The Effect of Phenazine Methosulfate on the Activity of Dehydrogenases as Studied by Starch Gel Electrophoresis

The demonstration of oxidative enzymes in tissue sections presents no difficulty when there is a fairly high level of activity present. In this instance, the insoluble colored formazans formed by the reduction of tetrazolium salts are easily visualized and the results obtained are easily reproduced. For the demonstration of weak activity, the utilization of a number of reagents which could act as efficient intermediary electron acceptors has been tried[1-5]. Phenazine methosulfate (PMS) is one of the many which have been utilized to improve the localization of succinic dehydrogenase (SDH) and lactic dehydrogenase (LDH) activity in tissue sections[6-9]. In the present study, we have utilized different concentrations of this compound to improve the demonstration of faint bands of enzyme activity as separated by starch gel electrophoresis.

The gray matter of the cerebral cortex from the chimpanzee (*Pan troglodytes*) was homogenized, and the concentrated supernatant was electrophoresed for 18 h at 4 °C with a constant voltage (3 V/cm) through the gels. The buffer systems and gel systems were previously reported from this laboratory and elsewhere[10,11]. The standard methods of NACHLAS et al.[12] and HESS et al.[13] were utilized for the localization of SDH and LDH respectively. The amount of PMS varied from 1.0–9.0 mg/100 cm^3 of incubation mixture. Substrates without the addition of PMS were used as controls.

5 distinct bands with strong LDH activity and 5–6 faintly colored bands were observed in the gels after 4 h incubation (Figure 1a). With the addition of 1.5 mg PMS/100 ml, these bands were seen in a matter of minutes. After 8 min incubation with PMS present, the staining corresponded approximately to that seen with $2^1/_2$ h of incubation in the absence of PMS. With 20–25 min incubation, in the presence of PMS, all enzyme bands which could be seen were demonstrated (Figure 1b). Longer incubation times, or increasing amounts of PMS, resulted not only in deeper staining of the bands in general but also in the non-specific deposition of the dye on and around the bands and in a diffuse staining of the gels.

In addition, the observation was made that, with an excess amount of PMS in the incubation mixture for the localization of SDH activity, one sees consistently the production of negative bands (Figure 2). It is extremely difficult for us at this stage of our program to interpret the production of colorless bands by an excess amount of PMS, a substance which in optimal amounts acts as an excellent mediator of electrons for the demonstration of dehydrogenases. We would have, in fact, expected more highly-colored formazans. The present studies agree with those of CONKLIN et al.[14] in that PMS in appropriate

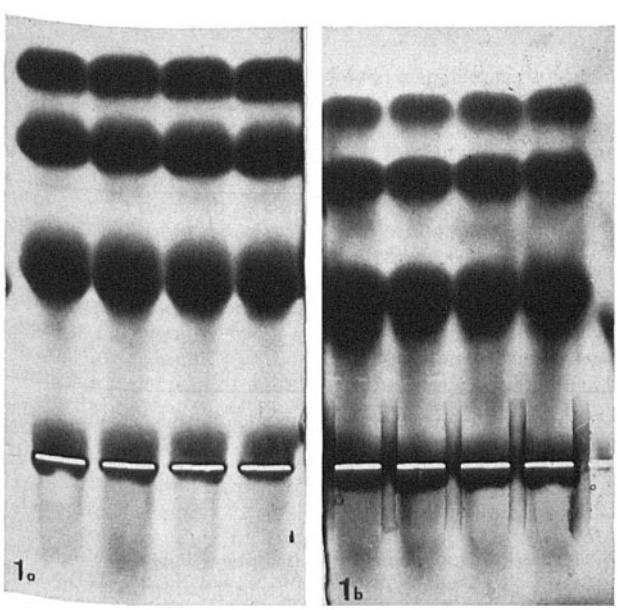

Fig. 1. Lactic dehydrogenase reaction (a) without the addition of PMS to the substrate and incubated for $4^1/_2$ h, (b) after adding 1.5 mg PMS/100 cm^3 substrate and incubating for only 30 min. The bands with very weak activity in (a) have darkened in spite of 1/8 time of incubation.

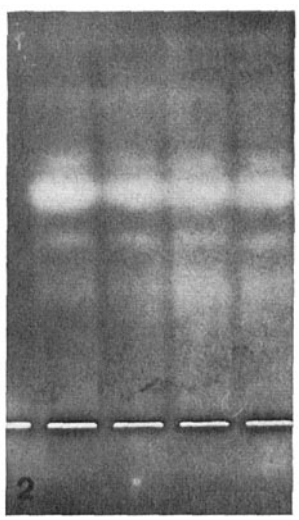

Fig. 2. Succinic dehydrogenase reaction, 6.5 mg PMS/100 cm^3 substrate, showing the 'negative bands', the composition and significance of which are not known at this time.

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quantities enhances the staining of enzyme bands. In addition, excess amounts of PMS results in (1) the adherence of fine formazan deposits produced close to the surface of the gel and resulting in a diffuse staining of the gels, and (2) the production of negative bands[15].

Résumé. L'effet de la phenazine methosulfate (PMS) sur l'activité de plusieurs enzymes oxydatifs a été étudié. En tant que transporteur d'électron, la PMS a un rôle important sur la coloration des isoenzymes séparés par électrophorèse en gel d'amidon. En ce qui concerne la déshydrogénase lactique, un excès de PMS provoque une coloration diffuse, alors que dans le cas de la déshydrogénase succinique on observe l'apparition de «bandes négatives» qui n'ont pas encore été décrites.

S. L. MANOCHA, G. H. BOURNE, and M. SANDLER

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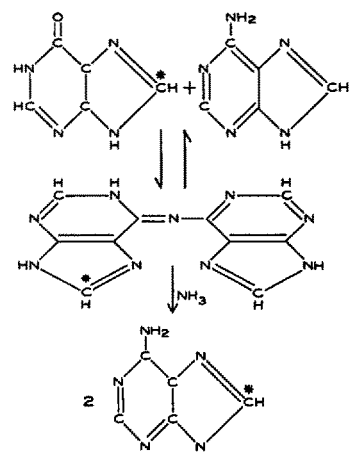
On the Interaction of Hypoxanthine with Adenine[1]

A non-enzymatic conversion of hypoxanthine to adenine was proposed on the basis of spectrophotometric observations obtained from solution mixtures containing adenine and hypoxanthine in 1–5 *M* ammonium phosphate[2]. The syntheses of the Schiff bases of 2-amino-4-methylpyrimidine were said[3] to support the reaction involving a condensation of the keto form of hypoxanthine and adenine through a Schiff base intermediate and its subsequent cleavage by the addition of ammonia to give 2 molecules of adenine. The results of these experiments have been presented to explain a mechanism that could account for the aberration of tetrads observed in nucleic acids.

Certain considerations should be taken into account, however. The concentration of the reactants (about 10^{-3} *M*) makes this reaction highly improbable. In the event that a certain amount of intermediate is formed, the low pH of the reaction mixture is certainly an unfavorable condition for the cleavage of the Schiff base by nucleophylic ammonia molecules (NH_3), which are non-existent at pH 6.

This communication is to verify whether the spectrophotometric measurements described by BOWNE actually represent a transformation of hypoxanthine to adenine. Under the conditions used by BOWNE[2], radioactive C^{14}-hypoxanthine was incubated with non-radioactive adenine in ammonium phosphate, pH 6. If the reaction of hypoxanthine and adenine was to take place, the presence of radioactive adenine would be expected at the end of the reaction (Figure). However, the results of several experiments showed the absence of radioactive adenine in the incubated reaction mixture. No trace of radioactive adenine could be detected by a combined paper chromatographic and X-ray procedure nor could any substantial amount of radioactivity be found in the adenine isolated from the paper (Table).

Radioactive contaminants were found on the paper between the spots of adenine and hypoxanthine which were responsible for the small amount of radioactivity found in adenine. This view was further supported by the fact that the radioactivity of the base was considerably reduced when the adenine isolated from the paper was treated with charcoal Darco G-60 (Table). Taking into consideration the specific activity of the hypoxanthine used in the reaction mixture, the specific activity of the



Mechanism proposed by BOWNE[2] for the conversion of hypoxanthine to adenine as it would take place in the presence of C^{14}-hypoxanthine.

Specific activity

Components	μmoles counted	Radioactivity cpm	cpm/μmole[a]
Adenine	2.3	133	0.0[b]
Hypoxanthine	0.4	$5.5 \cdot 10^4$	$1.37 \cdot 10^5$
Paper control No. 1		147	
Paper control No. 2		124	
Adenine (after charcoal)	3.0	50	6
Hypoxanthine (after charcoal)	0.5	$6.9 \cdot 10^4$	$1.38 \cdot 10^5$
Background		33	

[a] Background corrected. [b] After correction for the amount of radioactivity present in the paper controls No. 1 and No. 2.

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