older embryos proved unsuccessful.

Summary. Observations are reported on the infectivity of the murine SK strain for mice based on certain types of exposure to the virus. Some of the infected mice were embedded *in toto,* sectioned, and examined histologically for evidence of virus multiplication in non-nervous as well as nervous tissue. Although infections occurred readily under various types of exposures to the virus, no lesions suggestive of virus activity were observed in any tissues or organs apart from the CNS. Infections by the intranasal route could not be prevented by prior intranasal instillations of zinc sulphate solution used to block the olfactory pathway as a portal of entry. Mice could be readily infected with virus-contaminated drinking water. Virus cultivated in developing eggs proved less infectious for mice when supplied to the animals in drinking water or food than mouse brain-cord virus suspensions. Old mice showed themselves less susceptible than young mice to certain types of exposure, but to other types of exposure the difference was less marked. Young mice could be readily infected by housing them in battery jars contaminated with virus, but it required a heavy contamination to bring this about. While a virus suspension dropped into the conjunctiva after light scarification of the cornea induced infection in 70% of mice, only 10% infection resulted when this procedure was not preceded by scarification of the cornea. Infection was induced in 60% of mice by applying the virus to the skin and then introducing it by the multiple puncture method. Infection was induced in 30% of mice on introducing the virus into the rectum. Young mice can be easily infected by the intraperitoneal route, this route being only slightly less sensitive than the intracranial route. The average incubation period following intravenous inoculation did not differ significantly from that observed following inoculations by other routes with similar amounts of virus. The virus induced a typical poliomyelitic syndrome in guinea pigs. In these animals it became a low titer virus. It proved infectious for hamsters but not for rabbits or monkeys. Attempts to adapt it to baby chicks were unsuccessful.

16453 P

Effect of Desoxypyridoxine on Reproduction in the Rat.*

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It has recently been possible to show that an upset in the reproductive mechanism is quickly produced when normal adult female rats are shifted to purified diets deficient in pantothenic acid[1] or in pteroylglutamic acid.[2] The same purified diets in which the missing vitamins were present enabled normal reproduction to be evidenced. Such results induced inquiry as to the rapidity with which reproductive impairment might be evidenced by shifting similar healthy adult female rats to similar purified diets from which pyri-

* Aided by grants from the Board of Research and from the Department of Agriculture of the University of California, and the Rockefeller Foundation, New York City. The following materials were generously contributed: synthetic desoxypyridoxine, α-tocopherol, 2-methyl-1,4-naphthoquinone, and crystalline B vitamins from Merck and Company, Rahway, N. J.; synthetic d-biotin from Hoffman-La Roche, Inc., Nutley, N. J.; and synthetic pteroylglutamic acid from Lederle Laboratories, Inc., Pearl River, N. Y.

1 Nelson, M. M., and Evans, H. M., *J. Nutrition,* 1946, **31**, 497.

2 Nelson, M. M., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 289.

doxine had been withdrawn. Preliminary studies, however, on pyridoxine deficiency started the day of breeding or one to two months prior to breeding showed only slightly adverse effects and, furthermore, the addition of succinylsulfathiazole to repress intestinal synthesis did not result in greater reproductive impairment. Recently, there has become available a very potent inhibitor of pyridoxine, "desoxypyridoxine" (2,4-dimethyl-3-hydroxy-5-hydroxy-methylpyridine) which is an antagonist to pyridoxine for the chick,[3] the rat[4,5] and the mouse.[6]

In the present communication we wish to report the reproductive behavior of normal adult female rats placed either on the day of breeding or shortly before it, on a pyridoxine-deficient diet to which desoxypyridoxine had been added.

Experimental. Normal female rats (Long-Evans strain), 3 months of age, were bred with normal males and placed on the pyridoxine-deficient diet† containing 0.5 mg % desoxypyridoxine on the day of breeding. Other groups of rats were maintained on this diet for 10, 15, or 20 days and then bred as rapidly as possible. Control rats were given the identical diet supplemented with 0.5 mg % pyridoxine on the day of breeding after

TABLE I.
Reproduction of Rats Maintained on Purified Diets Containing Desoxypyridoxine (0.5 mg %).

Day exp. diets started	No. of rats bred	Failed implantations %	Resorptions of implantations %	Litters of implantations %	Total No. young	Young born dead %	Avg size of litter	Avg wt of young, g
Pyridoxine supplemented: Day of breeding*	10	0	0	100	93	0	9.3 (6-16)	5.6
Pyridoxine deficient: Day of breeding	21	0	10	90	143	3	7.5 (3-11)	5.0
12 days before breeding	11	0	36	64	34	0	5.7 (2-9)	5.2
16 " "	12	0	75	25	19	21	6.3 (5-8)	4.6
22 " "	7	29	100	0				

* These rats were given the pyridoxine-deficient diet containing 0.5 mg % desoxypyridoxine 14 days (11-22) previous to breeding.

[3] Ott, W. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 125.

[4] Porter, C. C., Clark, I., and Silber, R. H., *J. Biol. Chem.*, 1947, **167**, 573.

[5] Emerson, G. A., *Fed. Proc.*, 1947, **6**, 406.

[6] Stoerk, H. C., *Fed. Proc.*, 1948, **7**, 281.

† The pyridoxine-deficient diet containing the antagonist was composed of 24% alcohol-extracted casein, 64% sucrose, 8% hydrogenated cottonseed oil (Crisco), 4% salts No. 4, and 0.5 mg % desoxypyridoxine. Crystalline vitamins per kg diet were: 300 μg d-biotin, 5 mg 2-methyl-1,4-naphthoquinone, 5.5 mg synthetic pteroylglutamic acid, 5 mg thiamine HCl, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg nicotinic acid or amide, 50 mg calcium pantothenate, 400 mg inositol, and 1.0 g choline chloride. 5 mg pyridoxine HCl per kilo (0.5 mg %) was added to the diet for the control group. Each rat received weekly a fat-soluble vitamin mixture containing 800 U.S.P. units vitamin A, 115 chick units vitamin D, 6 mg synthetic α-tocopherol, and 650 mg corn oil (Mazola).

being maintained on the deficient diet containing the antagonist for 10 to 20 days. Vaginal smears were examined daily during gestation for the presence of erythrocytes, the sign that implantation has occurred; all rats were weighed at regular intervals.

Results. Table I shows that placing adult rats the day of breeding on the pyridoxine-deficient diet containing the antagonist resulted in a slight interference with reproduction, evidenced principally by the occurrence of resorptions in 10% of the group. When the rats were placed on this diet 10 to 20 days prior to breeding marked upsets in reproduction were observed. The percentage of resorptions increased directly with extension of the deficiency period, *i.e.* 36% of the rats resorbed after 12 days (11-13) of deficiency prior to breeding, 75% for those averaging 16 days (14-22) deficiency prior to breeding, and 100% of the rats showing the implantation sign, resorbed when the deficiency was extended to 22 days (18-27) before breeding. In the last group it may be noted that a failure of implantation also occurred to a significant extent (29%). The percentage of young born dead was markedly increased (*i.e.* 21%) in the group with 16 days deficiency prior to breeding and a significant decrease in the average weight of the young at birth was also observed. The tendency toward a decreased number of young per litter and decreased weight of the young at birth may be noted in all deficient groups. The normal reproductive behavior of rats supplemented with pyridoxine the day of breeding after 14 days (11-22) of deficiency previous to breeding, showed that the vitamin counteracted all the deleterious effects of the antagonist.

This study adds another dietary condition to those previously found to cause resorption in the adult female rat.[1,2] The rapidity with which the reproductive mechanism is disturbed in this experiment is similar to that previously found in the case of pantothenic acid deficiency. To be noted is the early appearance of the erythrocyte sign frequently observed in this study—on the 9th, 10th, and 11th days of gestation instead of the normal 13th, 14th and 15th days. In the rats not bred, there was an early suppression of the oestrous cycle despite the maintenance of body weight and an early appearance of the characteristic acrodynia on the paws (in 15 days for many rats), indicating the acuteness of the deficiency produced by the antagonist and the importance of pyridoxine for these functions.

Summary. The addition of the pyridoxine antagonist, desoxypyridoxine, to a pyridoxine-deficient diet resulted in marked reproductive upsets when normal adult female rats were placed on the diet only 10 to 20 days prior to breeding. Supplementation with pyridoxine on the day of breeding counteracted the adverse effects of the antagonist.

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Morphology of *Hemobartonella muris* as Revealed by Darkfield Microscopy.*

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During the course of studies on *Hemobartonella muris*, we have had occasion to observe the morphology of this organism with the aid of the darkfield microscope. Darkfield preparations of peripheral blood, made at the height of anemia in the rat, have been compared with blood smears made simultaneously and stained with one of the Romanowsky methods.

* Aided by a grant from the John and Mary R. Markle Foundation.