tion of the changes during healing in the organic matrix. To integrate these observations with present knowledge, a working hypothesis must take into account enzyme action, hydrogen ion concentration, and the concentrations of ions such as calcium, carbonate, and phosphate as well as other electrolytes participating in the transformation of the organic matrix. Further studies are indicated and are in progress. Because of the difficulties involved in obtaining large quantities of healing callus, the identification of the acid mucopolysaccharide is incomplete.

Summary. Chemical and histochemical observations concentrate on a mucopolysaccharide which appears to be important in bone healing.

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Effect of Dietary Restriction on Susceptibility of Mice to Infection with Theiler's GDVII Virus.* (17488)

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That nutritional deficiencies are frequently accompanied by anorexia is well known. Numerous reports(1-7) have been published which concern the relation of nutritional inadequacies both to bacterial and to viral infections, but the evidence as to whether or not the course of experimental infections is influenced by caloric restriction, or by restriction of an adequate diet, inanition, seems equivocal. Different infective agents, different host species, genetic status of host and of pathogen, different routes of infection, and many other important variables frequently make direct comparison impossible and analysis of conflicting results exceedingly difficult. The experiments here reported, query further the relative effects of reduced food intake resulting from loss of appetite and of a simple decrease in caloric intake on the course of Theiler's GDVII encephalomyelitis in mice.

Materials and methods. Mice: Webster Swiss mice, from our own colony, were used in all experiments. When 3 to 4 weeks old, they were removed from the colony, weighed and distributed in individual screen-bottom cages. Each group contained approximately equal numbers of male and female mice of similar weight and litter mates in any one group were kept to a minimum.

Diets: the diet referred to as "optimum" has the following percentage composition; casein 18† sucrose 72.2, salts IV 4,(8) vitamins 0.5,‡ choline chloride 0.3 and corn oil 5.§ The vitamins were made up with sucrose so

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† A hot alcohol-extracted, vitamin test casein (General Biochemicals, Inc., Chagrin Falls, Ohio).

‡ We are indebted to Merck & Co., Rahway, N.J., for the crystalline vitamins.

8. Lichstein, H. C., McCall, K. B., Kearney, E. B., Elvehjem, C. A., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 279. In these studies the salt mixture was modified by using $ZnSO_4$ insted of Zn Cl_2.

TABLE I.
Dietary Regimen.

Ration No.	Type of restriction	Ration fed per mouse per day
1	Caloric	1 g FPSV + 0.5 g sucrose
2	"	1 g FPSV + 1.5 g sucrose
3	Total	1 g optimum ration
4	"	2 g optimum ration
5	"	3 g optimum ration
6	None	Optimum ration *ad lib.*

that 0.5 g of the mixture contained the following: thiamine 0.3 mg, riboflavin 0.3 mg, pyridoxine 0.3 mg, niacin 1.5 mg, calcium pantothenate 2.0 mg, i-inositol 100 mg, sodium p-amino-benzoate 100 mg, biotin 10 γ, folic acid 25 γ. In the rations designed to determine the effect of caloric restriction, fat, protein, salts, and vitamins (referred to as FPSV) were fed in the same proportions as they occur in the optimum diet, and the indicated amounts of sucrose were added. Each gram of the FPSV mixture contained casein 0.65 g, salts IV 0.14 g, vitamins 0.029 g and corn oil 0.18 g, enough to reasonably insure the consumption of as much of each per mouse as would be consumed by mice receiving the optimum diet *ad lib.* This being the case, the only constituent deficient in rations 1 and 2 was sucrose. The dietary regimen of each group is listed in Table I. In previous experiments of a similar nature it was learned that mice would die if placed abruptly on the restricted rations, therefore a plan of progressive restriction as outlined in Table II was followed during the first ten days of the experiment. For caloric restriction the FPSV mixture and sucrose were measured separately and blended manually in each ration cup. Groups 1 through 4 received the same quantity of FPSV. For total restriction, decreasing amounts of the optimum diet were fed until the determined level of food consumption was reached. Water was always available. Five days after the start of the experiment, the individual weights of the majority of the animals had reached a maximum (except the optimum *ad lib.* group) and by the seventh day, they had begun to decline.

Virus: 16 days after beginning the experiment all the test groups were inoculated intracerebrally with 0.03 ml of a saline suspension (Series 98, 10^{-2}; Series 115, 10^{-3}) of Theiler's(9) GDVII virus infected brain and cord. All animals were observed daily and weighed twice weekly.

Results and discussion. The results are summarized in Table III. Uninoculated mice remained alive throughout the experiment and were alert and active in contrast to those receiving the virus. In the latter group, hindleg weakness, tremors, ataxia, humped back, and occasionally tonic convulsions, with extension of the hindlegs and flexion of the forelimbs, were observed. During the convulsions, the mice became rigid and cyanotic; death sometimes occurred during these seizures, but more often the mouse recovered. Similar convulsions have been seen in other nutritional deficiencies such as that of tryptophan. In the present experiments, the animals that were paralyzed and those that showed only signs of encephalitis were grouped together in calculating the percentage virus infection.

In *Series 98,* in which practically 100% of the mice in the control inoculated groups developed signs of the virus infection, Groups 2 and 6 showed this typical infection in only 50% and 43%, respectively, but showed total fatalities in 100% and 95% of the animals. This would seem to indicate that either a diet with low caloric value or a restriction of the optimum diet to 1 g per day definitely alters the signs of infection. But, in these same groups, animals dying without showing any signs of infection survived 5 and 7.5 days (average survival time, AST) respectively while the average incubation period (AIP) of mice which did show the typical picture was 9 and 8.8 days respectively. We assume that in most cases the mice which died without signs of infection actually succumbed to virus invasion. Several lines of evidence support this assumption. Gard(10) has presented evi-

§ Mazola, fortified with oleum percomorphum (Mead Johnson & Co., Evansville, Ind.) to supply *ca.* 1800 I.U. vitamin A and 260 I.U. vitamin D per 100 g ration.

9. Theiler, M., and Gard, S., *J. Exp. Med.,* 1940, v72, 49.

10. Gard, S., *J. Exp. Med.,* 1940, v72, 69.

TABLE II.
Plan of Restriction.

Group No.	Days after start of experiment: 0	3	4	5	7	10
1 and 2	Opt. *ad lib.*	Opt. 3 g	FPSV 1 g + sucrose 2 g	FPSV 1 g + sucrose 1.5 g	FPSV 1 g + sucrose 1 g	FPSV 1 g + sucrose 0.5 g
3 and 4	,,	,,	,,	,,	FPSV 1 g + sucrose 1.5 g	FPSV 1 g + sucrose 1.5 g
5 and 6	,,	,,	Opt. 2.5 g	Opt. 2 g	Opt. 1.5 g	Opt. 1 g
7 and 8	,,	,,	,,	,,	Opt. 2 g	Opt. 2 g
9	,,	,,	Opt. 3 g	Opt. 3 g	Opt. 3 g	Opt. 3 g
10	,,	*Opt. ad lib.*	Opt. *ad lib.*	Opt. *ad lib.*	Opt. *ad lib.*	Opt. *ad lib.*

TABLE III.
Summary of Fatalities and Virus Infection.

Series No.	Group No.*	Ration No.	No. of mice	Fatalities,† %	Virus infection, %	Total fatalities, %	AST,‡ days	AIP,§ days
98	1 U	1	7	0	—	0	—	—
	2 I	1	20	50	50	100	5.0	9.0
	3 U	2	7	0	—	0	—	—
	4 I	2	21	0	100	100	—	7.7
	5 U	3	7	0	—	0	—	—
	6 I	3	21	52	43	95	7.5	8.8
	7 U	4	7	0	—	0	—	—
	8 I	4	20	0	100	100	—	7.9
	9 I	5	14	7	93	100	12.0‖	8.9
	10 I	6	14	0	100	100	—	8.9
			138					
115	1 U	1	7	0	—	0	—	—
	2 I	1	21	14	86	100	10.0	9.5
	3 U	2	7	0	—	0	—	—
	4 I	2	21	5	95	100	7.0‖	9.1
	5 U	3	6	0	—	0	—	—
	6 I	3	20	35	65	100	9.0	10.2
	7 U	4	7	0	—	0	—	—
	8 I	4	20	5	95	100	10.0‖	9.9
	9 I	5	14	7	93	100	7.0‖	10.0
	10 I	6	14	14	86	100	8.0	11.0
			137					

* U = uninoculated; I = inoculated.
† Without signs of infection.
‡ Average survival time of mice dying without signs of GDVII infection.
§ Average incubation period of mice developing signs of GDVII infection.
‖ 1 mouse only.

dence that the AIP is an index of virus titer. On numerous occasions we have assayed the brains and cords of infected, undernourished mice at intervals after inoculation and when moribund but without typical signs of infection. This central nervous system material when subsequently inoculated into normal mice has shown virus activity indicating that the virus does proliferate in the CNS of deficient mice even though the typical signs of infection are not seen. Further, in some experiments, deficient inoculated mice which have survived beyond the normal incubation period without visible evidence of infection have succumbed to the disease with the typical picture when fed optimum rations. As criteria of infection, therefore, we are inclined to weigh not only paralysis and visible signs of encephalitis, but also the AIP, the AST and total fatalities.

For example, in this series 98, although groups 2 and 6 showed only 50 and 43% respectively of manifest virus infection, the AIP, the AST, and the total fatalities present a picture of almost 100% actual virus infection. The results obtained with Groups 9 and 10 are essentially alike with almost 100% manifest virus infection. A number of mice receiving 3 g of optimum diet per day did not consume this amount from one feeding to the next; it is inferred therefore that their appetites were satisfied, approximating the *ad lib.* group in food consumption. The groups receiving 1 g or 2 g of optimum diet per day devoured their ration completely. The remaining groups(4,8) with less severe restrictions, showed no significant differences in incidence of paralysis.

Series 115. Thirty-five % of the mice in group 6 (1 g optimum diet) died without any signs of the disease. However, the AST (9.0 days of these mice was less than the AIP (10.2 days) for this group and both the AST and AIP were less than the AIP (11.0 days) of the animals on the optimum ad lib. diet (Group 10). Among the remaining groups (2, 4, 8) in this series, typical infection levels ranged from 86% to 95%.

We have therefore almost identical pictures in the two series. In each, the groups receiving restricted amounts of food, whether low in calories, or low in amounts of an optimum diet, showed no significant decrease in infection as we have defined the term. The exact figures for the two series are not directly comparable since in series 98, the inoculum was a 10^{-2} suspension whereas in series 115, this was a 10^{-3} suspension. This difference resulted in a longer AIP and a slightly longer AST in the latter series. Our results are not precisely in accord with the reports of others who have found that restriction of the caloric value or reduction of food intake produces results similar to but less marked than those obtained with thiamine deficient diets. We would emphasize that our criteria of infection do not rest solely on visible signs and that the other authors used different viruses, either Theiler's FA or the Lansing mouse adapted poliomyelitis virus. Only with the Lansing strain did they obtain definitive results.

Although Theiler(11) has reported that the more obvious physical and chemical properties of mouse encephalomyelitis virus and human poliomyelitis are "identical", immunologically, and on the basis of species susceptibility and the pathological lesions produced, the viruses are definitely different. The dissimilarity in behavior of GDVII and Lansing poliomyelitis virus in mice when the animals are under various nutritional stresses appears to be another manifestation of differences between these viruses. Our experience indicates that the course of infection with the Lansing strain is more sensitive to nutritional stress than is infection with Theiler's GDVII virus.

Summary. Although diets restricted either calorically or by total food intake do influence the course of infection in mice inoculated with Theiler's GDVII virus, in that frank signs of infection, *i.e.* paralysis, encephalitis, are frequently not evident, yet the total fatalities, the average incubation period, and the average survival time are not modified appreciably by these deficiencies.

11. Theiler, M., *Med.*, 1941, v20, 443.

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