Previous research with the dba mammary tumor used in this study has provided both direct and indirect evidence for the tumor agent or virus concept of tumor etiology(6-9). It is suggested that the present results contribute to the same hypothesis. The protection afforded the embryo against vaccinia by the implanted tumor could have been due to the well-known blocking effect which one virus may have on another.

Summary. 1. Yolk sac implants of a dba mouse mammary tumor protected the host embryos against vaccinia virus. A dosage of the virus which killed embryos of nontumor-bearing eggs in 3-4 days gave no evidence of toxicity in the embryos bearing the dba tumor 7 days after inoculation. Tests demonstrated that the virus was present in the fluids of these embryos. 2. Comparable tests with eggs bearing a C_3H mammary tumor indicated the embryos were only slightly protected against the virus, and yolk sac implants of a rat sarcoma gave negative results in this respect. 3. None of the 3 tumors affected the toxicity of a strain of influenza. 4. It is suggested that the dba tumor's protective effect on the embryos was due to the neutralization of the vaccinia virus by the tumor agent.

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Effect of Fatigue on Susceptibility of Mice to Poliomyelitis. (20456)

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It is often observed that the paralysis of poliomyelitis is preceded by a period of exhausting physical exercise and the belief is prevalent that fatigue increases susceptibility to paralysis in persons already infected. Statistical studies(1-6) tend to confirm these clinical impressions but can be accepted only with reservations. For example, the use of non-paralytic cases for control(4) is subject to considerable error because of the difficulty in making an accurate clinical diagnosis in the absence of paralysis(7). Variables inherent in estimation of the degree of paralysis make judgment difficult on this basis alone(1-3). In one series, fatigue was associated with an increased incidence of paralysis in adults, but no significant difference was found in children(5). The association of increased paralysis with the distance of transportation of patients(6) may be due to other factors than fatigue.

To our knowledge, there has been only one previous report concerning the susceptibility of fatigued animals to experimental poliomyelitis(8). In these experiments, fatigue was induced in monkeys by forcing the animals to swim until exhausted. However, under these conditions it was difficult to distinguish between the effects of fatigue and chilling and use of monkeys limited the numbers of animals in each experiment. In our experiments, fatigue was induced by a technic that avoided immersion in water and the availability of mice permitted the use of relatively larger numbers of animals.

Materials and methods. In the beginning,

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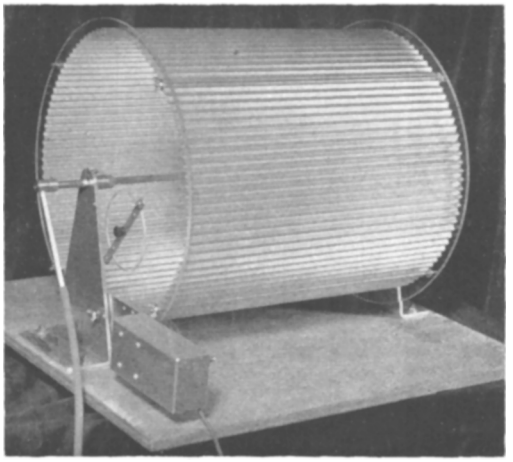

FIG. 1. Apparatus for inducing fatigue in mice. Dimensions of drum are 18 × 48 in. Corrugations are ⅜ in. wide. Ends of the drum are enclosed with plates of plexiglass ⅜ in. thick. Axle consists of a hollow tube of stainless steel with perforations to allow escape of air. Inlet for air is through a movable adaptor at one end. The drum is friction driven by contact between the edge of one plexiglass plate and a rubber stopper mounted on the drive shaft of a small motor. Rate of rotation is approximately 4 revolutions per min.

there was much difficulty devising an apparatus suitable for the purpose of inducing fatigue in mice. After a number of trials, the apparatus illustrated in Fig. 1 was developed. A drum of corrugated stainless steel was closed at both ends by discs of plexiglass, mounted on an axle, and rotated by a motor. Mice within the drum were forced to walk or run constantly since it was impossible for them to hold to the corrugated steel. For ventilation, a stream of compressed air was introduced into the drum through perforations in the hollow stainless steel axle. By this technic, mice could be exercised for many days at a constant rate and in such a way that there was no doubt of severe fatigue and yet the mice were not traumatized and did not die. After long periods of uninterrupted exercise, complete fatigue occurred and was recognized by the fact that the mice would roll in the drum instead of walking or running. In the experiments, the degree of fatigue was not allowed to reach this complete stage. The Lansing strain of poliomyelitis virus† was used in all the experiments. In preparation of each stock suspension of virus, the spinal cords and medullae of several mice showing paralysis were ground in a mortar with enough tryptose phosphate broth to make a 10% suspension. After centrifugation and culture for sterility, aliquots of the supernatant fluid were stored in a dry ice box and further dilution in broth was carried out on each aliquot just before use. Intracerebral injection of 10-fold dilutions into mice gave 50% infectivity titers of 10^{-4} to $10^{-4.5}$. The mice were of a single strain (CF1, Carworth Farms) and all mice of each experiment were young females of the same shipment.

In each experiment, the mice were divided into 2 groups. Mice of one group were exercised for 7 hours in the fatigue apparatus and immediately thereafter were injected intracerebrally under ether anesthesia with 0.03 ml of a dilution of viral suspension. Non-exercised mice in the other group serving as controls were injected at the same time with the same dilution of virus. Beginning the day after injection and continuing daily for a period of 14 days, the test groups of animals were exercised in the fatigue apparatus for a period of 8 hours. All animals were observed twice daily for paralysis over a period of 30 days. Animals in exercised groups showing paralysis were removed from the apparatus and not subjected to further exercise. Nevertheless, all such animals died. Mice found dead less than 48 hours after inoculation were thought to have died of trauma and were not included in the results. Mice found dead more than 48 hours after inoculation were considered to have died of viral infection even though paralysis had not been observed. Statistical analysis was carried out by the χ^2 method with a 4-fold table(9). In the experiments indicated, the Yates correction for small numbers was employed.‡

Results. Seven experiments were done in which a total of 223 mice were subjected to exercise and 328 mice were kept as controls. Viral dilutions were 1:5000 or 1:10,000. The incidence of paralysis and the fatalities in these experiments is shown in Table I and Fig. 2. It can be seen that larger numbers of

† Kindly furnished by Dr. Jonas Salk.

‡ Advice concerning the statistical method was kindly given by Dr. Goldine Glaser, statistician of the Department of Neuropsychiatry.

TABLE I. Effect of Exercise on Incidence and Incubation Period of Experimental Poliomyelitis in Mice. 7 exp.

Dilution of virus	Exercised: Injected	Exercised: Paralyzed	Exercised: Died*	Exercised: Mean time of survival, days	Control: Injected	Control: Paralyzed	Control: Died*	Control: Mean time of survival, days	Chi square†	P
1: 5000	31	28	28	11.7	35	20	26	13.3	2.60‡	.1
1: "	29	22	22	13.4	33	15	15	16.3	5.95	.02
1: "	32	26	26	15.3	35	18	21	15.4	3.70	.05
1: "	35	21	21	20.2	35	15	16	17.2	1.43	.2
1: "	25	24	24	12.0	66	49	53	10.7	2.26‡	.1
1:10000	31	17	17	13.2	64	28	28	17.0	1.02	.3
1: "	40	23	23	18.1	60	23	27	18.5	1.50	.2
	223	161	161		328	168	186		18.46	.02

* Includes all paralyzed mice and sometimes a few more that died without paralysis being observed.
† Based on number of deaths 2 to 30 days after inj.
‡ Yates correction for small numbers was used in these experiments.

exercised mice became paralyzed and died than did the control animals. The differences in each experiment were not great and, considered individually, the results of 5 of the 7 experiments were not within the range of statistical significance. However, the results were consistent and the accumulated values of χ^2 indicate that the observations as a whole are not likely to be due to chance.

The mean survival times of mice dying within 30 days are also shown in Table I. Since the differences in survival times between exercised and control animals were not considered significant, further details have not been recorded.

Discussion. It is important to note in these experiments that the differences between test and control groups were consistent. The difference, while small in the majority of the individual experiments, was statistically significant when the observations were considered as a whole. We believe, therefore, that the results are valid and that the small differences observed reflect the true influence of fatigue under these conditions.

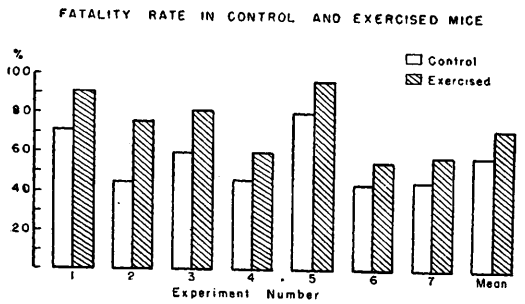

FIG. 2.

The mechanism by which fatigue increases susceptibility to poliomyelitis is not known nor does it appear likely that evidence concerning the underlying processes can be obtained from statistical studies of human beings(10). However, the reproduction of this phenomenon in mice offers a model in which experiments concerning the nature of the increased susceptibility can be carried out. The possibility exists that exercise affects the adrenal cortex causing increase or decrease of hormones which in turn influence susceptibility to this viral infection. Supporting this concept are reports that administration of adrenal or pituitary hormones to experimental animals may increase their susceptibility to experimental infection with poliomyelitis virus (11-15).§

Summary. In order to determine the effect of fatigue on susceptibility to experimental poliomyelitis, mice were forced to run in a revolving drum during the day preceding and for 8 hours each day subsequent to the intra-

§ Because of reports indicating that fatigue of nerve cells is associated with depletion of Nissl substance(16-19), an attempt was made to correlate this phenomenon with increased susceptibility to infection with poliomyelitis virus. Paraffin sections were prepared of spinal cords of normal and fatigued mice and stains for Nissl substance were done carefully by several methods. However, the amounts of Nissl substance in the nerve cells of a single section were found to vary so greatly that no conclusions could be drawn as to its depletion in fatigued mice.

cerebral injection of the Lansing strain of virus. Control animals inoculated at the same time with the same amount of virus were allowed to rest in their cages. In all 7 experiments, the incidence of the disease as measured both by paralysis and by death was greater in the exercised animals than in the resting controls.

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Oral Administration of Co^{60} Vit. B_{12} in Tropical Sprue and Hepatic Cirrhosis and Diarrhea.* (20457)

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Smith reported that in the stools of rats fed Co^{60} vit. B_{12} the radioactivity is present in a conjugated or degraded form of the vitamin but not as ionized cobalt(1). Rosenblum and his coworkers regarded this as unlikely and concluded that vit. B_{12} is destroyed in the feces, probably by bacterial action(2). Barbee and Johnson, by means of radioautographs of paper strip chromatograms, showed that the feces of rats contained considerable ionic Co^{60} after oral administration of the labelled vitamin, and that all the radioactivity in the feces after injection is in this form(3).

The present study was undertaken to determine fecal excretion of radioactive cobalt following oral administration of Co^{60} vit. B_{12}

TABLE I. Radioactivity of Stools Following Oral Administration of 0.5 μg of Co^{60} Vit. B_{12} to 4 Normal Subjects.

Patient	Age	Total fecal excretion of Co^{60} (%)
D.	26	78
O.	30	31
R.	30	39
S.	33	50

* Radioactive vit. B_{12} was supplied by Merck and Co., Rahway, N. J. Expenses for these studies were partially defrayed by grants from the Squibb Institute for Medical Research, New Brunswick, N. J. and the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.