these compounds is the cause of the increase in the force of contraction or of other effects on the function of the myocardium. More probably, the functional and metabolic changes are both products of a cellular alteration the nature of which still remains to be disclosed.

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The Reduction of Triphenyltetrazolium Chloride by Dehydrogenases of Corn Embryos

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The fact that triphenyltetrazolium chloride is reduced to the red formazan by enzymes of viable tissue has been shown by several authors (*1–3*). Jerchel and Möhle (*4*) reported that the apparent redox potential (E'_0 at pH 7.0 of 2,3,5-triphenyltetrazolium chloride was about – 0.08 v. This fact suggested that the compound might be reduced through the catalytic action of certain pyridine nucleotide dehydrogenases, for the redox potentials of most of these enzyme systems fall below – 0.08 v. One of these systems, glucose dehydrogenase, was found to reduce the tetrazolium salt (*2*). Kun and Abood (*1*) reported that succinic dehydrogenase, which does not require a coenzyme, also reduced the tetrazolium compound at pH 7.4 and that the amount of formazan produced could be used as a measure of succinic dehydrogenase activity. The reduction by succinic dehydrogenase is unexpected, for the redox potential of the succinate/fumarate system is 0.00 v at pH 7.0. Fred and Knight (*5*) found that sodium malonate, sodium azide, 2,4-dinitrophenol, sodium fluoride, and iodoacetic acid at *M*/100 concentration merely slowed reduction of the salt by *Penicillium chrysogenum*. From this lack of specificity of inhibitors, the authors of the latter article concluded that a number of enzyme systems are able to reduce the dye.

We have investigated several enzyme systems, prepared from corn embryos, as to their ability to reduce the tetrazolium reagent. The apoenzyme mixture was prepared by the following method: Corn kernels were placed in water overnight, and the embryos were removed from the remainder of the kernel, extracted with acetone, dried, and ground with water. The extract was pressed out, centrifuged, and dialyzed. The solution was brought to pH 5.7, centrifuged, adjusted to pH 7.0, and saturated with ammonium sulfate. The precipitate was filtered, dried on a porous plate, and finally dried *in vacuo* over sulfuric acid. Diphosphopyridine nucleotide (DPN) was prepared by the method of Williamson and Green (*6*), and triphosphopyridine nucleotide (TPN) was prepared by the method of Adler, Elliot, and Elliot (*7*).

The ability of pyridine nucleotide enzyme systems to reduce the tetrazolium salt was determined by preparing solutions containing equal volumes of 1% apoenzyme preparation, 0.1% coenzyme, 0.5% tetrazolium salt, 4% substrate, and *M*/15 phosphate buffer (pH 6.6). The solutions were placed in the dark at room temperature and examined for color formation after 45 min.

TABLE 1

REDUCTION OF TRIPHENYLTETRAZOLIUM CHLORIDE BY CORN EMBRYO DEHYDROGENASES

Dehydrogenase System	Coenzyme	Substrate	Redox Potential at pH 7.0		Color Developed in 45 Min
Succinate/fumarate	....	Sodium succinate	0.00	Kalckar (1941)	None
Glutamate/α-ketoglutarate	DPN	Sodium glutamate	– 0.03	" "	"
Malate/oxalacetate	DPN	Sodium malate	– 0.102	Lehmann and Jorgensen (1939)	Pink
Ethyl alcohol/acetaldehyde	DPN	Ethyl alcohol	– 0.163	Kalckar (1941)	"
Lactate/pyruvate	DPN	Sodium lactate	– 0.18	Barron and Hastings (1934)	"
Glyceraldehyde-3-phosphate/1,3-diphosphoglycerate	DPN	*Sodium fructose diphosphate	– 0.28	Ochoa (1946)	"
β-Hydroxybutyrate/acetoacetate	DPN	Sodium-β-hydroxybutyrate	– 0.293	Hoff-Jørgensen (1938)	"
Isocitrate/oxalosuccinate	TPN	Sodium isocitrate	– 0.30	Ochoa (1946)	Pink (after 24 hr)
Glucose/gluconate	DPN	Glucose	– 0.45	" (1946)	Pink
α-Glycerophosphate/triosephosphate	DPN	Sodium-α-glycerophosphate			"

* Aldolase was assumed to be present.

The dehydrogenase systems shown in Table 1 were tested under aerobic conditions. Similar results were obtained when the solutions were placed in evacuated Thunberg tubes. When the enzyme system reduced the tetrazolium salt, the omission of enzyme, coenzyme, or substrate resulted in no color formation.

The following pyridine nucleotide dehydrogenases requiring DPN reduced the tetrazolium salt: glucose dehydrogenase, alcohol dehydrogenase, malic dehydro-

genase, β-hydroxybutyric dehydrogenase, lactic acid dehydrogenase, 3-phosphoglyceraldehyde dehydrogenase, and α-glycerophosphate dehydrogenase.

Triphenyltetrazolium chloride was also reduced by isocitric dehydrogenase, a pyridine nucleotide dehydrogenase requiring TPN, but the reduction was very slow as compared to the DPN enzyme systems.[1]

When sodium glutamate was employed as the substrate, no color developed, although glutamic dehydrogenase has been shown to be present in seeds (*8*). Since the redox potential of this dehydrogenase system has been reported as −0.03 v, it is possible that the enzyme, even though present, would not reduce the tetrazolium salt.

The addition of sodium succinate to the enzyme mixture prepared from corn embryos did not result in the reduction of the tetrazolium salt at pH 6.6. At this pH the redox potential of the succinate/fumarate system probably is above the redox potential of the tetrazolium salt, and a reaction should not be expected. However, the addition of coenzyme and sodium succinate to the enzyme preparation resulted in a reduction of the salt. Possibly this indicates that succinic dehydrogenase was present in the enzyme mixture and that the reduction of the tetrazolium salt only took place through the action of pyridine nucleotide systems which were one or more steps removed from the succinate reaction.

It is probable that the reduction of triphenyltetrazolium chloride by viable corn embryos can take place if one or more dehydrogenase systems with favorable redox potentials are present. The presence of active enzyme systems does not necessarily indicate seed viability, but the absence of active dehydrogenases probably indicates loss of germinating ability.

Because of the simplicity of the equipment and procedure, the elimination of the use of flavoprotein and cytochrome preparations, and the stability of the formazan, the use of triphenyltetrazolium chloride provides a convenient method for determining the presence of certain dehydrogenases.

[1] The dimethyl ester of isocitric lactone was kindly supplied by H. B. Vickery, of the Connecticut Agricultural Experiment Station.

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Comments and Communications

Paper Ionophoresis

IN A recent communication by McDonald, Urbin, and Williamson (*Science,* **112**, 227 [1950]), and experimental technique was described for the separation of various ions on filter paper strips saturated with electrolyte solutions across which an electric potential had been impressed. They also used this same method to determine mobilities of certain ions. It is certainly true that this method is, in many cases, capable of giving good ionic separations, as these authors have concluded. However, it is open to question whether the experimental conditions reported are satisfactory for determination of ion mobilities.

In a recent publication (*J. Am. Chem. Soc.,* **72**, 2943 [1950]) we pointed out that the progression of certain ions along filter paper under conditions essentially similar to those described by McDonald *et al.* was not a linear function of time. Although it is true that our experimental conditions were not exactly the same as those reported by McDonald *et al.,* all the essential elements upon which the following discussion bears were present in both studies. That is to say, electrolyte was fed continuously to the filter paper by capillary forces from both its ends, which were immersed in electrolyte supply vessels during the time a current flowed through the paper; the paper hung free in a surrounding enclosed vapor space; and the mixture to be separated was applied to the paper at an intermediate point.

It is manifest that, for any method to be suitable for ion mobility studies, the following minimum conditions be met:

1. Adsorption must not be a factor, or must be corrected for if present.
2. Electroendosmosis must be taken into account in cases where appreciable zeta potentials are to be anticipated.
3. The path through which the ion migration is observed must be uniform with respect to the applied electrical field, with respect to its ionic composition, and with respect to temperature.

None of these factors apparently has been given consideration in the paper under discussion.

With respect to point 1, it is conceded that some ions behave on paper saturated with electrolyte solutions in a manner similar to their behavior in "free solution." However, this factor is strictly dependent upon the system under consideration and, in certain instances in our own experience (e.g., with the dye acridine orange in certain electrolytes), strong adsorption may altogether preclude the application of this technique.

With respect to point 2, the authors make the statement that electroendosmotic flow did not appear to be