the submicron aerosol was produced in the same manner as the larger aerosol.

Explosive volcanoes, such as St. Augustine, probably account for most of the aerosol emitted into the atmosphere from volcanoes (*6*). Total worldwide volcanic emissions have been estimated to be 25 to 150 $\times$ 10^6 metric tons per year for aerosol less than 40 μm in diameter (*7*). On the basis of the data presented here, we deduce that these total annual emission rates are equivalent to about 80 to 500 eruptions of the type that occurred at St. Augustine on 8 February. Since 57 eruptions of various intensities occurred from St. Augustine alone between 23 January and 14 February 1976 (*8*), it appears that the worldwide emissions of particles from volcanoes may have been underestimated.

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9. This report is dedicated to the memory of our late pilot, R. D. Spurling, whose untimely death occurred shortly after this research was completed. This research was supported by grants ATM-74-14726-A02 (Atmospheric Research Section, Meteorology Branch) and EAR76-15392 (Earth Sciences Section) of the National Science Foundation. Special thanks are due to Drs. William E. Benson and Frank H. Eden of the National Science Foundation; without their prompt action the results reported here would not have been obtained. Contribution No. 383 from the Atmospheric Sciences Department, University of Washington.

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Avian Muscular Dystrophy: Functional and Biochemical Improvement with Diphenylhydantoin

Abstract. *Chicks affected with hereditary muscular dystrophy were injected twice daily with 20 milligrams of diphenylhydantoin per kilogram of body weight on days 1 to 40 after hatching. The righting ability of dystrophic chicks treated with diphenylhydantoin was improved compared to that of untreated dystrophic chicks, and acetylcholinesterase activity was reduced to normal levels in the posterior latissimus dorsi muscles.*

Unlike a normal chicken, one affected with hereditary muscular dystrophy cannot right itself after being placed on its back (*1*). Although many biochemical and morphological abnormalities have been demonstrated in fast-twitch muscles of dystrophic chickens (*2*), the physiological basis of the righting difficulty is unknown. One possibility is that skeletal muscle rigidity, such as that found in myotonia congenita (*3*), prevents successful righting. This hypothesis is supported by the facts that dystrophic chicken muscle fibers have increased transmembrane resistance (*4*) and continue to fire abnormally in response to mechanical stimulation after neuromuscular blockade by *d*-tubocurarine (*5*). Both of these electrophysiological abnormalities are also found in myotonic mammalian muscle fibers (*6*). One might then expect an improvement in righting ability of dystrophic chickens after administration of diphenylhydantoin (DPH), a clinically useful antimyotonic drug (*7*). We have found that DPH dramatically improves the righting ability of dystrophic chicks and partially corrects abnormalities of acetylcholinesterase (AChE) (E.C. 3.1.1.7) and fiber morphology in posterior latissimus dorsi (PLD) muscles of dystrophic chicks.

The study was begun with 1-day-old New Hampshire chicks from normal line 412 and the related, homozygous dystrophic line 413 (*8*). Four groups of chicks were used. Group 1 included normal, untreated chicks; group 2, dystrophic, untreated chicks; group 3, dystrophic, exercised chicks; and group 4, dystrophic, DPH-treated, exercised chicks. Chicks were injected intraperitoneally with either diluent (group 3), or DPH, 20 mg/kg (group 4), twice daily at 12-hour intervals (*9*). The dose of DPH (20 mg/kg) was calculated from the mean body weight of chicks in group 4. The concentration of the stock DPH solution was increased as needed to deliver the 20 mg/kg dose in an injection volume of 0.1 to 0.25 ml. Chicks in groups 3 and 4 were "exercised" immediately before each injection by placing each chick on its back until it could no longer right itself. The number of consecutive times a chick could right itself was termed the exhaustion score. Exhaustion scores of chicks in groups 3 and 4 were determined just before the second daily injection on days 1 to 40 after hatching. Exhaustion scores of untreated chicks in groups 1 and 2 were determined at 5-day intervals in order to minimize the effects of exercise. Student's *t*-test was used to determine statistically significant differences in mean exhaustion scores.

Table 1 shows that untreated normal and dystrophic chicks differed greatly in their exhaustion scores from days 13 to 35 after hatching (*10*). Chicks in group 1 (normal) righted themselves an average of 19.5 times during each test period, whereas chicks in group 2 (dystrophic) averaged only 1.8 times. Injections of DPH had a dramatic effect on exhaustion scores. Chicks in group 4 (DPH-treated, exercised) had scores that were not statistically different ($P > .1$) from those of group 1. Chicks in group 3 (exercise alone) also showed improved exhaustion scores, but the mean score for this group was significantly lower ($P < .001$) than that for group 4. Regardless of how exhaustion scores were averaged (that is, whether as means of all chicks in a group at a specific test period or as means of single chicks for the entire experimental program) only the chicks in group 4 performed as well as normal chicks. In another experiment dystrophic chicks injected with DPH, but not exercised, had

Table 1. Effects of DPH and exercise on exhaustion scores of normal (line 412) and dystrophic (line 413) chicks. Test periods indicate the number of times each chick was exercised to exhaustion during days 13 to 35 after hatching. Exhaustion scores are grand means (± standard deviation) of the average score for each bird in the group. The number of birds per group is shown in parentheses.

Line	Group	Test periods	Exhaustion scores
412	1	5	19.5 ± 1.9 (12)*
413	2	5	1.8 ± 2.0 (16)†
413	3 (exercise)	46	8.9 ± 2.8 (15)*†
413	4 (exercise plus DPH)	46	20.3 ± 3.8 (11)*

*Statistically different from group 2 ($P < .01$). †Statistically different from group 1 ($P < .01$).

a mean exhaustion score of 15.5. This value was not statistically different ($P > .1$) from those of untreated normal chicks and DPH-treated, exercised dystrophic chicks.

The difference in exhaustion scores between chicks given exercise alone and chicks given DPH plus exercise is emphasized when the results are plotted as a function of age of the chicks (Fig. 1). Exhaustion scores of groups 1 and 2 were similar during the first week, but after this time the chicks in group 1 became progressively less able to right themselves (Fig. 1A). Chicks in group 3 had higher exhaustion scores than did those in group 2, particularly at days 18 to 20, but their scores were never as high as those in group 1 and they progressively decreased with time (Fig. 1B). In contrast, DPH-treated chicks had essentially normal scores from days 13 to 35 (Fig. 1B).

To determine whether characteristic abnormalities of dystrophic muscle had been altered by DPH, we obtained biochemical, histochemical, and histological data from PLD muscles of chicks aged 27 to 41 days. One PLD from each bird was homogenized for spectrophotometric determinations of lactic dehydrogenase (LDH) (*11*) and AChE (*12*) activities. The contralateral PLD was frozen in liquid nitrogen–cooled isopentane, and sectioned on a cryostat. The sections were stained histochemically for AChE localization (*13*) and with hematoxylin and eosin for gross morphology.

Table 2. Activities of LDH (*11*) and AChE (*12*) in PLD muscles from normal (line 412) and dystrophic (line 413) chicks on days 27 to 41 after hatching. Activities were determined as changes in absorbance per gram (wet weight) of muscle per minute. Values are means ± standard deviation. Numbers of muscles are shown in parentheses.

Line	Group	Activity (g/min)	
		LDH	AChE
412	1	1909 ± 297 (3)	1.37 ± 0.35 (3)
413	2	1023 ± 128 (4)	4.94 ± 3.12 (6)
413	3 (exercise)	*2150 ± 384 (4)	5.45 ± 2.82 (4)
413	4 (exercise plus DPH)	*1643 ± 427 (4)	*1.40 ± 0.54 (6)

* Not statistically different ($P > .4$) from group 1 and statistically different ($P < .05$) from group 2.

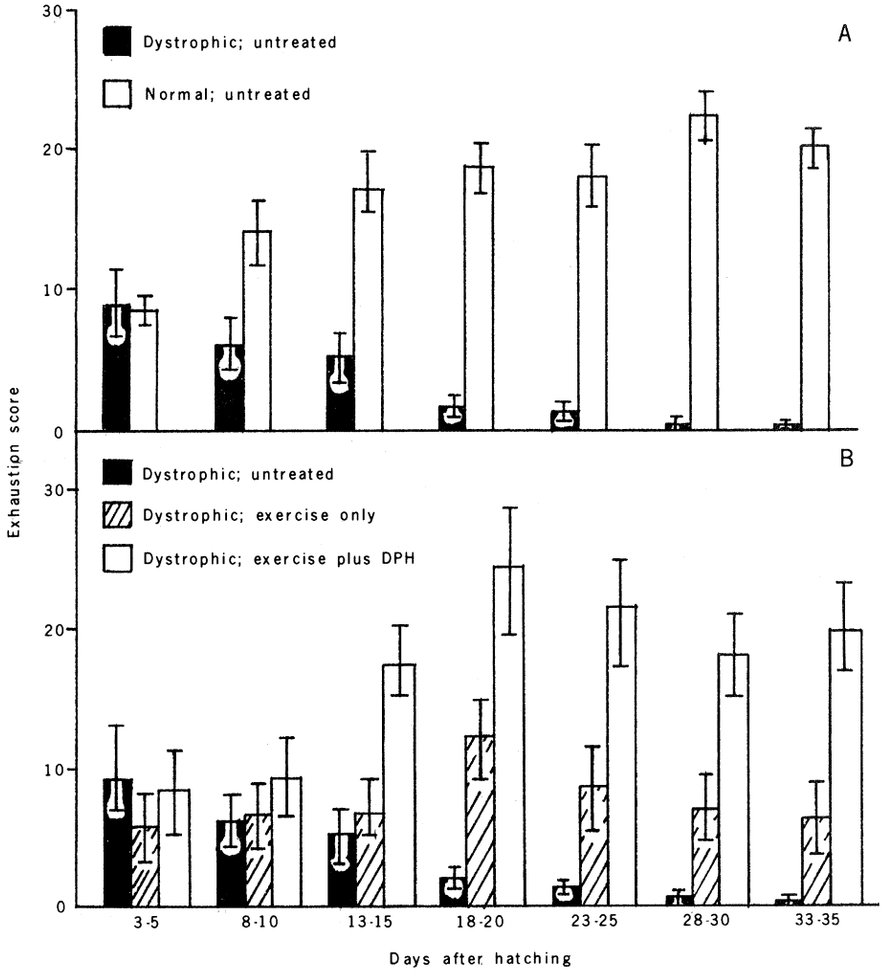

Fig. 1. Mean exhaustion scores plotted against age of chicks. Scores were obtained on days indicated. Each bar represents 11 to 16 chicks. Vertical lines on each bar are standard errors of group mean scores.

As shown previously (*2*), dystrophic muscles had lower LDH and higher AChE activities than did normal muscles (Table 2). The amount of LDH activity was increased in muscles from chicks both of group 3 and of group 4, whereas AChE activity was reduced to normal levels only in muscles from chicks in group 4 (Table 2). The high variability in AChE activity of untreated dystrophic muscles reflects the large increase in AChE levels from days 27 to 41. Histochemically, AChE activity is observed in the sarcoplasm and around the nuclei in dystrophic muscle fibers and is not localized to motor end plates as in normal muscle fibers (*14*). Fibers from chicks in group 3 had high sarcoplasmic and nuclear AChE activity. In contrast, fibers from chicks in group 4 had low sarcoplasmic AChE activity, although perinuclear staining was still higher than in normal fibers. Treatment with DPH plus exercise, but not exercise alone, also improved the gross morphology of dystrophic PLD muscles. Muscles from chicks in group 4 had more tightly packed fibers and less interfiber connective tissue than those from chicks in groups 2 and 3.

The results show that the combination of DPH and moderate exercise corrects several abnormalities associated with inherited dystrophy of the chicken, and that exercise alone produces transient improvement in righting ability. The mechanism of action of DPH in dystrophy of the chicken and in myotonia of humans is unknown. Treatment with DPH does alter the membrane fluidity of erythrocytes from patients with myotonic dystrophy (*15*), and it has a stablizing effect on neuronal membranes (*16*). Penicillamine (*17*) and the serotonin antagonists, methysergide and cyproheptadine (*18*), also improve righting ability and enzyme activities of dystrophic chickens. Whether or not these chemically dissimilar drugs exert their actions at the same site in the dystrophic chicken is a matter for future study.

Our data do not indicate whether DPH will produce long-term alleviation of the symptoms of dystrophy of the chicken.

They do emphasize the need to determine the relation between the electrophysiological abnormalities of the dystrophic muscle fiber membrane and the biochemical abnormalities of dystrophic muscles.

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9. The DPH (Dilantin, Parke-Davis) was administered in a diluent of 40 percent propylene glycol and 10 percent alcohol in water adjusted to *p*H 12. The dose of DPH, high by comparison to the typical human dose [see (*7*)], was chosen on the basis of preliminary trials with 50 chicks in which a single injection of 20 mg of DPH per kilogram produced rapid improvement in righting ability with no behavioral signs of involvement of the central nervous system.
10. This time period was chosen because exhaustion scores on days 1 to 12 after hatching were not sufficiently different to distinguish among the four groups (see Fig. 1).
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Effect of Delta-9-Tetrahydrocannabinol on Uterine and Vaginal Cytology of Ovariectomized Rats

Abstract. *The estrogen-like activity of delta-9-tetrahydrocannabinol (Δ^9-THC), an active component of marihuana, as measured by uterine weight gain and vaginal smear techniques in ovariectomized rats, is reflected in histological examination of uterine and vaginal tissues. Doses of 1, 2.5, and 10 milligrams of Δ^9-THC per kilogram elicit hypertrophy and hyperplasia of the uterus; the dose of 2.5 milligrams per kilogram is most effective. There is an increase in stratification of vaginal epithelium with doses of 2.5 and 10 milligrams per kilogram; cornifying cells are seen with 2.5 milligrams per kilogram.*

A recent report (*1*) demonstrates that administration of delta-9-tetrahydrocannabinol (Δ^9-THC), one of the major components of marihuana, in doses within ranges used in human male studies (*2*), has significant estrogenic activity in ovariectomized rats as measured by uterine weight gain and vaginal smear bioassay techniques. We now report the results of histological examination of tissues obtained in the earlier study (*1*) from ovariectomized rats treated with Δ^9-THC. Cytologic studies of these tissues show that Δ^9-THC induces hyperplasia and hypertrophy of the uterus. Doses of 2.5 and 10 mg of Δ^9-THC per kilogram elicit an increase in the number of cell layers in the vaginal epithelium; cornifying cells are seen at the 2.5 mg/kg dose.

The effects of estradiol benzoate (EB) or Δ^9-THC, or both, were studied in ovariectomized rats by using the following parameters: the uterine weight gain and vaginal smear analyses for estrogens (*3, 4*) and histologic examination of uterus and vagina (*5*). Estrogen dosage was based on requirements for estrogen in spayed rats (*3, 4*). The doses of Δ^9-THC were based on our preliminary studies.

At 75 days of age, 55 female rats of the Charles River (CD) strain were bilaterally ovariectomized by removing the coiled fallopian tubes and ovarian capsule with the surrounding fat to assure complete removal of ovarian tissue. The animals were grouped so that the average body weight of each group was between 184 and 189 g, and treated as follows: group 1, sham ovariectomized rats treated intraperitoneally with saline to serve as controls for ovariectomy. Groups 2 to 5, ovariectomized rats treated with either sesame oil intraperitoneally or subcutaneously, or with saline intraperitoneally or subcutaneously to serve as controls for injection trauma, vehicle, and route of vehicle administration. The experimental groups were: group 6, EB, 2 μg/kg per day; groups 7 to 9, Δ^9-THC, 1, 2.5, or 10 mg/kg per day, respectively; group 10, EB (2 μg/kg per day) plus Δ^9-THC (10 mg/kg per day). Treatment with 0.2 ml of the appropriate compound was begun on the day of operation and continued daily for 14 days. Estradiol benzoate and Δ^9-THC were administered in sesame oil according to common usage: estrogens administered subcutaneously (*3, 4, 6*), Δ^9-THC administered intraperitoneally (*7*). Daily vaginal smears were obtained according to the method described by Zarrow *et al.* (*3*), and the animals were weighed twice weekly.

At the termination of the experiment, on the 14th day, the animals were killed by guillotine and examined for complete removal of both ovaries. Reproductive tissue, from the vagina to the tip of the uterine horn, was excised. Uteri were removed, stripped of adhering fat and connective tissue, and weighed. The tissues were fixed in Carnoy's solution (*8*). Paraffin sections were cut at 5 μm and stained with hematoxylin and eosin (*8*). Step sections from comparable levels of mid-horn and vagina of each group were examined. Vaginal smears were coded, and read independently by two individuals.

Histologic examination of mid-horn sections obtained from control rats treated intraperitoneally with sesame oil (Fig. 1) show the effects of ovariectomy in the rat: the uterus is small and poorly

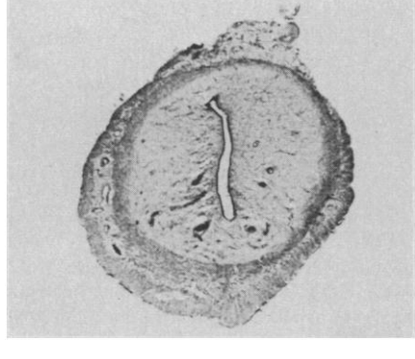

Fig. 1. Uterus of control ovariectomized rat. The organ is small and poorly vascularized. The lumen is slit-like, and the surface epithelium, the endometrium, and the myometrium are atrophied. Endometrial glands are collapsed (×22).