either the undifferentiated adenocarcinoma Eo771 or the myeloid leukemia C1498. Dosage of the compounds to be screened was arbitrarily set at 40% of the MLD/50 dose, except in cases where the animals reacted unfavorably to this amount.

Recently Skipper (*7*), working with Ak 4 mouse leukemia, reported that substitution of the 8-position of 2,6-diaminopurine eliminates its antileukemic activity. These results are confirmed by our data. Studies are now in progress with purine derivatives, similar to those in Table 1 (6–11), in the adenine, guanine, and isoguanine series.

References

1. FILDES. *Lancet*, **1**, 955 (1940).
2. STOWELL. *Symposia of the Society of Experimental Biology. I. Nucleic Acids*, 190 (1947).
3. HITCHINGS *et al. J. Biol. Chem.*, **174**, 765 (1948).
4. BURCHENAL *et al. Cancer*, **2**, 119 (1949).
5. ROBLIN *et al. J. Am. Chem. Soc.*, **67**, 290 (1945).
6. KIDDER *et al. Science*, **109**, 511 (1949).
7. SKIPPER *et al. Cancer Research*, **10**, 166 (1950).

Utilization of C^{14}-labeled Glucose by Cardiac Muscle Treated with a Cardiac Glycoside

Albert Wollenberger[1]

Department of Pharmacology,
Harvard Medical School, Boston, Massachusetts

Investigations in several laboratories have shown that cardiac muscle tissue exposed *in vitro* to a cardiac glycoside, or isolated following administration of the drug *in vivo*, respires at a markedly elevated rate (*1*). Because this increase in respiration is restricted to intact tissue and is a function of the concentration of appropriate exogenous substrate, it has been attributed to an increase in the rate of permeation of the substrate into the cell. This paper provides evidence that the phenomenon requires a different explanation.

Ouabain in a final concentration of 5×10^{-7} *M* was added simultaneously with C^{14}-labeled glucose to slices of dog myocardium respiring in substrate-free medium. The $C^{14}O_2$ evolved in the oxidation of the sugar was absorbed by the alkali in the center cup of the respirometer vessel. Table 1 shows that the stimulation of respiration by the cardiac glycoside was not accompanied by a greater uptake of glucose; on the contrary, somewhat less glucose was taken up than in the absence of the drug. However, the rate of glucose oxidation was doubled. According to calculations based on the data in Table 1, the fraction of glucose consumed which was oxidized to carbon dioxide and water rose from 16% to 38%. Comparison of the $Q_{C^{14}O_2}$ and Q_{CO_2}

[1] This work was supported by a grant from the Life Insurance Medical Research Fund. The author wishes to express his thanks to Robert E. Olson for a gift of glucose evenly labeled with carbon 14 and for his interest in the problem, and to Elizabeth White for measuring the radioactivity of the samples.

TABLE 1

EFFECT OF OUABAIN ON THE UTILIZATION OF C^{14}-LABELED GLUCOSE BY SLICES OF DOG VENTRICULAR CARDIAC MUSCLE

Treatment	$-Q_{glucose}$	$Q_{lactate}$	$Q_{keto\ acid}$	$Q_{C^{14}O_2}$	Q_{CO_2}	$-Q_{O_2}$
Control	3.03	1.61	0.07	2.97	7.80	8.33
5×10^{-7} *M* Ouabain	2.69	0.63	0.02	6.08	11.07	11.18

~150 mg of slices (wet wt) per vessel, shaken in 2.85 ml modified Krebs-Ringer-phosphate solution (*4*); O_2 atmosphere, 38° C. Glucose (16.1 micromoles) and ouabain added after 45 min; 2-hr incubation period in glucose. All quantities are expressed as µl ideal gas (N.T.P.) absorbed or evolved /mg dry tissue/hr.

values shows that the acceleration of cardiac respiration by ouabain is quantitatively accounted for by the increase in the rate of glucose oxidation.

Table 1 indicates further that lactic acid production by cardiac slices during aerobic incubation in glucose is greatly reduced by ouabain. The $Q_{lactate}$ declined from 1.61 to 0.63. The change in keto acid production was minor in comparison. Since endogenous lactate formation was nil, one can calculate from the data that the amount of glucose consumed which was converted to lactate decreased from 28% to 11%. And, since the decrease in lactate appearance was not accompanied by synthesis of glycogen,[2] it is also evident that most of the extra glucose oxidized under the influence of ouabain would, in the absence of drug, have been glycolyzed.

Suppression of aerobic glycolysis by ouabain has also been observed in smooth muscle (*2*). In brain cortex, on the other hand, which is the only animal tissue besides heart muscle that has been found to respond to cardiac glycosides with an increase in respiration, these drugs strongly stimulate aerobic glycolysis (*3*). The greater abundance of the Krebs cycle phorase enzymes relative to the glycolytic enzymes in heart muscle as compared to brain cortex may be responsible for the difference in the metabolic response of these two tissues to cardiac glycosides.

It has previously been reported (*4*) that in the presence of pyruvate, which increases the rate of oxygen consumption of cardiac slices to the level attained following addition of a cardiac glycoside in glucose or in lactate, the drug causes no further increase in metabolism. One may infer that in cardiac muscle the cardiac glycosides accelerate the formation of pyruvate from glucose and lactate. Experiments aimed to test this hypothesis are now in progress in this laboratory.

Little can be said at this stage concerning the significance of the present findings with regard to the action of the cardiac glycosides on cardiac function. As has been pointed out before (*4*), it seems unlikely that the stimulation of cardiac energy metabolism by

[2] The glycogen content of the slices declined rapidly during the preliminary incubation and remained uniformly very low (about 20 mg/1,100 g) during the experimental period.

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.

References

1. WOLLENBERGER, A. *Pharmacol. Rev.*, **1**, 311 (1949).
2. WERNER, G. *Arch. Intern. Pharmacodynamie*, **79**, 323 (1949).
3. WOLLENBERGER, A. *Federation Proc.*, **9**, 326 (1950).
4. ———. *J. Pharmacol. Exp. Therap.*, **91**, 39 (1947).

The Reduction of Triphenyltetrazolium Chloride by Dehydrogenases of Corn Embryos

C. O. Jensen, W. Sacks, and F. A. Baldauski

Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pennsylvania

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-