gests that a low ceiling limits the number of avoidance-type responses in the animal's repertoire and in turn promotes the noninstrumental act of freezing. The resultant effect is a decrease in the probability of making the final shuttling response. The observation of relatively frequent freezing behavior with onset of buzzer in the low-ceiling groups supports this interpretation.

It further follows that to the extent a rat in the low-ceiling box learns the avoidance response, freezing must extinguish. This may account for the relatively better performance after a 1-hour delay for the low-ceiling rats. The 1-hour delay, according to the incubation-of-anxiety hypothesis, would, in general, increase the tendency to freeze, but less so in the low-ceiling conditions than in the high-ceiling because the animals in the high-ceiling box have not been freezing and thus the freezing response has had little opportunity to extinguish. Therefore, after 1 hour the rats in the high-ceiling box are at a disadvantage and show no improvement.

In contradistinction to the rats under low-ceiling conditions, rats under high-ceiling conditions can initially make more responses to the stimulus situation —leaping, jumping, standing, and such responses—that can readily chain in with the correct response. At first glance, the results pose a paradox. For by lowering the ceiling one might expect an increase in the specification of the correct response of shuttling. Instead it appears that a low ceiling promotes a response (freezing) which is incompatible with shuttling and incompatible with responses allied with shuttling.

The less important variable of length is more difficult to theorize about but may operate as follows. Under low-ceiling conditions the escaping rat can only run, which means it runs through shock to escape shock, being consistently punished during the early stages of making the correct response. Thus learning, despite the greater motivation for avoiding, tends to be poorer the longer the box (the greater the punishment). In high-ceiling boxes where escape is possible in a number of ways, this factor is not as critical, and the longer the alley the greater the motivation for avoiding.

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Dehydrogenase Activities in Dystrophic Mice

Abstract. The levels of activity of glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, glutathione reductase, lactic dehydrogenase, and α-glycerophosphate dehydrogenase have been studied in the gastrocnemius muscle of mice with "dystrophia muscularis." The activity of enzymes requiring triphosphopyridine nucleotide as a cofactor is increased relative to the control littermates, whereas the activity of those enzymes requiring diphosphopyridine nucleotide is decreased.

In 1955 Michelson *et al.* (*1*) described a hereditary disease in mice which is similar to human progressive muscular dystrophy. Since that time numerous investigators have attempted to determine whether or not this disease is related to a primary biochemical lesion. The present study is a report on the levels of activity of several dehydrogenases in the gastrocnemius muscle of mice with "dystrophia muscularis."

Dystrophic mice (genotype *dydy*) and control littermates (genotype *Dydy*) of the Bar Harbor strain 129 were used. At the time of sacrifice the gastrocnemius muscle was rapidly isolated, frozen in liquid nitrogen, and stored at −70°C. Transverse sections (15μ thick) were obtained and lyophilized at −30°C under vacuum, and the dry sections were stored at −20°C. At the time of analysis each transverse section (20 to 40 μg [dry weight]) was trimmed free of mounting material under a dissecting microscope, weighed on a quartz fiber balance, and placed in the appropriately sized test tube. Procedures for preparation and handling of the small pieces of tissue have been previously described (*2*).

The use of frozen-dried transverse sections of an entire muscle has several obvious advantages over the usual homogenate preparations. First, the stability of the preparation allows repeated analysis of the same tissue over a long period of time without deterioration of enzymatic activities or chemical constituents. Also, the morphology is preserved so that the extent of degenerative changes in each animal can be determined. Further, sampling errors due to a nonhomogeneous distribution of dystrophic fibers within the muscle are decreased. Whether or not the biochemistry of the "obviously dystrophic" fibers is markedly different from that of the "normal-appearing" fibers within an affected muscle, or whether the biochemical alterations are equally distributed among all of the fibers is a question for further study.

The present study confirms the fact that the lipid content of dystrophic muscle is increased (*3*). Therefore, the enzymatic results are based on the fat-free dry weight (*2*) determined separately for each muscle.

The activities of glucose-6-phosphate dehydrogenase (*4*), glutathione reductase (*5*), isocitric dehydrogenase (*6*), lactic dehydrogenase (*7*), and α-glycerophosphate dehydrogenase (*8*) were measured by the fluorescence of the oxidized or reduced pyridine nucleotides (*7*).

All of the enzymes requiring triphosphopyridine nucleotide (TPN) increased in activity in the muscle of dystrophic mice (Table 1). The activity of glucose-6-phosphate dehydrogenase was found to be approximately 400 percent ($P < .01$) of the value observed in the control littermates. Preliminary results indicate that 6-phosphogluconic dehydrogenase, the second step in the glucose-6-phosphate shunt, exhibits a similar increase in dystrophic mice. The activities of isocitric dehydrogenase and glutathione reductase also increased, and were approximately 180 percent ($P < .02$) and 170 percent ($P < .01$) of the control, respectively.

In contrast, the activities of the dehydrogenases requiring diphosphopyridine nucleotide (DPN) decreased in dystrophic mice. The lactic dehydrogenase and α-glycerophosphate dehydrogenase activities were approximately 75 percent ($P < .01$) and 50 percent ($P < .01$) of the control, respectively.

Previous reports (*9*) have indicated that the activities of the Krebs-cycle enzymes are not markedly altered in muscular dystrophy. However, the activity of isocitric dehydrogenase, a TPN-requiring dehydrogenase in this cycle, increased almost twofold here. Rosenkrantz (*10*) previously reported no change in the activity of this enzyme in control and dystrophic mice. The reason for this apparent discrepancy may be due in part to different assay

Table 1. Dehydrogenase activities in mouse muscle. Activities are expressed as micromoles of product formed per gram of fat-free dry weight per hour, except for those marked * which are expressed as millimoles per gram of fat-free dry weight per hour. Results were obtained on five samples from each animal. G-6-PDH, glucose-6-phosphate dehydrogenase; ICDH, isocitric dehydrogenase; Gl-R, glutathione reductase; α-GPDH, α-glycerophosphate dehydrogenase; LDH, lactic dehydrogenase.

Dehydrogenase	Control	Dystrophic
TPN-requiring:		
G-6-PDH (10)	100 ± 3	387 ± 30
ICDH (4)	393 ± 48	603 ± 62
Gl-R (4)	551 ± 25	819 ± 35
DPN-requiring:		
α-GPDH (2)	7930 ± 14	3210 ± 14
LDH (4)	168 ± 7*	131 ± 10*

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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.

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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 observation 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*).

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	$3 \times 10^{-4}M$ 3-AT
Pea	3.80	2.02
Pea	3.35	1.15
Corn	2.02	0.01
Corn	2.42	0.01

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-Summerson spectrophotometer at a wavelength of 420 mμ.

The data of Table 1 indicate that 10^{-5}, 10^{-4}, and $10^{-3}M$ 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 $3 \times 10^{-4}M$ 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.

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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-