In many cases, pronounced changes in the colors of solutions take place near the polarographic critical concentration. The normal deep blue of the hydroxytartrato-copper(II) complex is changed to greenish by dodecyltrimethylammonium bromide, while gelatin gives (as with the corresponding citrate complex) a light purplish-blue succeeded, at higher concentrations, by pure violet. Hexadecyltrimethylammonium bromide alters the light greenish-blue of the copper(II) hydrogen tartrate complex to a deep green. A pink solution of cobalt(II) in *F* potassium thiocyanate becomes blue on the addition of about $5 \times 10^{-3}\%$ Triton X-100, a color change long associated with the removal of water from cobalt (II) complexes.

While this strongly suggests some sort of reaction in the bulk of the solution, the observed polarographic effects vary so widely in nature, magnitude and direction as to make it appear that their complete interpretation must await a much more detailed study of the phenomena occurring both in the solutions and at the electrode surface.

The work here described was begun in collaboration with Dr. Eugene L. Colichman. It is a pleasure to acknowledge his coöperation and assistance in that portion of the experimental work dealing with the several copper tartrate systems.

Summary

When any of a number of surface-active materials is added to an aqueous solution, the drop time of a capillary producing approximately 4-sec. drops is substantially unaffected up to some sharply defined concentration, and then decreases nearly linearly with the logarithm of the concentration. The concentration at which the discontinuity occurs is identical with that at which the diffusion coefficient of methyl red suddenly decreases by a factor of about 100. Because these phenomena are closely similar to those characterizing the classical "critical concentration for micelle formation," this point is termed the "polarographic critical concentration."

Also occurring at this concentration are discontinuities in the effects of these materials on the polarograms of a wide variety of substances. The effects observed are divided into six classes. Two earlier explanations of isolated examples of these types of behavior are shown to be inadequate: a complete explanation will probably have to take into consideration both adsorption phenomena and direct or indirect (*i.e.*, activity effects) chemical reactions in the body of the solution.

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[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

Chain Reactions Induced by Enzymic Systems

BY GIUSEPPE PARRAVANO

Recent work on induced polymerization at catalytic surfaces[1] offered the opportunity to test whether enzymic and biological systems are able to initiate chain polymerization reactions of a vinyl monomer. This note describes some results obtained in this study.

Exploratory work was begun with the use of washed suspensions of *B. coli*. During formic acid decomposition by *B. coli* an aqueous solution of methyl methacrylate (MMA) was added, and turbidity detected in the system over a period of a few days, at room temperature. However, in view of the difficulties involved in the use of a living system work has been continued with pure enzymes.

The enzymatic reaction chosen was formaldehyde dehydrogenation by xanthine oxidase (Schardinger enzyme) (XO). The enzyme was obtained from fresh milk by the method of Dixon and Kodama.[2] Polymerization reactions were carried out in test-tubes filled with appropriate amounts of reactants, thoroughly degassed by high vacuum technique. Results are collected in Table I (MB = methylene blue; AN = acrylonitrile).

Experiments were made to ascertain the influence of oxygen on the system. For this purpose samples were prepared which were not degassed. Results are collected in Table II.

(1) THIS JOURNAL, **72**, 3856 (1950).

(2) Dixon and Kodama, *Biochem. J.*, **20**, 1104 (1926).

TABLE I

POLYMERIZATION INDUCED BY ENZYMATIC DEHYDROGENATION OF 0.4% SOLUTION FORMALDEHYDE, t = 22°

Enzyme soln., cc.	HCHO soln., cc.	MMA, cc.	MB, soln., cc.	Polymer ppts., hr.	Remarks
5	5	...	2	..	Color fades
4	5	0.2	..	~15	Two samples
6	5 (4%)	.3	..	~10	
2	1	.1	..	~24	+5 cc. H_2O
4	5	.2	1	>5 days	
4	.....	.2	..	No polymer	+5 cc. H_2O; two samples
4	5 (4%)	1 (AN)	..	~12	
16	10 (4%)	1.5	..	~2 days	

TABLE II

POLYMERIZATION INDUCED BY ENZYMATIC DEHYDROGENATION OF 0.4% FORMALDEHYDE SOLUTIONS, t = 22°, SOLUTION NOT DEGASSED

Enzyme soln., cc.	HCHO soln., cc.	MMA, cc.	MB soln., cc.	Remarks
4	5	...	1	Color does not fade
4	5	0.2	..	Some polymer after 5 days
4	5	.2	1	No polymer after 10 days
4	..	...	1	+5 cc. H_2O, color does not fade

Discussion

From the results of Table I it can be concluded

that monomer can replace MB as a hydrogen acceptor from the system XO + HCHO. This conforms with the known fact that XO has no pronounced specificity so far as hydrogen acceptors are concerned.

The mechanism for the initiation of addition polymerization reactions by the system XO + HCHO + MMA can therefore be explained following the accepted scheme for the interaction of the system XO + HCHO + MB. The reaction occurring in the system XO + HCHO + MMA can be represented by the generalized equation

$$H_2C(OH)_2 + 2m \underset{+2H}{\overset{-2H}{\rightleftharpoons}} XO \quad 2Hm^* + HCOOH \quad (1)$$

where m is a monomer molecular and Hm* the monomer free radical formed. Reaction (1) can be visualized as occurring through the possible steps

$$+ H_2C(OH)_2 \rightleftharpoons XO - H + H_2C(OH)O\cdot \quad (2)$$

$$XO - H + m \longrightarrow Hm^* + XO \quad (3)$$

$$H_2C(OH)O\cdot + m \longrightarrow Hm^* + HCOOH \quad (4)$$

Reactions (3) and (4) can initiate polymerization chains. The occurrence of reaction (3) depends mostly on the thermodynamic oxidation–reduction potential of the system XO–H + m. The mechanism represented by equation (1) is in accord with the Haber and Willstätter[3] theory for chain processes in enzymic systems. The outstanding features of this theory are: (1) hydrogen atom transfer should occur in single steps; (2) reaction is propagated by radicals produced by monovalent dehydrogenation. Both requirements are strikingly demonstrated by the present experiments. It should be added that it has already been shown by Michaelis, *et al.*,[4] that during the two stages of reduction of riboflavine to leucoriboflavine free radicals of the semiquinone type are involved. From the results obtained in the presence of MB it can be concluded that it is a faster or more specific hydrogen acceptor from XO than MMA.

As can be seen from Table II no polymer can be obtained in the presence of oxygen. This can be accounted for on the assumption that the action of XO is blocked by hydrogen peroxide formed by reduction of molecular oxygen, unless catalase is present, as is the case for living systems.

(3) Haber and Willstätter, *Ber.*, **64**, 2844 (1931).

Summary

1. Enzymic systems such as *B. coli* in formic acid and xanthine oxidase in formaldehyde can initiate polymerization of methyl methacrylate present in aqueous solutions freed from oxygen.

2. Methylene blue is a better acceptor than methyl methacrylate in xanthine oxidase–formaldehyde solutions.

3. Oxygen inhibits the polymerization in presence of xanthine oxidase.

4. The mechanism proposed for the polymerization process is in accord with the Haber–Willstätter theory of chain processes in enzymic systems.

(4) Michaelis, *J. Biol. Chem.*, **116**, 587 (1936); *Chem. Revs.*, **16**, 243 (1935); **22**, 437 (1938); THIS JOURNAL, **60**, 1678 (1938); Kuhn and Ströbele, *Ber.*, **70**, 753 (1937).

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[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Partial Synthesis of Compounds Related to Adrenal Cortical Hormones. XIV. Preparation of the Dihydroxyacetone Side Chain; 17α-Hydroxyprogesterone and "Substances L and P"[1]

BY THEODORE H. KRITCHEVSKY AND T. F. GALLAGHER

Current investigations have focused attention on the profound influence of the structure of the side chain on the biological activity of the adrenocortical hormones. The dihydroxyacetone structure present in cortisone and related hormones has from a biochemical and medical standpoint the greatest interest because these compounds exhibit the most striking chemotherapeutic action on the metabolic processes altered by disease. The primary problem in the synthesis of the side chain of these compounds is the formation of a tertiary alcohol in the proper orientation at C-17 with the retention of a ketone function at C-20. A corollary problem, then, is the introduction of a hydroxyl group at C-21. We have been able to accomplish both of these objectives smoothly and in high yield from readily available materials. We have exemplified these procedures by the partial synthesis of the representative adrenal hormones, 17α-hydroxyprogesterone and Reichstein's "Substances L and P."[2] The reactions involved are generally applicable and permit the preparation of a series of cortical hormones with or without oxygen at C-11.[3] We have utilized them for the elaboration of isotopically labeled compounds for future biochemical investigation. At the same time, however, they afford a convenient means for the technical preparation of biologically important hormones and structurally related compounds of immediate interest in medical research.

We found[4] that when an enol ester of a 20-keto-

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(2) The isolation and chemical identification of these hormones have been reviewed by Reichstein and Shoppee in Harris and Thimann, "Vitamins and Hormones," Vol. 1, p. 359, New York, N. Y., 1943. The "recorded constants" in the experimental section have been taken from this article.

(3) Koechlin, Garmaise, Kritchevsky and Gallagher, THIS JOURNAL, **71**, 3262 (1949).

(4) Kritchevsky and Gallagher, *J. Biol. Chem.*, **179**, 507 (1949).