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

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

TABLE I.
Food Consumption and Changes in Body Weight.

	l-Serine Group		*dl*-Serine Group	
	Before Admin.	During Admin.	Before Admin.	During Admin.
Food consumption, g	33; 37; 36; 41; 32	18; 19; 20; 19; 26	37; 28; 42; 28; 29	9; 14; 13; 11; 10
Individual values*	33	27	30	9
Avg	35.3	21.5	32.2	11.0
Changes in body wt, g	+0.5; —1.0; +2.0	—3.5; +1.0; —2.5	—2.0; 0; 0; —2.5	—8.1; —7.2; —7.0
Individual values†	—4.0; —0.5; +6.0	—5.5; +4.0; +9.0	+8.0; +5.0	—10.0; —5.5; —6.5
Avg	+0.5	+0.4	+1.4	—7.4

* Total food consumption of each rat for a 3-day period.
† Increase (+) or decrease (—) in the body weight of each rat for the 3-day period.

in 10% formalin, cut at 6 microns and stained with hematoxylin and eosin.

The urine was tested qualitatively for protein (Heller's, Esbach's, trichloracetic acid, heat coagulation tests) and for reducing sugar (Benedict's test). When the urines reacted positively for protein, its amount was estimated approximately with the quantitative Esbach's test as commonly used clinically.

A more extensive study was made on the urines collected from 2 rats receiving *l*-serine for 3 days and from 2 others receiving *dl*-serine for the same length of time. In these experiments, modifications of published methods were employed for the determination of bisulfite-binding substances,[4] of reducing substances (ceric sulfate titrimetric procedure),[5] of ammonia (steam distillation), and of serine (periodate oxidation).[6] These modifications are described in more detail in the following paper.

Results. The clinical appearance of the animals receiving *l*-serine was uniformly good in contrast to those animals receiving *dl*-serine, which, after 2 or 3 doses of the racemic amino acid, became definitely ill.

Data on food consumption and changes in body weight during the first 3 days of serine administration are recorded in Table I. It appears that the food consumption is reduced in rats receiving both the *l*- and *dl*- compound, but to a smaller extent in the *l*-serine group. No significant change in body weight was observed after the administration of *l*-serine, whereas all the animals receiving the *dl* amino acid showed a marked loss in weight.

The pathological findings in animals receiving *dl*-serine corresponded to those previously described in detail.[3] Severe renal necrosis followed by rapid tubular regeneration and fibrosis, was seen in the kidneys of these animals. In the *l*-serine group, however, there were no demonstrable alterations of the normal renal architecture. Abnormal fatty changes were seen in livers of both groups of animals, but this was to be expected, since the experimental diet was poor in choline or choline precursors.

Protein reactions were all negative or at most doubtfully positive in the urine of rats receiving *l*-serine. On the other hand, in agreement with our previous results,[1,3] significant amounts of protein were present in the urines from rats on *dl*-serine. The proteinuria was most marked during the first 3 days of serine administration, the amounts of protein eliminated in this period ranging between 5 and 13 mg per rat per day.

The findings in the urines of the animals employed in our more detailed investigation are recorded in Table II. In both groups of animals receiving either *dl*- or *l*-serine, the ammonia and the urine volume were increased. After *dl*-serine, in addition to the protein, the urine contained large amounts of unchanged serine for the whole 3-day period. Furthermore, the first day's collection after *dl*-serine showed a marked increase in bisulfite-binding and reducing substances. A strongly positive Benedict's test was obtained on this urine. None of these changes was apparent in the urine of the rats receiving *l*-serine.

Discussion. In the present experiments the

[4] Wortis, H., Bueding, E., and Wilson, W. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 279.

[5] Miller, B. F., and Van Slyke, D. D., *J. Biol. Chem.*, 1936, **114**, 583.

[6] Artom, C., *J. Biol. Chem.*, 1945, **157**, 585.

TABLE II.
Analytical Data on Rat Urine.*

	l-Serine Group					*dl*-Serine Group				
	Before admin. 3-day avg	During administration			3-day avg	Before admin. 3-day avg	During administration			3-day avg
		1st day	2nd day	3rd day			1st day	2nd day	3rd day	
Vol., cc	3.7	3.3	5.7	6.7	5.2	3.8	6.3	4.5	8.4	6.4
Proteins (qualitative)	—	±	—	—		—	++	+++	++	
"Sugar" (Benedict's qualitative)	—	—	—	—		—	+++	±	-	
"Serine" (micromoles)	36	42	23	21	29	21	231	250	201	227
NH_3 (micromoles)	166	243	238	199	227	143	197	214	237	216
Reducing substances (glucose equiv. mg)	15	16	13	14	14	15	39	17	11	22
Bisulfite binding substance (micromoles)	27	21	24	19	21	24	55	24	22	34

* Per rat, per day.

same amounts of *dl*- or *l*-serine were given by stomach tube to rats maintained on an experimental diet. Severe clinical disturbances and pathological lesions of the kidney were observed in the animals receiving the racemic mixture. Except for a decrease in appetite (less marked than in the *dl* group), rats receiving the natural isomer did not show injurious effects. It may therefore be reasonably assumed that, under the conditions of our experiments, it is the *d* component which is mainly responsible for these effects.‖

As for the urinary findings, the values in the *l*-serine group (except for NH_3 and urine volume) were almost identical to those found before the administration of the amino acid. On the other hand, in the *dl*-serine group, the urine contained protein, sugar-like substances and larger amounts of substances which bind bisulfite. This last finding may be compared to the observation of an increase of keto-acids (determined as 2,4-dinitrophenylhydrazones) in the urines of rats receiving various racemic amino acids, including *dl*-serine.[7]¶

The greater elimination of unchanged serine after giving the racemic mixture is in line with previous observations on the differences in the utilization of *l*- and *dl*-amino acids observed in humans,[8] in animals,[9] and

‖ One cannot exclude the possibility that the difference noted is one of degree and that some noxious action may become evident, should much larger doses of the *l*-compound be administered. In the present study, the animals received twice as much of the *l* compound as compared with the amount of the *d* component in the racemic mixture.

[7] Waelsch, H., and Miller, H. K., *J. Biol. Chem.*, 1942, **145**, 1.

¶ Waelsch and Miller[7] state: "Only 2 of the 12 amino acids fed as the natural isomers, namely tyrosine and lysine, induced a definite increase in keto acid excretion, whereas all 9 amino acids fed as the racemic compounds did so."

[8] Albanese, A. A., *J. Biol. Chem.*, 1945, **158**, 101; Albanese, A. A., and Frankston, J. E., *J. Biol. Chem.*, 1944, **155**, 101; Albanese, A. A., *Bull. Johns Hopkins Hosp.*, 1944, **75**, 195.

[9] Wohlgemuth, J., *Ber. Chem. Soc.*, 1905, **38**, 2064; Abderhalden, E., and Tetzner, E., *Z. physiol. chem.*, 1935, **232**, 79; Dakin, H. D., *J. Biol. Chem.*, 1910, **8**, 25; Kotake, Y., Matzuoka, Z., and Okagawa, M., *Z. physiol. chem.*, 1922, **122**, 166;

in microörganisms.[10] However, with respect to serine, whereas only the *l* form is utilized by *Lactobacillus*,[11] both *d* and *l* isomers appear to be attacked by *Proteus*,[12] *Pseudomonas*,[13] and *B. coli*.[10,14]

On the basis of the present results, it is not yet possible to decide which one of the general hypotheses, previously suggested,[2] would best explain the injurious action of serine. The present evidence suggests that the *d* isomer is less easily metabolized. Accordingly, one would understand a toxic action due to the increased concentration in the tissues of the unmetabolized *d*-serine (perhaps by a competitive inhibition of certain enzyme systems).

However, according to the alternative hypothesis, the injury may be due instead to products of the metabolism of serine. It may be that *d* serine, although metabolized more slowly, is degraded through a pathway different from that of the natural isomer, giving rise to abnormal toxic compounds.

On the other hand, even if *d*- and *l*-serine are metabolized alike, the normal intermediates may become toxic if they accumulate. The finding of an increased elimination of bisulfite-binding substances in animals receiving *dl*-serine may be pointed out in this connection. One might postulate that after the administration of the unnatural isomer, toxic concentrations of intermediates (such as, possibly, pyruvic acid[14,15,16]) occur in the kidney. This may be the result of an overproduction of the metabolite (*e.g.*, because of the high activity in the kidney of *d*-amino acid oxidase, or other enzymes, specific for *d*-amino acids**) or the consequence of its retarded disposal (*e.g.*, through a diminution in the activity of kidney cocarboxylase).[20]

Summary. A comparison has been made of the relative toxicity of *l*- and *dl*-serine in rats. Under the same experimental conditions, the administration of the natural *l* isomer was not accompanied by the clinical and pathological alterations and by the chemical changes in the urine, characteristically found in animals receiving the racemic mixture. It appears, therefore, that the unnatural *d*-isomer is chiefly responsible for the injurious action of *dl*-serine.

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13 Webster, M. D., and Bernheim, F., *J. Biol. Chem.*, 1936, **114**, 265.

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** *d*-serine is acted upon by *d*-amino oxidase at a slower rate than other amino acids,[17] but it is likely that certain *d*-amino acids may be oxidized by different enzyme systems in tissues.[18] The role of the "dehydrase," to which the anaerobic deamination of *dl*-serine by microörganisms and liver has been ascribed,[14,16] remains uncertain. In fact, no specific difference in the action of this enzyme on the *d*- and *l*- component has yet been noted. Moreover kidney slices do not deaminate serine under anaerobic conditions.[19]

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