromine unexpectedly showed in this experiment a higher reactivity than chlorine. While in the system chlorine/water the hypochlorous acid (1), HOCl, has been identified as the actual agent, in the system iodine/water (in the presence of additional iodine) it is the free molecular iodine, I<sub>2</sub> (2,3,4).

In view of the wide spread application of povidone-iodine preparations in human medicine, e.g. at the therapy of burns or as an antiseptic in operating wounds, where generally high protein loads (wound fluids, blood) can occur, it was of interest, how iodine containing disinfectants behave in vitro in the presence of blood, serum, plasma and defined protein constituents of low molecular weight. In this connection especially the change of free iodine and titrable (total) iodine should be investigated since these parameters largely correlate with disinfection rate and disinfection capacity.

## EXPERIMENTAL

### Methods:

The oxydation capacity ( $C_{Ox}$ ) has been determined by iodometric titration with 0,1 N sodium arsenite. The endpoint in the case of experiments with blood was recognized potentiometrically (turning point of the function  $E_{Pt/Cal} = f$  (titrant volume)). Free iodine has been measured according to Lit. (5). All experiments have been conducted at  $25,0 \pm 0,1^\circ C$ .

### Reagents:

Blood: heparinized, not more than 12<sup>h</sup> old.

Plasma: by centrifuging fresh heparinized blood.

Amino acids, nucleotides and albumine: degree of purity "for biochemical purposes".

Povidone-iodine solutions: Commercial preparations and pure solutions of povidone-iodine in water adjusted to pH 4,0

Iodine-iodide solutions: 0,05 M/l iodine, 0,17 M/l potassium iodide in water and 1% Lugol's solution (fig. 7).

## Procedure:

The iodine-containing solutions were mixed with the reactants which were undiluted in the case of blood and plasma. The amino acids were added either dissolved or in solid form. The latter was essential to prevent a dilution of the iodine-system which would result in an unwanted augmentation of the concentration of free iodine (see (5)).

For the determination of the oxydation capacity aliquots were taken at definite times, while free iodine has been measured continuously.

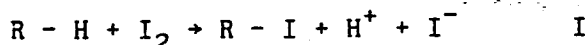
## RESULTS

Fig. 2 shows the relative decrease of  $C_{Ox}$  expressed as the consumption of grams iodine per ml reactant in a 10% povidone-iodine solution after addition of blood (plasma). As can be seen, plasma reacts stoichiometrically with iodine, while blood does not, as the relative decrease of  $C_{Ox}$  gets smaller when the amount of blood is increased.

Moreover the reaction with blood was not good reproducible but showed throughout a significant higher consumption of iodine than with plasma.

In Fig. 3 the percental decrease of  $C_{Ox}$  and free iodine ( $[I_2]$ ) at the reaction of blood, plasma and water with 10% povidone-iodine is reproduced. It indicates that the free iodine is more affected than  $C_{Ox}$ .

This can be explained by the increase of the iodide concentration: Iodide is formed at the reaction with protein constituents (I) and as a consequence the triiodide-equilibrium is shifted to the right (II)



Plasma consumes significantly less  $C_{Ox}$  than does blood. The surprising effect that  $[I_2]$  remains unchanged has to do with a special property of concentrated povidone-iodine solutions, that is the rise of free iodine on dilution (5) as can be seen too in Fig. 3. At the reaction with plasma coincidentally two opposite effects are compensated so that free iodine is not changed.

The effect that  $\Delta \% [I_2] > \Delta \% C_{Ox}$  is more pronounced when a reducing agent is added in solid form, as Fig. 4 shows. Here sodium thiosulfate is added to commercial povidone-iodine-preparations. As can be seen significant differences in the relative decrease of free iodine exist at the same decrease of  $C_{Ox}$ . In the case of preparation B the initial  $[I_2]$  was very high, which requires that the concentration of free iodide is very low, so that the formation of iodide at the reduction of iodine results in a comparatively high decrease of  $[I_2]$ .

This influence of iodide is demonstrated in Fig. 5, where the effect of the reaction of the amino acid cysteine with both a 10% povidone-iodine-solution and a iodine-iodide solution of the same concentration of free iodine and  $C_{Ox}$  but with a very high iodide concentration is reproduced. As can be seen there is no

difference between the relative decrease of  $[I_2]$  and  $C_{ox}$  if the system contains much iodide.

#### Kinetic measurements:

Fig. 6 shows that the main reaction with blood is completed nearly immediately, a small portion needs some minutes while the further reaction is very slow and its rate is independent of the amount of blood.

It was now of interest, which constituents of proteins are reacting with iodine. In Table 1 are listed the investigated pure protein constituents according to their reactivity against iodine in aqueous solution. Cysteine and methionine react nearly immediately, histidine, cytosine, thyrusine, uracil and albumine reacted slowly, while arginine, alanine, glycine, asparagine, lysine, guanine and adenine did not react under the chosen conditions.

Fig. 7 shows the speed of iodine exhaust of the slow reacting protein constituents while Fig. 8 demonstrates the reaction of cysteine, which can react with iodine depending on the conditions in the ratio 1:0,5 to 1:3.

#### CONCLUSIONS

- 1) Blood has a relative high iodine demand, so that it can effect a significant reduction of the disinfection performance of povidone iodine preparations (Tab. 2).

Tab. 2: Effect of blood on  $C_{ox}$  and  $I_2$  of 10 % povidone-iodine solutions (average values)

% blood	$C_{ox}$	$[I_2]$
10	- 20 %	- 25 %
25	- 40 %	- 50 %
50	- 65 %	- 80%

- 2) The main portion of the iodine consumption by blood takes place immediately and can be attributed to protein bound sulfur (amino acids with - SH and -  $SCH_3$  groups).
- 3) The relative decrease of free iodine is always more extensive than the one of  $C_{ox}$  and is bigger in preparations with a high content of free iodine.
- 4) Serum or albumine which are commonly used as model substances for testing in vitro the influence of a protein load on the activity of antiseptics and disinfectants (6), are not suited for this purpose in the case of iodine containing preparations because they do not simulate sufficiently the iodine demand which actually can occur in general practice.

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FIGURE 1

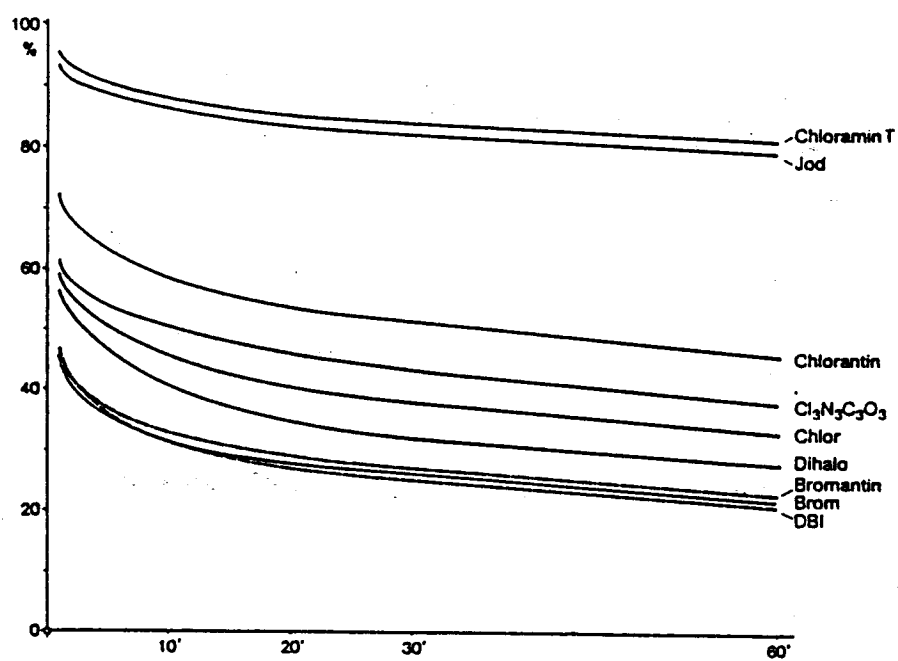


Fig. 1. Course of the reaction of  $\text{Cl}_2$ ,  $\text{Br}_2$ ,  $\text{J}_2$  and some N-chloro- and N-bromo compounds with peptone (pH = 7.00;  $5 \times 10^{-3}$  M active halogene/l; 0.5 g peptone/l).

FIG. 2 REACTION OF BLOOD AND PLASMA WITH 10 % POVIDONE-  
 IODINE SOLUTION: EFFECT OF THE AMOUNT OF BLOOD  
 (PLASMA) ON THE RELATIVE DECREASE OF TITRABLE  
 IODINE (COX)

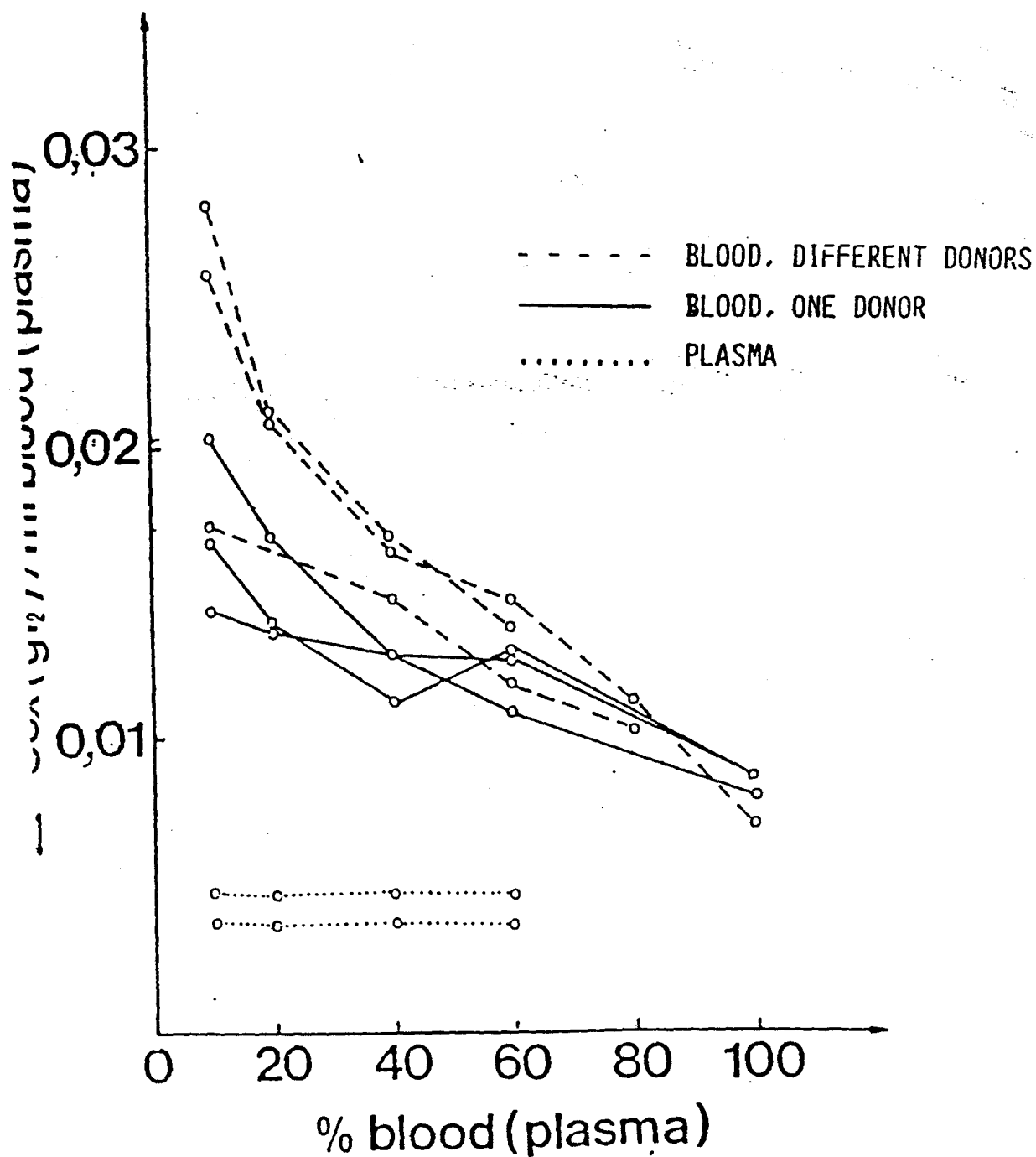


FIG. 3 REACTION OF BLOOD (PLASMA, WATER) WITH 10 % POVIDONE-IODINE SOLUTION: EFFECT OF THE AMOUNT OF REACTANT ON FREE IODINE ( $[I_2]$ ) AND TITRABLE IODINE (COX)

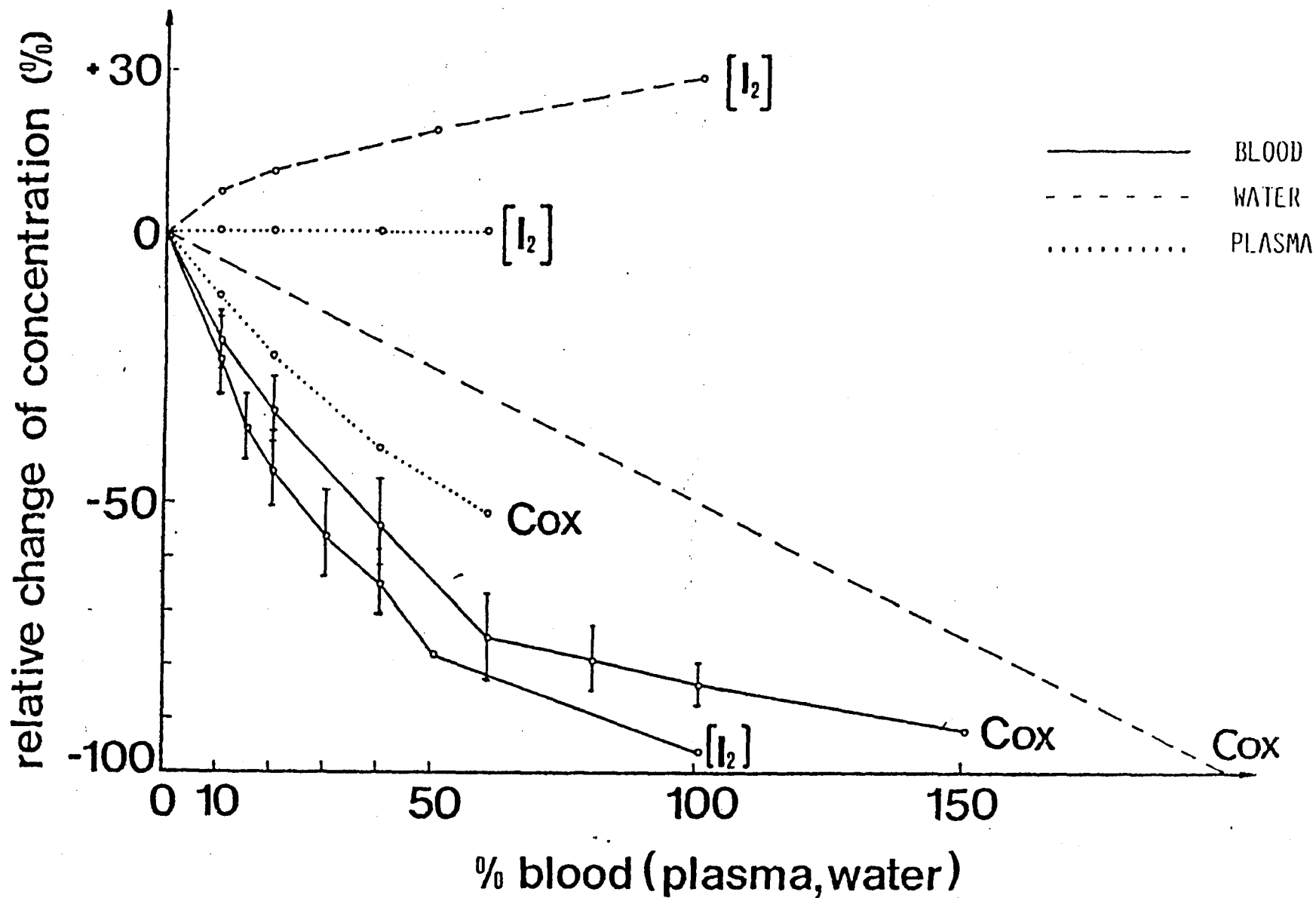


FIG. 4 REACTION OF  $\text{Na}_2\text{S}_2\text{O}_3^{\text{x)}$  WITH POVIDONE-IODINE PREPARATIONS:  
RELATIVE DECREASE OF FREE AND TITRABLE IODINE

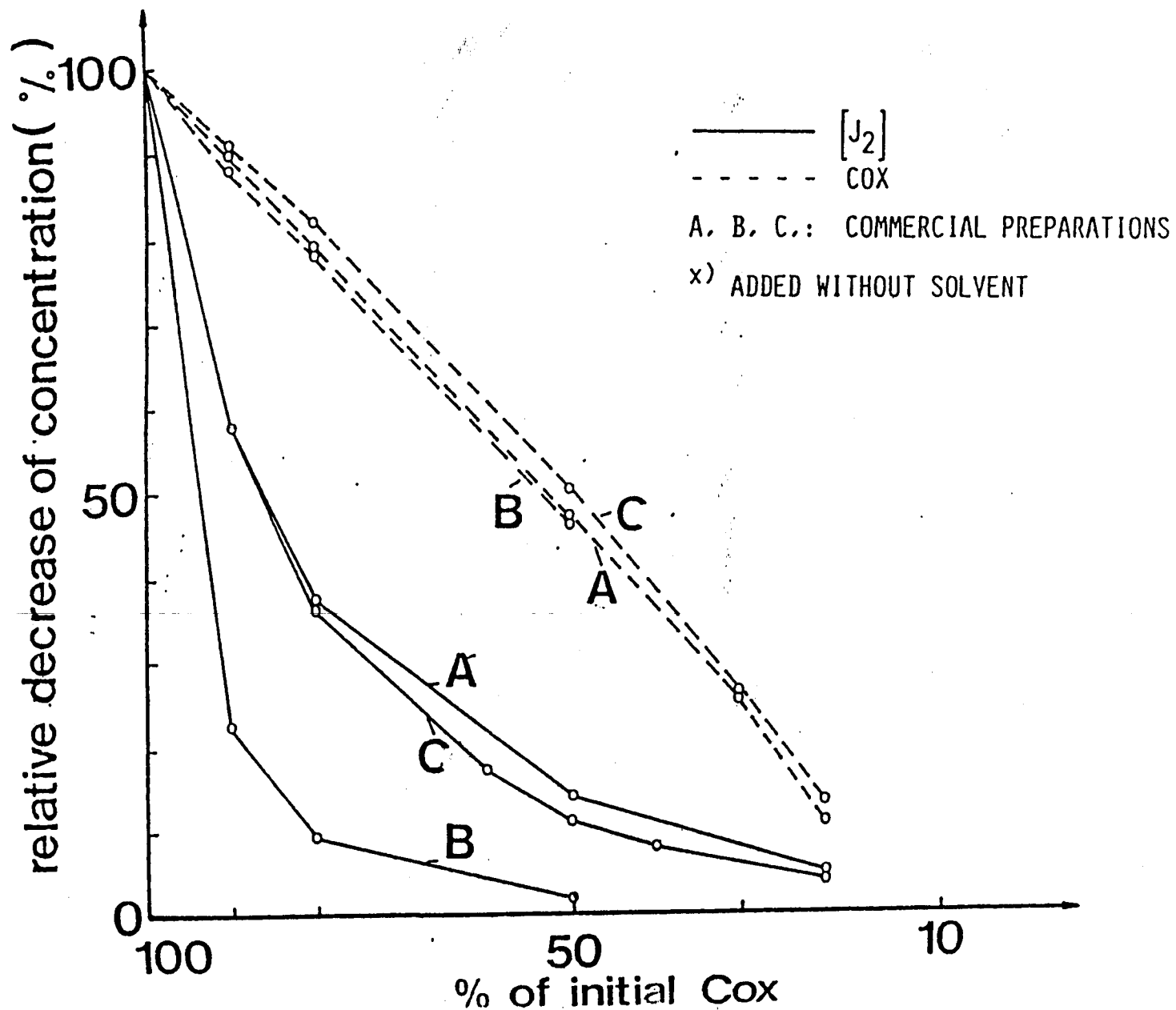


FIG. 5 REACTION OF CYSTEINE <sup>x)</sup> WITH AQUEOUS SOLUTIONS OF POVIDONE-  
 IODINE AND J<sub>2</sub>/KJ: RELATIVE DECREASE OF FREE AND TITRABLE  
 IODINE

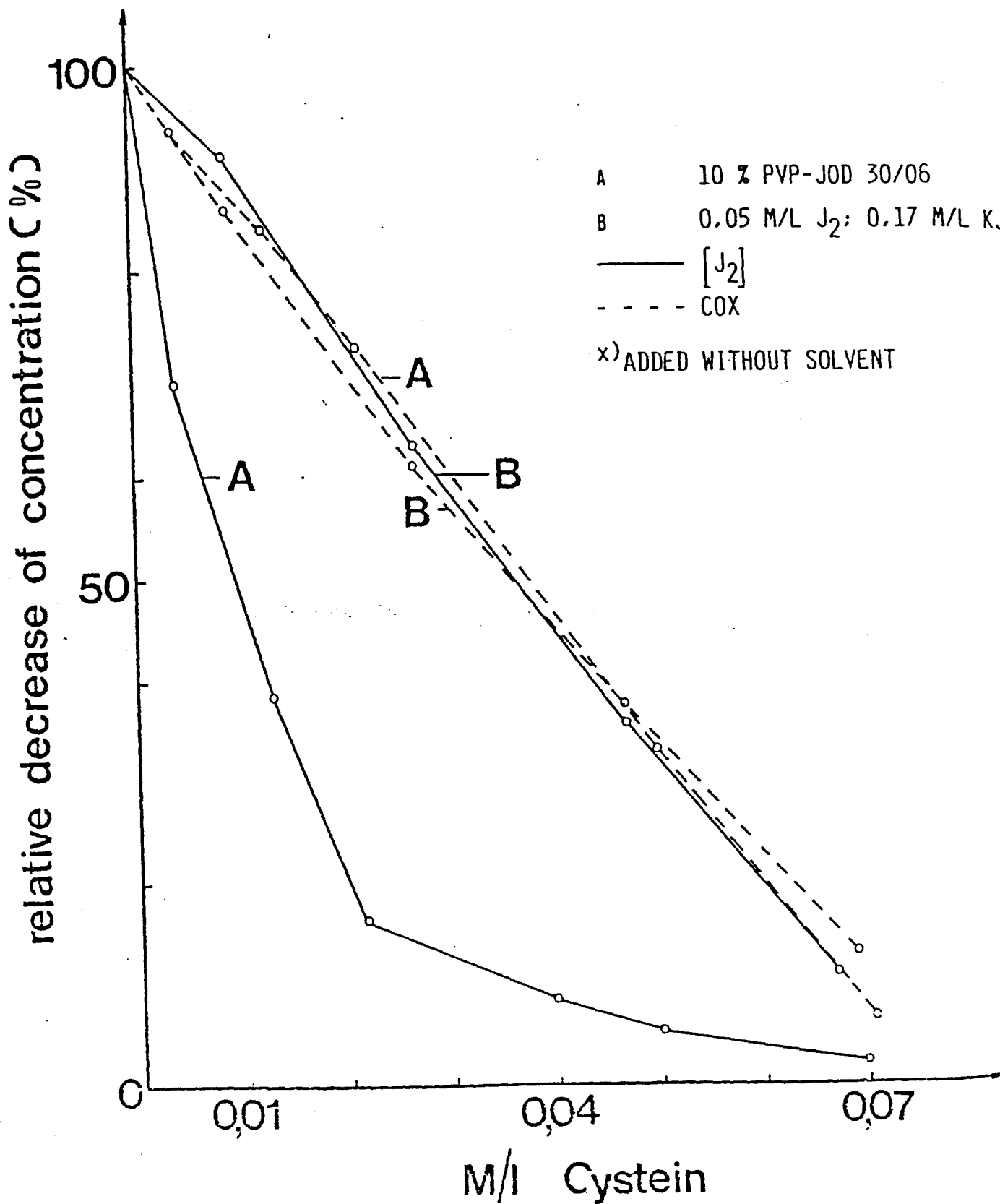


FIG. 6 RATE OF THE REACTION OF 10, 25 AND 100 % BLOOD WITH 10 % AQUEOUS POVIDONE-IODINE SOLUTION:  
DECREASE OF FREE AND TITRABLE IODINE.

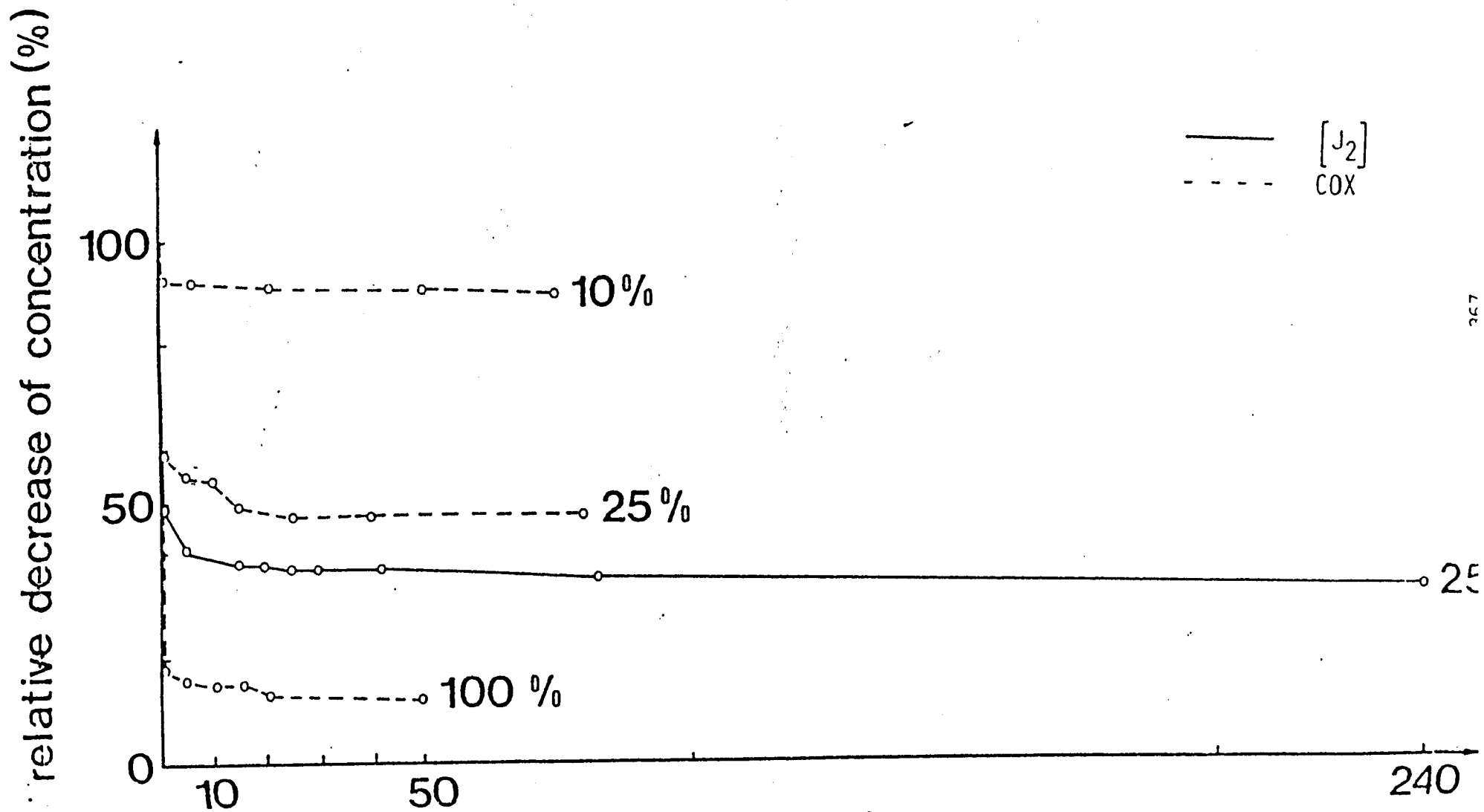


FIG. 7 RATE OF THE REACTION OF IODINE WITH SOME PROTEIN CONSTITUENTS

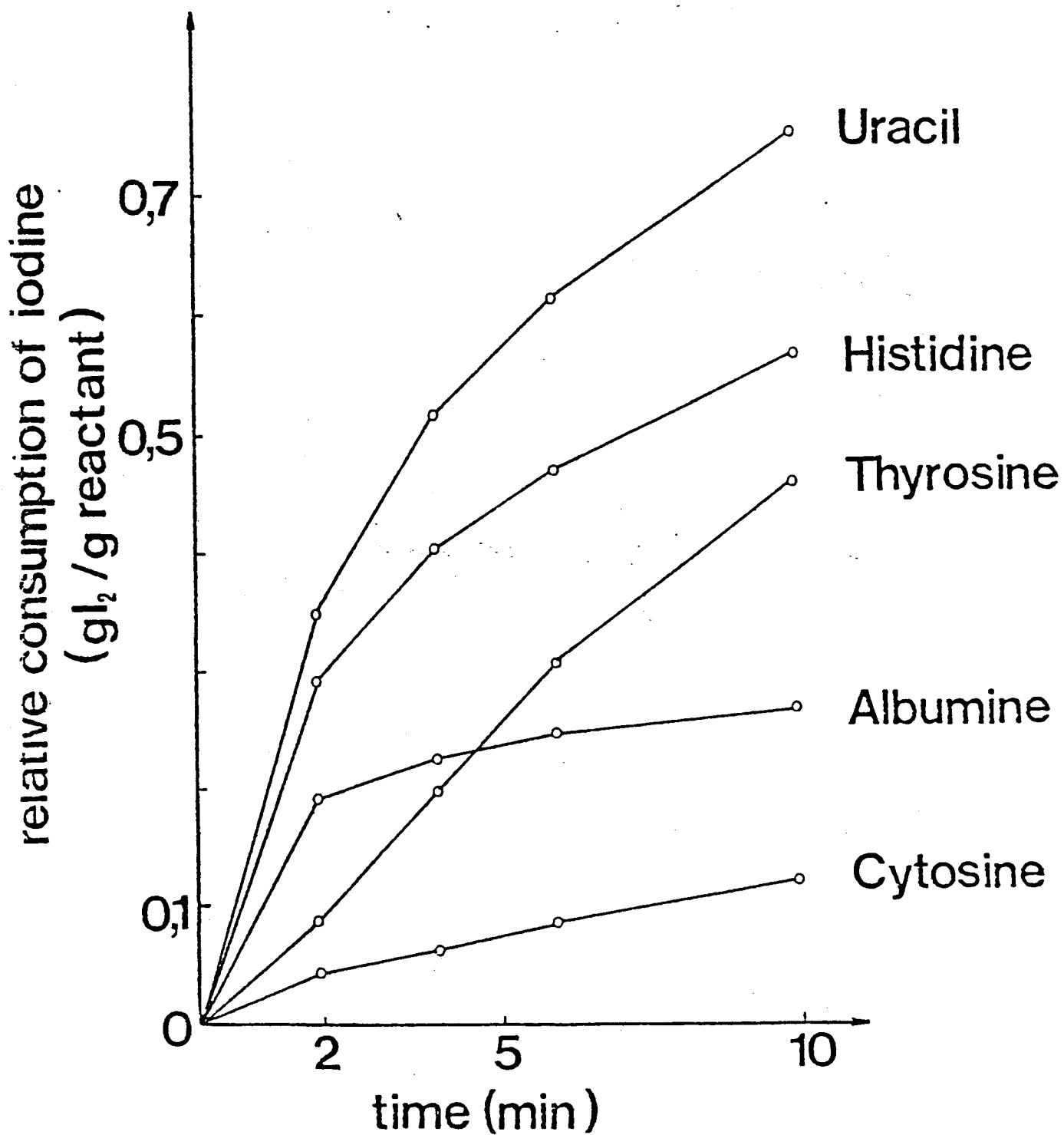
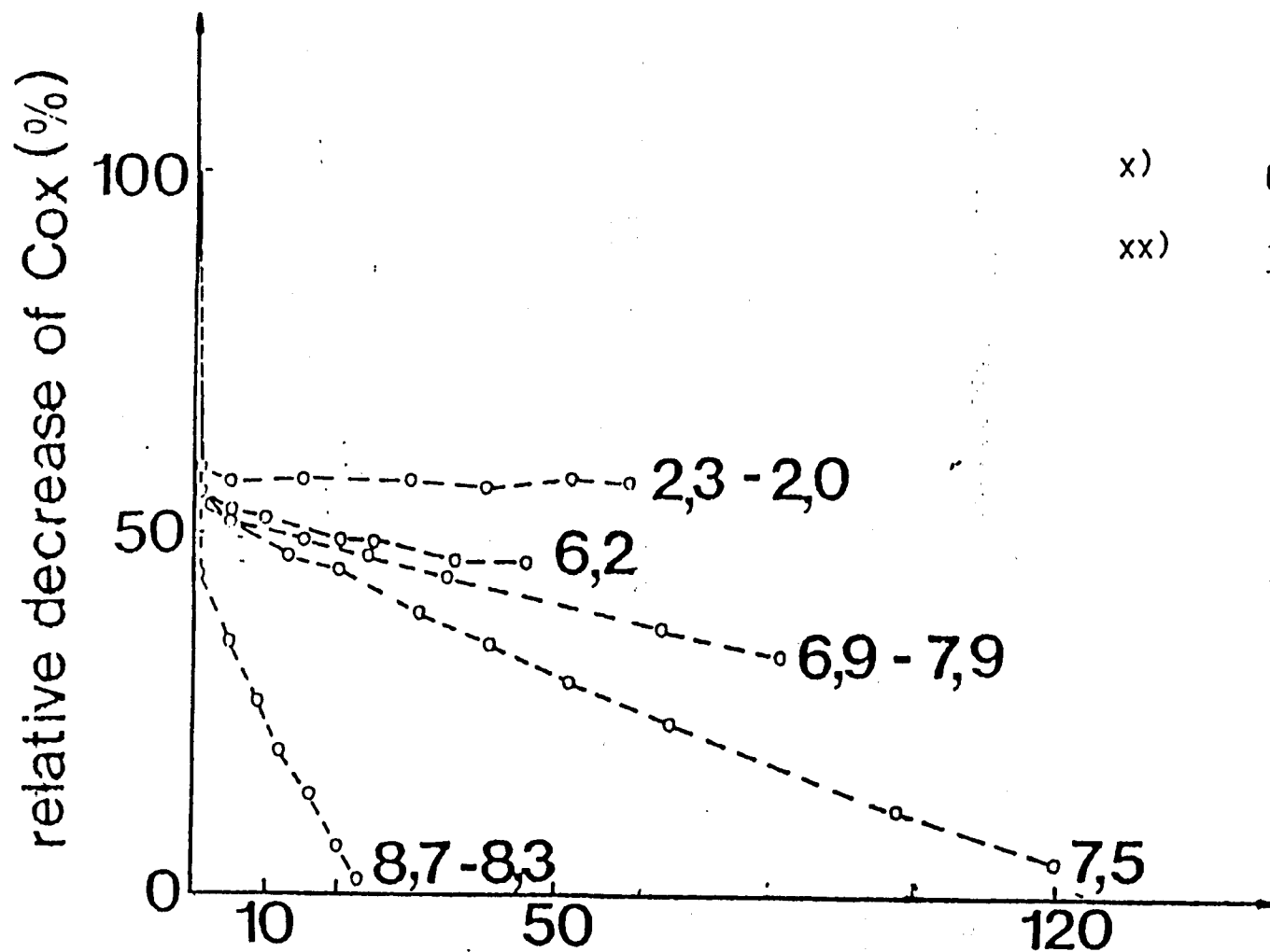


FIG. 8 REACTION OF CYSTEINE<sup>x)</sup> WITH POVIDONE-IODINE<sup>xx)</sup>:  
RATE OF REACTION AT VARIOUS pH-VALUES



x)  $6.6 \times 10^{-2}$  M AQUEOUS SOLUTION

xx) 10 % POVIDONE-IODINE 30/06

TABLE 1: RATE OF THE REACTION OF PROTEIN CONSTITUENTS WITH  
IODINE IN AQUEOUS SOLUTION (0.05 M/L  $I_2$ ,  
0.17 M/L KJ, PH = 4, 25°C)

VERY FAST	SLOW	NO REACTION
CYSTEINE	HISTIDINE	ARGININE
METHIONINE	CYTOSINE	ALANINE
	THYROSINE	ASPARAGINE
	URACIL	LYSINE
	ALBUMINE	GLYCINE
		GUANINE
		ADENINE

\* \* \* GOTTARDI DISCUSSION \* \* \*

Q: (Conferee) Quick question on your use of blood. When you say blood, you mean blood containing viable cells? Could the cells be participating in any of your iodine reactions?

A: (Gottardi) The first slide shows that fresh blood, has a significantly greater demand for iodine than the plasma. The reason is that red blood cells contain a lot of substances that consume iodine. But why there's bad reproducibility, we can not yet explain fully.

Q: (Conferee) Some of these commercial povidone concentrates, after several years of storage, tend to degrade. Do you have any explanation as to the reactions that take place here? In other words, when you buy a commercial povidone and put it on the shelf, for several years and some of them dissipate down to practically zero, even though there is still a brown solution there, what has happened?

A: (Gottardi) Povidone concentrates are not fully stable but we do know why. It is proven that on storage, solutions become acidic and iodine levels decrease.

Q: (Fina) In that lower equation you produce a lot of triiodide. Isn't triiodide a very poor disinfectant?

A: Yes.

Q: (White) We have done some work with blood where we mixed viable counts of microorganisms into the blood to try to determine killing effect vs. protein inactivation. We observed some very interesting phenomena in terms of the localization of the microorganisms and almost a preference of those cells compared to the whole blood cells and/or blood fragments. Have you done any work in mixed blood work with the iodine?

A: No, only fresh blood and plasma.

Q: (White) In fact, if your work generally is done with whole blood, you don't take out the clotting materials. Is that correct?

A: That is right.