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-

tenuated polioviruses, an unexpected low rate of virus isolation from the CNS of the inoculated monkeys was observed (*1*). Sabin and Goffe have reported similar inability to isolate attenuated polioviruses from monkey CNS (see *2*). Furthermore, when infected CNS tissues were plaque assayed on monkey kidney cell cultures more virus plaques appeared at higher dilutions of CNS tissue suspensions than at lower dilutions, strongly indicating virus inhibition. A study was therefore undertaken to determine the nature of the inhibition.

Ten-percent suspensions of lumbar or cervical cord or 30-percent suspensions of brain were prepared by vigorously shaking a thick-walled glass tube containing the CNS specimen, glass beads, and Earle's balanced salt solution at *p*H 7.5. Poliovirus isolations and plaque assays were performed as previously described (*1, 3*).

In previous studies, poliovirus was readily recovered from tissues of the CNS of monkeys which demonstrated positive CNS histopathology after inoculation with virulent virus (type 1, Mahoney strain) (*4*). In the present study, however, virus was recovered from the CNS of only 30 percent of monkeys in which histologic lesions of poliomyelitis were observed to follow intraspinal and intrathalamic injections of attenuated polioviruses. Table 1 shows the results of plaque assays of some of these CNS specimens infected with attenuated polioviruses. It may be seen that the higher concentrations of CNS tissue suspensions inhibited up to 900 plaque-forming units of poliovirus.

When attenuated poliovirus was added to suspensions of brain and spinal cord tissues from normal monkeys similar inhibition occurred, indicating that previous infection of the monkey was not necessary to produce the inhibition of virus multiplication in cell cultures.

Furthermore, virulent Mahoney strain of type 1 poliovirus, when mixed with brain or spinal cord suspensions, was inhibited to the same degree as the attenuated strains. This result gives rise to a seeming paradox: if the infection of monkey kidney cell cultures by virulent and avirulent poliovirus was inhibited to the same degree by CNS tissue suspensions, why was there such a disparity between the isolation rate of virulent and avirulent viruses from the CNS tissues of inoculated monkeys? A possible answer to the question may lie in the fact that virulent poliovirus when inoculated into the CNS of monkeys may multiply to a much greater degree than the avirulent types. Thus, when CNS tissue suspensions from monkeys inoculated with virulent virus are placed on monkey kidney cell cultures the higher concentration of virus may be capable of overcoming the inhibition. Some experimental support for this hypothesis comes from the work of Bodian (*5*).

Table 1. Poliovirus plaque assay of CNS tissue from infected monkeys.

Monkey number	CNS specimen	Dilutions of CNS	Plaque-forming units per 0.2 ml		
			Observed	Calculated titer in undiluted CNS	Deficit
		virus: Lederle-MEFL type 2 No. 7-1232-243			
V540	Cervical	1:10	6	60	94
	cord	1:100	7	700	3
		1:1000	1	1000	0
V538	Brain	1:3	6	18	194
	cortex	1:30	5	150	15
		1:300	2	600	0
V572	Lumbar	1:10	0	0	20
	cord	1:100	2	200	0
		1:1000	0	0	0
		virus: Sabin L Sc type 1 2ab			
V312	Lumbar	1:10	38	380	962
	cord	1:100	31	3100	69
		1:1000	10	10000	0

Table 2. Effect of monkey CNS tissue suspensions on the infectivity of three enteric viruses.

Inoculum	Plaque forming units per 0.4 ml		
	Type 1 poliovirus Mahoney strain	Coxsackie A9	ECHO 12
0.4 ml virus + 0.4 ml diluent	18	10	83
0.4 ml virus + 0.4 ml CNS suspension	3	18	66

To determine whether the inhibitor acted directly upon the poliovirus, CNS tissue suspensions or diluting fluid were each mixed with 1000 plaque-forming units of Mahoney strain poliovirus, incubated for 6 hours at 36°C, diluted 1:100 (beyond the level of inhibition) and plaque assayed. The same amount of virus was recovered from the CNS-virus mixture as from the control fluid-virus mixture indicating that the inhibition was not directed against the virus but may affect viral multiplication by altering the host cell.

When equal volumes of poliovirus types 1, 2, and 3 were each mixed with CNS tissue suspensions and then plaque assayed, all were inhibited to the same extent. In contrast Coxsackie A9 and ECHO 12 viruses were not significantly inhibited under the same conditions (Table 2), indicating some degree of specificity of inhibition. Other properties of the inhibitory material include inactivation at 56°C over 30 minutes, stability at —20°C for at least 4 months, inability to be dialyzed and ability to be sedimented at 1000 rev/min for 30 minutes. In addition, inhibition for poliovirus type 1 was not demonstrated in guinea pig brain.

Although a number of interpretations are possible, these data suggest that suspensions of rhesus monkey CNS tissues contain a material that is not an antibody to poliovirus and that inhibits poliovirus multiplication by acting on the host cell.

Studies are now in progress to determine the mechanism of action of the inhibitor, its properties, its relationship to previously described viral inhibitors and its significance in the pathogenesis of infection (*6*).

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