

conditions (for example, triphenyl tetrazolium chloride instead of TPN as electron acceptor).

The increase in activity of glucose-6-phosphate dehydrogenase is one of the most dramatic biochemical changes in dystrophic muscle which has been reported. A similar increase in the activities of other TPN-requiring dehydrogenases, although not so great, may indicate a general pattern of metabolic alteration in this tissue. This increased activity of the TPN-requiring dehydrogenases, coupled with a decreased activity of the DPN-requiring dehydrogenases, may produce abnormally high levels of reduced TPN or reduced glutathione and, thus, an altered intracellular metabolism. Tissue levels of the oxidized and reduced pyridine nucleotides and glutathione in control and dystrophic muscle are under investigation.

M. W. McCAMAN

*Institute of Psychiatric Research,
and Department of Neurology,
Indiana University Medical
Center, Indianapolis*

References

1. A. M. Michelson, E. S. Russell, P. J. Harman, *Proc. Natl. Acad. Sci. U.S.A.* **41**, 1079 (1955).
2. O. H. Lowry, *J. Histochem. and Cytochem.* **1**, 420 (1953).
3. H. L. Young, W. Young, I. S. Edelman, *Am. J. Physiol.* **197**, 487 (1959).
4. R. Kuhlman, O. H. Lowry, *J. Neurochem.* **1**, 173 (1956).
5. E. Racker, *Methods in Enzymology*, vol. 2 (Academic Press, New York, 1955), p. 722.
6. O. H. Lowry, in preparation.
7. O. H. Lowry, N. R. Roberts, J. I. Kappahn, *J. Biol. Chem.* **224**, 1047 (1957).
8. D. McDougal, R. Schimke, in preparation.
9. J. C. Dreyfus, G. Schapira, F. Schapira, J. Demos, *Clin. Chim. Acta* **1**, 434 (1956); N. Baker, M. Tubis, W. H. Bland, *Am. J. Physiol.* **193**, 525 (1958).
10. H. Rosenkrantz, *Federation Proc.* **18**, 312 (1959).

31 May 1960

Effect of 3-Amino-1,2,4-Triazole on the Synthesis of Riboflavin

Abstract. The production of riboflavin by *Eremothecium ashbyii* is appreciably reduced by 3-amino-1,2,4-triazole at concentrations of inhibitor which do not inhibit growth. Corn and pea leaf tissues which are albinistic as a consequence of treatment with this compound have a greatly lowered riboflavin content.

Studies in this laboratory (1) have shown that the inhibition of growth and chlorophyll development caused by 3-amino-1,2,4-triazole (3-AT) in the apex of tomato plants can be reversed if riboflavin and certain of its derivatives are supplied to the plant simultaneously with the inhibitor. This obser-

Table 1. Effect of 3-amino-1,2,4-triazole (3-AT) on mycelial weight and riboflavin production by *Eremothecium ashbyii*.

Concn. of 3-AT (M)	Mycelial wt. (mg [dry wt]/flask)	Riboflavin (mg/flask)
0	28.5	0.42
10 ⁻⁵	28.6	0.35
10 ⁻⁴	28.4	0.23
10 ⁻³	2.2	0.067

Table 2. Effect of 3-amino-1,2,4-triazole (3-AT) on the riboflavin content of pea and corn leaves.

Tissue	Riboflavin (μg/gm [fresh wt])	
	Control	3 × 10 ⁻⁴ M 3-AT
Pea	3.80	2.02
Pea	3.35	1.15
Corn	2.02	0.01
Corn	2.42	0.01

vation suggests that the phytotoxicity of 3-AT involves the inhibition of riboflavin synthesis. To substantiate this view the effect of 3-AT on the production of riboflavin by *Eremothecium ashbyii* and on the riboflavin content of pea and corn plants has been investigated (2).

For studies with the yeast 50 ml erlenmeyer flasks which contained 15 ml of Yaw's defined medium (3) were inoculated with cultures of *E. ashbyii* (NRRL No. Y 1363). The flasks were incubated at 25°C in a reciprocating shaker for 8 days. After incubation, the mycelial fragments were collected by filtration, dried at 80° to 100°C, and weighed. The riboflavin content of the filtrate was estimated from its optical density measured with a Klett-Summers spectrophotometer at a wavelength of 420 mμ.

The data of Table 1 indicate that 10⁻⁵, 10⁻⁴, and 10⁻³M 3-AT inhibit the production of riboflavin by 17.5, 46.2, and 83.2 percent, respectively. Growth was significantly inhibited only at the highest concentration. It is evident that at the appropriate concentration 3-AT inhibits the riboflavin synthesis but not the growth of *E. ashbyii*.

For studies with plants, pea and corn seedlings were germinated in the light in moist vermiculite and transferred, when about 3 inches tall, to beakers containing vermiculite which had been treated with a mineral nutrient solution. This solution included 3 × 10⁻⁴M 3-AT for the treated plants but none for the control plants. The plants were grown for an additional 3 days at 25° to 30°C with about 200 ft-ca of illumination from fluorescent lights until a moderate amount of newly developed albinistic tissue had appeared in the treated plants. Several samples of such

leaf tissue, each weighing about 1 gm, were collected from the treated plants for analysis. Comparable samples of green leaves were taken as controls from the untreated plants. The riboflavin content of this tissue was determined by a fluorimetric method (4).

From Table 2 it is evident that, in two different experiments, the albinistic pea tissue which developed subsequent to exposure of the plant to 3-AT had 55 and 33 percent of the riboflavin content of the control plant tissue. The reduction by 3-AT of the riboflavin content was even more striking for corn, for the riboflavin content of albinistic leaves from treated plants was practically zero.

The inhibition by 3-AT of riboflavin production by *E. ashbyii* and the deficiency of riboflavin in leaf tissues from treated plants is in accord with previous evidence (1) that the phytotoxicity of the inhibitor may be related to its inhibitory effect on riboflavin production.

KENNETH A. SUND*

HENRY N. LITTLE

*Department of Chemistry,
University of Massachusetts,
Amherst*

References and Notes

1. K. A. Sund, E. C. Putala, H. N. Little, *Agr. and Food Chem.* **8**, 210 (1960).
2. This work was supported by grants from the National Science Foundation (NSF G 4022) and the estate of Lotta Crabtree.
3. K. R. Yaw, dissertation, Yale University (1948).
4. K. Paech and M. V. Tracey, *Modern Methods of Plant Analysis* (Springer, Berlin, 1955), vol. 4, p. 645.

* Present address: Experiment Station, Hawaiian Sugar Planters' Association, Honolulu.

7 July 1960

Poliovirus Inhibitor from the Central Nervous System of the Rhesus Monkey

Abstract. Suspensions from the central nervous system of rhesus monkeys inhibited the infection of monkey kidney cell cultures by types 1, 2, and 3 poliovirus, whereas inhibition of Coxsackie A9 and ECHO 12 viruses could not be readily demonstrated. Failure of suspensions of tissues of the central nervous system to irreversibly neutralize poliovirus indicated that the inhibition was not directed against the virus but affected viral multiplication by altering the host cells.

In this laboratory, isolation in cell cultures of virulent poliovirus from the tissues of the central nervous system (CNS) of monkeys is an established procedure. During studies in which rhesus monkeys were inoculated intracerebrally and intraspinally with at-