Vitamin D Intake and Susceptibility of Mice to Experimental Swine Influenza Virus Infection.*† (17545)

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(Introduced by Jerome T. Syverton)

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Considerable interest has been manifested in recent years in the influence of nutrition on experimental infection. Reviews on the subject have been written by Aycock and Lutman,(1) Schneider,(2) and more recently by Clark *et al.*(3) More specifically related to the results presented in this paper is the work of Sprunt(4) who demonstrated that mice, maintained on low protein diets, showed, after injection with methionine, an increased susceptibility to swine influenza virus infection. Similarly, Midzuno(5) was able to produce influenza in rats by maintaining them on diets deficient in vitamin D. He also carried out similar experiments with avitaminotic D mice. In this paper results are presented which indicate that mice fed diets low in vitamin D are more susceptible to experimental swine influenza virus infection than are mice that receive vitamin D. These findings confirm those reported by Midzuno.(5)

Materials and methods. Experimental animals. The mice were obtained by 3 successive matings of 75 females and 35 males of second generation stock derived from Bittner's ZBC strain(6) with a commercial strain of Swiss mice. The breeding colony was maintained on Purina Fox Checkers and water. To assure uniformity in the ages of the test mice, all breedings for each replicate of the experiment were carried out within a period of 7 days. Mice were weaned at 21 days, and then placed on the experimental diets for 28 days. Thus, at the time of inoculation with swine influenza virus, the mice were at least 49 days old and not more than 56 days old.

Experimental diets. The basal diet was prepared by hand mixing all the ingredients listed in Table I, except the brewers' yeast, which was added later as the vehicle for vitamin D. The source of vitamin D was Fleischman's FIDY yeast type 9F containing 9000 U.S.P. units of vitamin D per gram. It was premixed with Fleischman's 2019 dry brewers' yeast. The desired levels of vitamin D were obtained by adding different quantities of the mixture to the diets. For the first experiment, the following amounts of vitamin D were incorporated into the diets: 0, 2.5, 5, 10, 50, 100, 250, 500 and 1000 U.S.P. units per 100 g of feed. In the second replicate experiment, 0, 10, 50, 100 and 500 U.S.P. units per 100 g of feed were used and in the third replicate experiment, 0, 2.5, 5, 10 and 100 units were fed. The diets were fed for 38 days including the 10 days following intranasal instillation of swine influenza virus into 593 test mice. Controls consisted of 123 mice also kept on the different diets for 38 days.

Mouse infectivity. The test virus was swine influenza virus (Shope 15) adapted to both eggs and mice. The mice were infected intranasally with 0.05 cc of a virus suspension

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5. Midzuno, H., *Nagoya Igakkai Zasshi*, 1941, v53, 655, through *Chem. Abst.*, 1948, v42, 963. We have been unable to secure the original paper as it was among the journals held by the Central Intelligence Agency of the Washington Document Center that were in process of being distributed to government libraries in Washington.

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TABLE I.
Basal Experimental Diet.

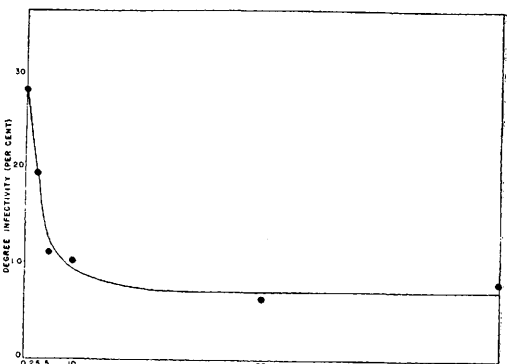
Corn starch			72%
Casein (Borden 52 edible)			18%
Corn oil			4%
Salts			4%
$CaHPO_4$	46.3%	$FeSO_4$	2.10
$CaCO_3$	16.9	KI_4	.22
NaCl	13.5	ZnO	.04
$MgCO_3$	8.5	$CuSO_{14}$	.02
KCl	6.7	$MnCl_2$	.02
KH_2PO_4	5.7		
Vitamins			Trace
Vit. A	900 U.S.P. units	100 g diet	
Choline chloride	200 mg	,,	
Inositol	21.6 mg	,,	
Pantothenic acid	4.4 mg	,,	
Vit. E	4.0 mg	,,	
Niacin	4.0 mg	,,	
Paraminobenzoic acid	4.0 mg	,,	
Riboflavin	1.6 mg	,,	
Thiamine hydrochloride	0.8 mg	,,	
Pyridoxine hydrochloride	0.8 mg	,,	
Brewers' yeast			2%
			100%

diluted in buffered saline to approximately the minimum infective $dose_{50}$. By the method of Reed and Muench,(7) the dilution for the ID_{50} was found to be $10^{5.4}$ for mice on a diet containing 100 U.S.P. units of vitamin D. In the first and third experiments, a dilution of $10^{5.7}$ of allantoic fluid was used, obtained from embryonated eggs harvested 48 hours after inoculation with 10^3 dilution of stock virus. The dilution of virus used for infecting mice was determined by preliminary tests on a small number of mice on a diet containing 100 U.S.P. units of vitamin D. In the second experiment, the preliminary virus titration was misleading, resulting in a $10^{7.0}$ dilution for inoculation of the mice.

The method for determining the extent of mouse infectivity was essentially that of Horsfall(8) and of Lauffer and Miller.(9) Mice dying of influenza within the 10-day test period were each given a score of 4, and those that showed consolidation of ¾, ½, ¼, or a trace of the lung area at autopsy on the 10th day were given scores of 3, 2, 1, and 0.5, respectively. The final positive scores differed from those of Lauffer and Miller in that the total score for each test group was divided by four times the number of mice rather than by the total number of mice. This variation made it possible to represent infectivity as a percental number of the maximal possible score that would result from death of all inoculated mice.

Results. Fig. 1 shows graphically the relative reactivity of the mice which had been maintained on graded doses of vitamin D to experimental infection with swine influenza virus. This curve, based on the infection of 593 mice, indicates clearly that mice fed for 28 days on a diet containing essentially no vitamin D were more susceptible to experimental infection than mice receiving some vitamin D in their diet. As little as 2.5 U.S.P. units of vitamin D per 100 g of diet afforded definite protection, and 5.0 units gave nearly maximal protection. The differences in relative infectivity of mice on the O diet as compared to those on the 2.5 diet, and the mice on the 2.5 diet as compared to those on the 5.0 diet were significant ($\chi^2 = 9.36$ and 7.74, df = 1, P less than 1%). There were no significant differences between the other adjacent points on Fig. 1. Points for 250, 500, and 1000 units of vitamin D are not shown on the curve, as they would only extend the curve parallel to the abscissa.

The control mice which were fed the same



DIFFERENCES IN SUSCEPTIBILITY OF MICE TO SWINE INFLUENZA INFECTION WHEN FED VARIED LEVELS OF VITAMIN D

FIG. 1.

7. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

8. Horsfall, F. L., Jr., *J. Exp. Med.*, 1939, v70, 209.

9. Lauffer, Max A., and Miller, Gail Lorenz, *J. Exp. Med.*, 1944, v79, 197.

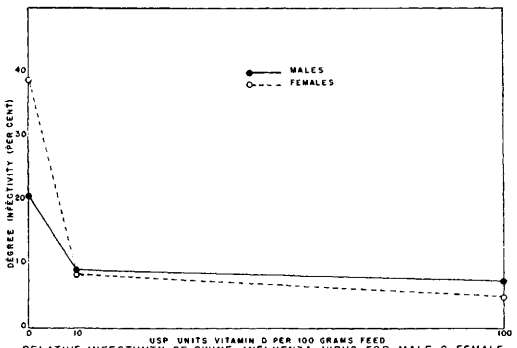


FIG. 2.

graded amounts of vitamin D as the test animals but which were given no virus failed to show evidence of pulmonary consolidation, although the lungs of mice on the lower levels of vitamin D appeared hyperemic. Evidence for vitamin D deficiency was not observed. The mean weight for all mice at the time of weaning was 9.3 g and at the time of infection 22.4 g, the gain in weight having been progressive for all mice to the time of inoculation.

The evidence presented graphically in Fig. 2 shows that susceptibility to infection reflects a sexual difference, as well as a relationship to the dosage of vitamin D. The females were more susceptible than males on diets with no added vitamin D. The differences between males and females on diets containing 10 and 100 U.S.P. units of vitamin D were negligible. Statistically, females on an avitaminotic D diet were significantly more susceptible than males on the same diet, or than females on a diet containing 10 units of vitamin D per 100 g of feed ($\chi^2 = 12.6$ and 43.4, df $= 1$, P less than 0.1%). Likewise males on the diet containing no added vitamin D were more susceptible than males on the 10 units diet ($\chi^2 = 8.6$, df $= 1$, P less than 1%).

Summary. Experimental evidence is presented which indicates that mice fed diets containing little or no vitamin D for 28 days after weaning showed increased susceptibility to experimental swine influenza virus infection. Females were more susceptible than males.

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Histochemical Localization of True Lipase.* (17546)

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In the course of experiments on the histochemical specificity of esterases (lipases) 35 different water-soluble substrates† were tried. Chemically, the substrates were long-chained (C_{12} to C_{18}) fatty acid esters of polyglycols or of sorbitan in which the remaining hydroxyl groups were etherified with ethylene oxide chains of various lengths. The fatty acids of these compounds were mostly saturated (lauric, palmitic, and stearic); however, a number of them contained unsaturated acids, either practically exclusively (undecylenic, oleic, and ricinoleic) or predominantly (fatty acids of linseed oil).

Experimental. Tissues of several species (man, dog, cat, guinea pig, rabbit, rhesus monkey, rat, mouse, and pigeon) were used. They were fixed in chilled acetone and embedded through acetone, alcohol, alcohol-ether, 4% collodion in alcohol-ether (12 hr) and 2 changes of chloroform, 1 hr each, in paraffin. As many as 20 different tissues were included in a single paraffin block. The in-

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