been used. The local tissue necrosis which occasionally results from intramuscular and subcutaneous administration of mercury bichloride thus cannot be the cause of the hyperlipemia previously described.

(3) The same hyperlipemic reaction is observed in rats after intramuscular and intravenous injection of mercury bichloride. However, no immediate rise in serum lipids was observed after the oral administration of mercury bichloride. It remains to be investigated whether an effect of this agent upon the liver might explain the different action of enteral and parenteral administration of mercury bichloride.

(4) It was found in 6 dogs that the oral administration of carbon tetrachloride leads to hypolipemia involving cholesterol, phospholipids, and total lipids. This makes it clear that the hyperlipemia previously observed after it had been injected intramuscularly was not due to carbon tetrachloride but to the necrosis of the tissues in which it had been injected.

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Influence of Pyridoxine, Inositol, and Biotin on Susceptibility of Swiss Mice to Experimental Poliomyelitis.*

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Previous reports from this laboratory[1,2,3,4] have shown that the nutritional state of the Swiss mouse plays a role of varying importance in the susceptibility of this host to experimental poliomyelitis. Similar results with thiamine have been reported by the Pennsylvania group.[5] The most striking data were obtained with thiamine,[1] namely, mice fed a synthetic ration deficient only in this vitamin, exhibited a marked protection against infection with the poliomyelitic viruses, and indeed, in some series this resistance was 100%.[4] An attempt to ascertain the fundamental reason for these results[4] showed that the addition of pyruvate to thiamine optimum diets prolonged the incubation period of the infection in some instances, but did not manifest the marked resistance noted in frankly thiamine-deficient mice.

The specificity of the effect of nutrition in this disease is suggested by the fact that in pantothenic acid deficiency there is a definite increased resistance to Theiler's encephalomyelitis, but little or none to the Lansing strain of poliomyelitis virus,[2] while mice deficient in riboflavin manifest no altered susceptibility to Theiler's infection and a very slight increased resistance to the Lansing virus.[3] These results as well as those obtained with thiamine deficiency[1] suggest that factors such as inanition play a less significant role than the specific vitamins.

In the present report studies are presented for pyridoxine, biotin, and inositol deficiencies.

Methods and Materials. The synthetic optimal diets employed were composed of the following parts per 100: sucrose, 73; vitamin-free casein, 18; salt mixture, 4;[6] and corn oil, 5. The vitamin supplements per 100 g of diet

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2 Lichstein, H. C., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 3.

3 Rasmussen, A. F., Jr., Waisman, H. A., and Lichstein, H. C., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 92.

4 Waisman, H. A., Lichstein, H. C., Elvehjem, C. A., and Clark, P. F., *Arch. Bioch.*, 1945, **8**, 203.

5 Foster, C., Jones, J. H., Henle, W., and Dorfman, F., *J. Exp. Med.*, 1944, **80**, 257.

6 Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

TABLE I.
Effect of Pyridoxine Deficiency on Susceptibility of Swiss Mice to Experimental Poliomyelitis.

Series No.	No. of days on diet before inoculation	No. of days after inoculation	% of mice paralyzed			
			Lansing (2%)			
			Deficient (21)*	Optimum† (21)		
28	35	5	19	10		
		6	29	24		
		7	39	24		
		9	52	57		
		10	57	62		
		13	81	71		
		15	81	76		
		28	86	81		
			Lansing (5%)		Theilers GDVII (1%)	
			Deficient (35)	Optimum (34)	Deficient (35)	Optimum (34)
40	27	6	3	15	0	0
		7	6	35	0	0
		8	14	44	0	0
		9	26	47	14	24
		10	31	47	29	35
		11	46	50	51	47
		14	66	62	63	79
		16	66	68	66	79
		18	69	68	71	82
		28	69	74	77	82
			Lansing (5%)			
			Deficient (25)	Optimum (27)		
44	33	6	16	26		
		7	24	37		
		8	28	44		
		9	48	52		
		10	52	56		
		12	56	67		
		18	68	70		
		28	68	70		

* No. of mice inoculated.

† This optimal diet contained 200 μg pyridoxine per 100 g diet, while in the other series, the optimum contained 300 μg.

unless otherwise stated were: thiamine, 0.3 mg; riboflavin, 0.3 mg; nicotinic acid, 0.5 mg; calcium pantothenate, 2 mg; pyridoxine, 0.3 mg; *i*-inositol, 100 mg; choline, 300 mg; para-aminobenzoic acid, 100 mg; and biotin, 5 μg. Through inadvertence in a previous paper[2] the thiamine content of the ration was not mentioned. Actually, 0.3 mg of thiamine was present in this ration as in all our diets save when otherwise stated. For the several deficiencies the respective vitamin was omitted from the ration. Each mouse received adequate amounts of oleum-percomorphum *per os* twice weekly.

Swiss mice bred in our own laboratory were used in all experiments, and split litter technic with consideration for sex and weight of mice was employed.

The viruses used, Lansing strain poliomyelitis and Theiler's encephalomyelitis (GDVII strain), were given by the intra-cerebral route using an inoculum of 0.03 ml and in the concentrations indicated. A comparison of the clinical picture obtained with our stock of GDVII with that reported by Olitsky[7] suggests that our virus is more nearly like their TO strain but of even greater virulence. However, until further verification is made the strain will be called GDVII. Uninoculated mice were maintained on both deficient and optimum diets to ascertain the effect of nutrition alone, and also to decide the optimum time for virus administration so as to obtain

[7] Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 77.

TABLE II.
Effect of Biotin Deficiency on Susceptibility of Swiss Mice to Experimental Poliomyelitis.

Series No.	Days on diet before inoculation	Days after inoculation	% of mice paralyzed					
			Theilers GDVII (10%)					
			Deficient (41)*		Optimum (42)			
41	48	8	17		2			
		9	22		12			
		10	46		33			
		12	78		62			
		14	83		74			
		20	88		83			
		28	88		83			
			Theilers GDVII (10%)					
					Regular opt.			
			Deficient (28)		(33)			
46	40	8	21		6			
		9	29		33			
		10	32		39			
		11	50		55			
		12	54		64			
			Theilers GDVII (1%)					
					Regular opt.			
			Deficient (31)	Opt. (28)	(29)			
48	30	7	13	0	0			
		8	19	4	10			
		9	26	28	28			
		10	32	54	59			
			Theilers GDVII (1%)			Lansing (5%)		
					Regular opt.			Regular opt.
			Deficient (24)	Opt. (25)	(18)	Deficient (30)	Opt. (30)	(19)
50	34	8	4	0	11	13	13	5
		9	12	4	11	13	20	5
		10	21	12	22	17	29	16
		11	29	24	39	33	29	21
		12	29	24	56	33	29	21
		13	42	44	72	43	30	26
		14	50	56	89	47	33	32
		16	50	56	89	53	40	37
		19	50	56	89	53	43	53
		28	50	60	89	53	47	53

* No. of mice inoculated.

Optimal diets contained 100 μg of biotin per 100 g except in Series 41 where only 20-50 μg was present; in Series 41 the optimum diet contained 10% egg white and 10% casein, while in Series 48 and 50 the egg white was present as the sole source of protein in a concentration of 20%.

maximum paralysis at the peak of the deficiency.

In all experiments the animals were observed twice daily after inoculation for signs of flaccid paralysis; the experiments were terminated 28 days after administration of the virus.

Results. Pyridoxine. Three series involving 302 mice were studied using both Theiler's and Lansing viruses (Table I). One observes a lesser growth rate in mice deficient in this vitamin but no other gross manifestations of the dietary inadequacy are seen even after 6 to 8 weeks on the pyridoxine-free diet.

The end results in all series indicate no difference in susceptibility of the deficient or optimally fed mice to either virus. However, in Series 40 the results suggest an increased resistance to infection with Lansing virus on the part of the deficient mice during the early portion of the incubation period (5 to 10 days). Although there is a slight suggestion of

this same trend in Series 44 the differences are too small to be considered significant especially since the reverse phenomenon is seen in Series 28.

Biotin. Five series totalling 632 mice were studied with this deficiency. Four of these are listed in Table II, the fifth being omitted because of the low infectivity of the virus employed in that experiment.

For the production of biotin deficiency a diet containing powdered hen's egg albumin was employed. This was added to the diets in quantities sufficient to allow the avidin to combine with the biotin synthesized by intestinal microörganisms (usually 20%). In Series 41, egg albumin was present in the optimal diet as well as in the deficient ration, but in Series 46 the optimal diet contained no albumin. A change was made in the diets of Series 48 and 50 so that the egg albumin was present as the sole source of protein replacing the vitamin-free casein.

Biotin deficiency signs appeared in the mice after 4 to 8 weeks on the deficient ration. The first signs were a weight plateau, a roughening of the fur coat, a distinct dermatitis, and a halting high-stepping gait followed by the appearance of "spectacled eyes." Later the lips and nose became swollen and remained thickened, while incrustations of an exfoliative nature appeared over the entire body but more especially on the denuded areas. Similar findings have been reported by other investigators.[8,9] Curvature of the spine[10] appeared after 4 or 5 weeks and persisted throughout the deficiency. Control mice receiving adequate biotin remained in good health throughout the experiment. Mice showing extreme signs of biotin deficiency which were given as much as 50 to 100 μg of crystalline biotin per 100 g of diet exhibited gains in weight and improvement in the encrusted and denuded areas of the skin, but did not manifest complete return to normal despite 6 weeks of this therapy. Alleviation of the dermatitis upon the addition of biotin to the deficient diets was not as rapid in those mice receiving 20% egg white (sole source of protein) as in those animals receiving 10% each of egg white and casein.

The results of these experiments indicate no striking effect of biotin on the course of infection, but do suggest a slight but questionable tendency towards increased resistance of the optimally fed mice to Theiler's infection. In Series 50, a lowered incidence to Theiler's infection by both groups receiving egg albumin as compared with the optimal diet without egg white is seen, but the reason for this is obscure. The data of Series 46 and 48 are tabulated only up to 12 and 10 days respectively, since death of the mice due to an intestinal infection made the subsequent results impossible to correlate. The signs of this infection were an acute diarrhea, the anal region and tail becoming covered with fecal material which caused at times the complete closure of the anus. Cultivation of the stools of these mice on suitable media revealed large numbers of organisms which were identified as probable *Salmonella typhimurium,* and postmortem examination of the animals showed enlargement of the spleen and an acute inflammation of the gastro-intestinal tract. While this condition was observed in only 2 of the 5 biotin series, it suggests that mice deficient in this vitamin are more susceptible to naturally occurring salmonella infections than are animals maintained on a diet optimum in biotin.

Inositol. Both synthetic and natural diets were used to produce an inositol deficiency in 3 series of animals (478 mice). No marked signs of deficiency were found in animals fed the inositol-free synthetic diet save for a slightly soiled and roughened fur coat and an occasional area of alopecia. This is in contrast to the findings of Woolley[11] who noted marked alopecia in mice on a similar diet. In addition, this author has pointed out the importance of the level of pantothenate in the diet on the development of alopecia, but our attempts to produce denudation by varying the level of this vitamin were unsuccessful. The natural inositol deficient diet employed

8 Nielsen, E., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 349.

9 Emerson, G. A., and Keresztesy, J. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 358.

10 Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 405.

11 Woolley, D. W., *J. Biol. Chem.*, 1941, **139**, 29.

TABLE III.
Effect of Inositol Deficiency on Susceptibility of Swiss Mice to Experimental Poliomyelitis.

Series No.	Days on diet before inoculation	Days after inoculation	% of mice paralyzed			
			Theilers GDVII (10%)		Lansing (5%)	
			Deficient (29)*	Optimum (31)	Deficient (35)	Optimum (34)
45	31	8	7	10	11	9
		9	14	26	14	15
		10	24	39	23	24
		11	52	55	26	24
		12	62	71	29	27
		14	86	90	34	30
		16	93	93	40	35
		18	97	93	51	38
		20	97	97	51	38
		28	97	97	51	41

Series No.	Days on diet before inoculation	Days after inoculation	Theilers GDVII (2%)		
			Deficient (25)	Opt., (25)	Synthetic opt. (17)
49	35	8	4	4	6
		9	20	8	18
		10	36	20	47
		11	56	48	76
		12	60	52	76
		14	72	68	94
		16	80	76	94
		17	84	84	94
		28	84	92	94

Series No.	Days on diet before inoculation	Days after inoculation	Theilers GDVII (10%)			
			Synthetic deficient (38)	opt. (38)	Natural deficient (39)	opt. (42)
51	44	8	0	5	8	7
		9	3	16	21	14
		10	18	24	26	26
		11	34	61	56	60
		12	47	79	74	71
		13	61	79	82	79
		14	63	84	90	81
		16	68	89	95	83
		17	71	89	95	83
		20	74	89	95	86
		28	74	91	95	86

* No of mice inoculated.
Series 45: synthetic ration.
Series 49: natural ration.

was the one suggested by Cunha *et al.*[12] which consisted of ground yellow corn 75.35, soybean oil meal 17.50, alfalfa meal 5.00, $CaHPO_4$ 1.00, NaCl (iodized) 0.50, $MnSO_4$ 0.01, haliver oil 2 drops weekly, a "folic acid" concentrate equivalent to 2 g liver extract[13] and 300 μg pyridoxine. While rats show alopecia on such a regimen, mice fed this diet did not develop any loss of hair throughout the experimental period. However, the use of this diet presented an opportunity to compare the effects of synthetic and natural foodstuffs on the course of virus multiplication.

With this deficiency as in the 2 previous ones there was no consistent difference between deficient or optimally fed mice to infection with either virus. Only in Series 51 was there a consistent lowered incidence of paralysis exhibited by those mice fed a synthetic ration deficient in inositol. The reasons for the increased resistance of both deficient and

12 Cunha, T. J., Kirkwood, S., Phillips, P. H., and Bohstedt, G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 236.

13 Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521.

optimally fed mice in Series 49 as compared with the synthetic optimum diet are not clear especially since this was not true in Series 51.

Summary. The influence of pyridoxine, inositol, and biotin deficiencies on the susceptibility of mice to experimental poliomyelitis has been studied in more than 1400 animals. No striking or consistent difference with reference to susceptibility to either Lansing strain poliomyelitis or Theiler's encephalomyelitis was noted between animals fed diets deficient or optimum in these vitamins.

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The Relative Toxicity of *l*- and *dl*-Serine in Rats.*

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An injurious action of *dl*-serine has been reported previously by the authors.[1,2,3] Thus, the administration of *dl*-serine (100 mg daily) by stomach tube or by injection to rats on certain experimental diets produces anorexia, loss in weight, and albuminuria, leading frequently to death. The most constant pathological finding is the presence of extensive necrotic lesions in the renal tubules. It has been pointed out[2] that the injury may have been due to either one or both of the components of the racemic *dl*-serine. Accordingly, in the present experiments a comparison has been made of the relative toxicity of *l*- and *dl*-serine.‡

Experimental. In most of our previous experiments, rats in groups of 20 were maintained on experimental diets for 4 weeks and received serine (100 mg) daily by stomach tube during the second and the third weeks. In the present study, because of the limited supply of *l*-serine, it would have been impossible to carry on long term experiments on a comparable number of animals so that complete data on weight changes and on mortality§ have not been secured. On the other hand, since the most characteristic clinical and pathological changes develop in the first few days of serine administration, our interest was centered on the alterations appearing during this period, only a few experiments being of longer duration.

In all the present experiments, male rats (100 g) were kept on diet 4 only (no B vitamins),[2] and the amino acid was administered by stomach tube beginning with the eighth day. Of the 8 rats receiving *l*-serine daily in 100 mg amounts, 2 were killed after 1 dose, 3 after 3 doses, 1 after 5 doses, 1 after 9 doses, and 1 a week after the completion of 14 days of serine administration. Usually, for each rat receiving the *l* isomer, another simultaneously received the *dl* compound. The animals were kept in individual metabolism cages. The urine was collected under toluene and was stored in the refrigerator. The urine volume, body weight, and food consumption were recorded daily. The animals were killed by decapitation and the autopsies were performed immediately. The tissues were fixed

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1 Fishman, W. H., and Artom, C., *J. Biol. Chem.*, 1942, **145**, 345.

2 Artom, C., and Fishman, W. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 239.

3 Morehead, R. P., Fishman, W. H., and Artom, C., *Am. J. Path.*, 1945, **21**, 803.

§ No spontaneous deaths occurred in rats receiving *l*-serine.