Data presented in Fig. 1 indicate that the drug inhibited production of RSV foci. A concentration of 18.4 μg/ml of amantadine HCl elicited 50 percent viral inhibition. Although the results were less consistent than with RSV, regression curves obtained for ESV also show activity against the virus (Fig. 1). Approximately 11.3 μg/ml (range 4.8 to 17.7) of the compound caused 50 percent viral inhibition. The slopes of the regression curves were different in experiments ESV 3, 4, and 5 as compared with ESV 1 and 2 (Fig. 1), but in every instance the influence of amantadine was evident.

Table 1. Effect of amantadine HCl on suspensions of Rous sarcoma (RSV) and Esh sarcoma (ESV) viruses at 37°C for 2½ hours.

Virus	Amantadine HCl (μg/ml)	Focus forming units (per ml)
RSV	6.25	6.2×10^4
	12.50	6.5×10^4
	25.00	7.1×10^4
	50.00	6.1×10^4
	0	6.4×10^4
	0*	7.6×10^4
ESV	6.25	2.6×10^3
	12.50	2.8×10^3
	25.00	2.5×10^3
	50.00	2.3×10^3
	0	2.9×10^3
	0*	2.2×10^3

* 2°C for 2½ hours.

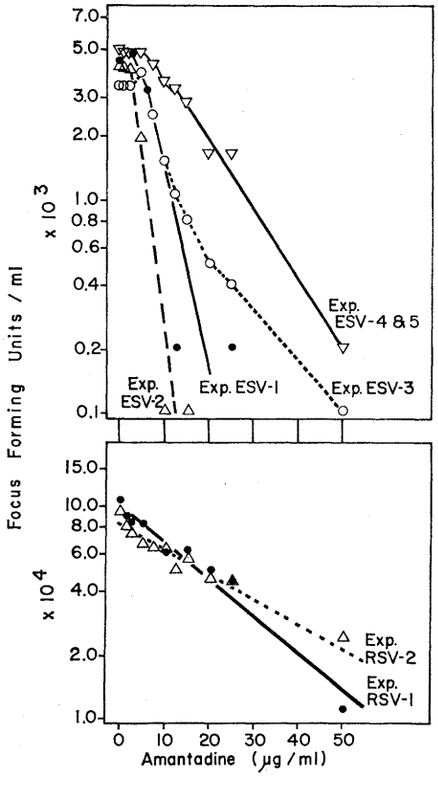

Fig. 1. Number of foci per milliliter produced by Rous sarcoma virus (RSV) and Esh sarcoma virus (ESV) in chick embryo fibroblasts in the presence of amantadine HCl. The points were identical for experiments ESV 4 and 5.

In each experiment with RSV and ESV, cells grown in the various concentrations of amantadine HCl were compared with both normal and virus-infected cells. Occasionally at drug concentrations of 25 μg/ml, and more often at 50 μg/ml, it was difficult to count the foci. This difference from the usual appearance of foci suggests that chick cells were affected in some manner by the compound without being rendered completely impregnable to virus invasion.

To eliminate the possibility of preventing viral replication by direct toxic effect of amantadine on chick cells, Newcastle disease virus (NDV), GB strain, a member of the myxoviruses known to be resistant to amantadine (*2*), was employed to infect secondary chick embryo cells treated with this compound at concentrations of 25, 50, and 100 μg/ml. The agar overlay also contained the amantadine. Cells and media used in this test were similar to those used in tests with tumor viruses. Control cells and amantadine-treated cells gave the same titer with NDV.

A virucidal effect was ruled out by adding amantadine to a viral suspension. Data in Table 1 show that amantidine HCl, at the concentrations used in these studies, was not virucidal to either RSV or ESV in suspension. There is good evidence to show that amantadine acts by blocking the penetration of virus into the cell (*3*).

Previous work indicates that amantadine elicits no effect on RSV pock-production on chorioallantoic membranes (*2*) or in a cell culture–drug screening system (*7*) and is ineffective against Friend virus in mice (*8*). The special significance of our results is that this is the first evidence that amantadine inhibits oncogenic viruses.

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Cytochemical Localization of Lactate Dehydrogenase in Muscular Dystrophy of the Mouse

Abstract. *By use of phenazine methosulfate and the "incubation mixture film method," lactate dehydrogenase activity has been demonstrated in the dystrophic muscle fibers of strain 129 mice. The results indicate that for demonstration of lactate dehydrogenase activity in dystrophic muscle fibers phenazine methosulfate is necessary. This finding is typical for the "white" muscle fibers in the normal muscle and suggests that the dystrophy affects primarily the "white" muscle fibers.*

The biochemical lesion in the striated muscles of dystrophic mice has been the subject of histochemical and biochemical study. The contents of lactate dehydrogenase (LDH) and of α-glycerophosphate dehydrogenase (linked to nicotinamide adenine dinucleotide, NAD) in the muscles of such strain 129 mice have been reported to decrease to about 75 and 50 percent of the control values, respectively (*1*). Subsequent histochemical studies, however, have not confirmed these biochemical findings (*2*).

Investigation of cytochemical localization of LDH in skeletal muscle (*3*) is complicated by the diffusion of LDH from tissue sections into the aqueous incubation media and also by the dependence on diaphorase activity of the final electron transfer from NADH to the tetrazolium salts. An "incubation mixture film" technique (*3*) has overcome the problem of enzyme diffusion, and the use of phenazine methosulfate

as an intermediate electron carrier has made the staining system independent of NADH-diaphorase. In our study this modified method for demonstration of LDH was applied to the dystrophic muscles of the strain 129 mouse.

Vastus lateralis and rectus femoris muscles were obtained from adult, homozygous (*dydy*) strain 129 dystrophic mice (Jackson Memorial Laboratory, Bar Harbor, Maine) and their heterozygous (*Dydy*) littermates. The animals were killed when about 5 weeks old, and the clinical signs of dystrophy served to differentiate the dystrophic animals from their littermate controls. The freezing, sectioning, and staining for LDH have been described (*3*). All sections were fixed briefly in acetone to avoid the interference of phenazine methosulfate with the staining of "red" muscle fibers (*3, 4*). Serial sections from the same blocks were stained with and without the methosulfate.

A few sections were counterstained with the Feulgen method for demonstration of nuclei. Control sections were stained in the same manner, but the specific substrate, lactate, and/or the coenzyme (NAD^+) were omitted from the incubation medium.

In the absence of phenazine methosulfate, muscles of the control nondystrophic mice (*Dydy*) stain for LDH more prominently in the small "red" fibers than in the larger "white" fibers (Fig. 1). In the presence of the phenazine methosulfate both fiber types stain with about the same intensity and a fine reticular network is visualized in the sarcoplasm of the muscle fibers (Fig. 2). In dystrophic muscles, in the absence of the methosulfate, the staining appears quite regular, and there is a tendency toward uniform staining of different types of muscle fibers (Fig. 3). Most of the fibers which stain well in the absence of the methosulfate have a normal histological appearance at this phase of the disease. In contrast the serial sections from the same material stained in the presence of the methosulfate reveal many fibers with small and large diameters (Fig. 4). When the latter sections were counterstained by the Feulgen method, centrally located nuclei were found in many of these abnormal fibers (Fig. 4, arrow). At higher magnification these same fibers also showed foci in the sarcoplasm that stained prominently (Fig. 5). These characteristics correspond to the morphologic descriptions of dystrophic muscle fibers in the mouse (*5*).

The control preparations stained in the absence of the substrate, lactate, and/or the coenzyme (NAD^+), showed

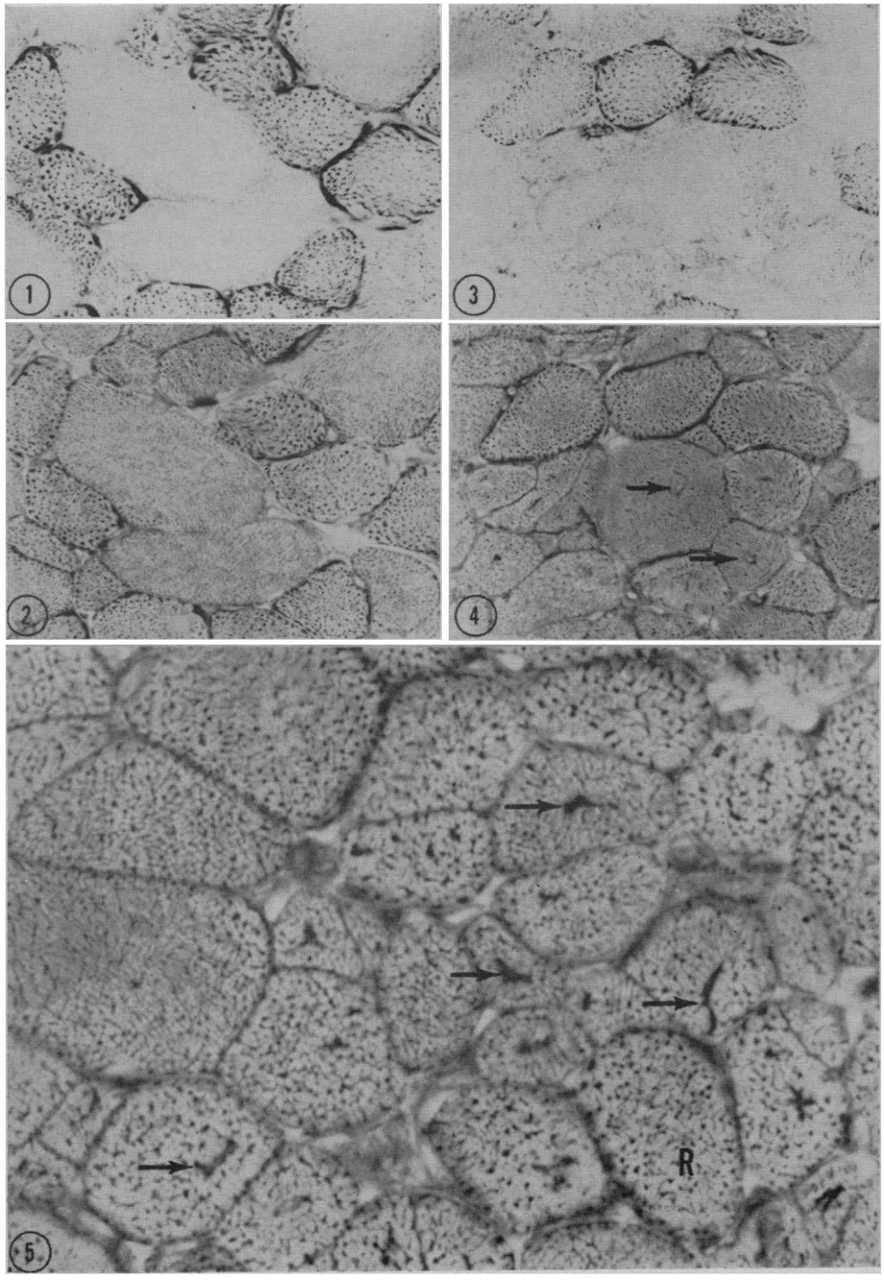

Fig. 1. Normal muscle from nondystrophic mice stained in the absence of phenazine methosulfate. "Red" muscle fibers stain prominently for LDH activity, while the "white" fibers remain unstained (× 350).

Fig. 2. Same field as in Fig. 1 from a serial section stained in the presence of phenazine methosulfate. "White" fibers stain, and all fibers show a reticular pattern in their sarcoplasm (× 350).

Fig. 3. Dystrophic muscle stained in the absence of phenazine methosulfate. Only "red" fibers are stained, and these appear to be normal (× 350).

Fig. 4. Same field as in Fig. 3 from a serial section stained in the presence of phenazine methosulfate. Many of the fibers which are now stained are either atrophic or hypertrophic with some nuclei (arrows) centrally located (× 350).

Fig. 5. Dystrophic muscle stained in the presence of phenazine methosulfate. By comparing this section with serial sections stained in the absence of methosulfate only the fiber labeled "R" stained. All other fibers in this field were visualized only when the methosulfate was added to the incubation medium, indicating that they are of the "white" type. Most of these "white" fibers appear abnormal and some show foci (arrows) of prominent staining in their sarcoplasm (× 600).

only a trace of a diffuse and generalized staining after prolonged incubation. Control experiments, confirming the specificity of the cytochemical method used herein for LDH have been described (*6*).

With phenazine methosulfate, and the application of the "incubation mixture film" method, LDH activity is demonstrable in dystrophic muscle fibers. In the absence of the methosulfate, dystrophic fibers remain unstained or stain only faintly. This may explain the failure of Fennel and West (*2*), who did not use this agent, to observe marked differences in the staining of dystrophic and normal muscles.

Phenazine methosulfate transfers the electrons from NADH to nitro blue tetrazolium and thus can substitute for the low content of endogenous NADH-diaphorase in "white" muscle fibers (*3, 6*). This essential role of the methosulfate in revealing cytochemically the LDH activity in normal "white" muscle fibers has been demonstrated previously (*3*) and also here in Figs. 1 and 2. It may therefore, be assumed that in the dystrophic muscles (Figs. 3 and 4) the majority of dystrophic fibers, which stain only in the presence of phenazine methosulfate, are of the "white" type. "White" fibers depend primarily on glycolysis for their energy metabolism (*7*), and if they were the fibers primarily involved in muscular dystrophy, the glycolytic activity would be expected to be affected in the dystrophic muscles. McCaman (*1*) has presented biochemical evidence of such an effect on glycolysis in dystrophic muscles.

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Antarctic Asteroid Odontaster validus: Constancy of Reproductive Periodicities

Abstract. *The fact that samples of* Odontaster validus *from the Balleny Islands (67°S) and Robertson Bay (71°S) closely resembled reproductively samples taken from McMurdo Sound (77°S) indicates reproductive synchrony in this species over much or all of their circumcontinental antarctic distribution. This synchrony suggests that the reproductive periodicities of* O. validus *are both adapted to and synchronized by the summer period of phytoproduction and that neither light nor temperature changes have any direct synchronizing role.*

The reproductive periodicities of the omnivorous antarctic asteroid *Odontaster validus* Koehler in two populations at McMurdo Sound (77°S, 166°E) have been worked out in detail (*1*). The populations were in reproductive synchrony, the beginning of gametogenesis (differentiation of spermatocytes or oocytes from gonial cells) occurring between May and March, with a peak of activity between August and January. Oocyte growth to full growth required between 18 and 24 months, while the differentiation of spermatozoa from spermatocytes required about 9 months. Spawning occurred during midwinter and late winter (June to mid-September). These periodicities probably result from adaptations to ensure a summer food supply for the slowly developing demersal bipinnaria larvae.

The main difference between the two populations was related to difference in amounts of available summer plant food. Different ice conditions resulted in much more phytoproduction at one locality, Cape Evans, than at the other, McMurdo Station, and the animals at Cape Evans produced about twice as many gametes as did those at McMurdo Station. Although food in quantitative terms did not effect the timing of reproduction, it was still considered likely that the marked seasonality of phytoproduction could synchronize the reproductive periodicities.

A sample taken in October from 12 m at Hallett Station (72°S, 170°E), 560 km north of McMurdo Sound, was in reproductive synchrony with the McMurdo Sound samples. Difference in light between Hallett Station and McMurdo Sound, as well as similar difference between Cape Evans and McMurdo Station, made photoperiodic control of the reproduction of *O. validus* very unlikely, but more observations were needed, especially from more northerly locations.

Slight but definite seasonal changes in sea temperatures were noted in McMurdo Sound. Because of the importance of changes in sea temperature to reproductive periodicities of north-temperate species, and because of the apparent unimportance of photoperiods and quantitative food differences, it was suggested that the slight seasonal changes in sea temperature might be important in the synchronization of reproduction in *O. validus*.

On 12 February 1965, eight specimens of *O. validus* were dredged from 274 m off Young Island, Balleny Islands (66°37′S, 162°38′E), nearly 1200 km north of McMurdo Sound. Three specimens were also dredged on 27 January 1965 from 274 m in Robertson Bay (71°24′S, 170°10′E), near Hallett Station. The gonads of these specimens were fixed for histologic preparation, as the McMurdo Sound samples had been treated. The reproductive conditions of all gonads were very similar to those collected from less than 40 m in McMurdo Sound during late January and February 1961. Three of the specimens from the Balleny Islands and two of those from Robertson Bay were males; their testes were filled with growing spermatogenic papillae containing mostly spermatids and spermatocytes, like comparable 1961 samples from McMurdo Sound. The females in both samples had two size groups of oocytes: one below 75 μ in diameter, with a mean diameter of about 30 μ; the other ranging between about 80 and 130 μ in diameter. These oocyte size frequencies were almost identical with those in comparable 1961 samples from McMurdo Sound (Fig. 1).

The similarity among the samples from the Balleny Islands, Robertson Bay, and McMurdo Sound in late January and February, as well as among the earlier October samples from Hallett Station and McMurdo Sound, indicates that reproduction in individuals of *O. validus* is in synchrony throughout much or all of their circumcontinental antarctic distribution. Such synchrony is not surprising because the winter breeding sea-