contraction was investigated. 2. In the presence of menadione the synthesis of acetylcholine is decreased. 3. The activity of choline esterase was not modified by concentrations of menadione up to $1x10^{-3}$ mol. and was decreased by higher concentrations (manometric method and striated muscle). 4. Menadione increased the effect of potassium in inducing muscle contraction. 5. In high concentrations ($2.5x10^{-3}$ mol.) it induced a contraction of the striated muscle. 6. The effect of methylene blue is similar to that of menadione except that methylene blue is a potent inhibitor of the choline esterase. 7. The mechanisms of the effects of menadione are discussed in relation to its ability to react with -SH groups, to its positive oxidation-reduction potential, and to its action on the aerobic glycolysis.

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Relation of Route of Administration to Toxicity of *dl*-Serine.*

CAMILLO ARTOM AND WILLIAM H. FISHMAN. (Introduced by Arthur Grollman.)

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In a previous report,[1] we have described an injurious action of *dl*-serine when administered by stomach tube. In male rats on an experimental diet, this procedure resulted in severe clinical disturbances (anorexia, loss in weight, albuminuria), demonstrable pathological lesions in the kidney, and a high mortality. It was thought that a comparison of the effects of administering the amino acid by routes other than by stomach tube might throw some light on the mechanism of the toxic action of *dl*-serine.

Experimental. Male albino rats (Rockland) weighing 95-105 g were transferred from the stock diet to an experimental diet (Diet 4), containing "Labco" vitamin-free casein 10 parts, dextrin 37, sucrose 37, Crisco 5, cod liver oil 5, "Ruffex" 2, salt mixture (Osborne and Mendel) 4.† Water was supplied *ad libitum*. After 7 days had elapsed, the daily administration of *dl*-serine by stomach tube, by intraperitoneal injection, or by admixture in the diet was initiated and continued for 14 days. The animals were maintained afterwards for an additional week on the experimental diet. A control group of rats received water by stomach tube for the same length of time. Body weight was recorded 3 times a week and food consumption daily.

Results. Mortality is recorded in Table I and composite curves of the changes in body weight are presented in Fig. 1. Where fatalities occurred, two separate curves are shown, one for the animals dying (Curves IIA and IIIA) and one for those surviving (Curves IIB and IIIB). The data on food consumption have been omitted for the sake of brevity but the values paralleled the changes in body weight.

Control animals receiving water only by stomach tube (Group I) showed no visible ill-effects and they all survived. Thus, even in animals on a deficient diet, prolonged stomach tubing is not harmful. Both the slow growth and the failure to gain weight during the 4th week may be ascribed to the low protein content of the diet, as well as to the probable development of a B-vitamin deficiency.

In conformity with our previous results, in Group II (receiving serine by stomach tube), a sudden loss in weight and serious disturb-

* This work was aided by a grant from the John and Mary R. Markle Foundation. A preliminary report appeared in *Federation Proceedings*, March, 1944, **3**, 10.

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

† This diet is identical to diet 1,[1] except that the small amounts of vitamin B complex were omitted.

TABLE I.
Route of Administration of *dl*-Serine and Mortality.

Group No.	Mg of *dl*-serine supplied daily*	Route	No. of rats	No. of deaths	Mortality in %
I	None	Stomach tube	12	0	0
II	100	" "	21	13	62
III	100	Injection	16	7	44
IV	100	Admixture in diet	11	0	0
V	200	" " "	12	0	0

* Group I received 3 cc of water daily. The daily dose of serine given to group II was dissolved in 3 cc of water and that to group III in 2 cc of water.

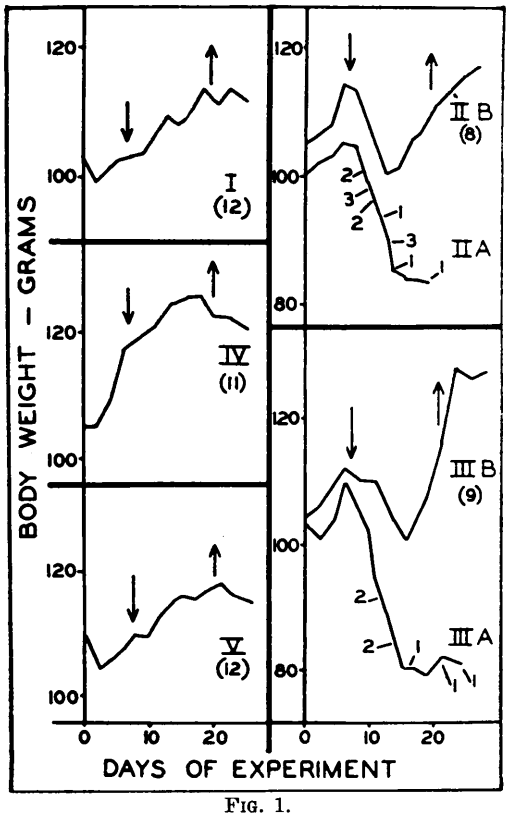

FIG. 1.
The influence of serine administration by various routes on body weight. For the periods between the arrows, the rats received either water by stomach tube (I) or *dl*-serine [by stomach tube (II); by injection (III); in the diet (IV = 100 mg daily; V = 200 mg daily)]. The number of rats surviving in each group is indicated in parentheses. The number of deaths in each day of experiment is recorded on curves IIA and IIIA.

ances were apparent during the first week of serine administration. Here it was that many deaths occurred. Later a number of these animals showed a marked recovery, in spite of the continued administration of the amino acid. The recovery was evidenced by the disappearance of clinical symptoms and by the resumption of growth. Approximately the same picture was obtained when the amino acid was injected parenterally (Group III). Here the mortality was also considerable.

When the same amount of serine was mixed in the diet (Group IV), no deaths resulted: at all times the animals appeared healthy and growth curves were very similar to those of the controls not receiving serine. Even when the amount of serine added to the diet was doubled (Group V), no ill-effects were observed.

Discussion. The same amount of *dl*-serine which is highly toxic when administered once daily either by stomach tube or by parenteral injection, is apparently harmless when it is absorbed from the diet in refracted doses during a 24-hour period. It is clear, therefore, that a sudden elevation of the level of serine in the blood and tissues is prerequisite to the production of the injury.

As for the mechanism of serine toxicity, many working hypotheses could be outlined. However, in our opinion, they may all be based on 2 general concepts. First the whole unmetabolized molecule of serine may be responsible. Thus, high concentrations of the amino acid may interfere with some essential metabolic processes by a mass action effect. This may possibly be exerted through a competitive inhibition by serine of certain enzymatic systems of the cells.

In the second interpretation, the injury may be due to products of serine metabolism. Normal intermediary products, which are present physiologically only in minimal amounts, may become injurious when they accumulate in

the tissues. Moreover, it is also possible that when the serine level is suddenly elevated, the formation of abnormal products through side reactions takes place to a large extent.‡

‡ Thus, ethanolamine, which in sufficient amounts is toxic for rats,[2] could possibly be formed by decarboxylation of serine. In this regard, the action of the intestinal flora should of course be considered. However, since serine by parenteral injection, as well as by stomach tube, is highly injurious, a role of the intestinal bacteria in the mechanism of serine injury appears unlikely.

It should be pointed out that these interpretations may apply to one or both of the components of the racemic *dl*-serine.

Summary. *dl*-Serine in 100 mg amounts daily is injurious to rats on an experimental diet when supplied by stomach tube and by parenteral injection, but is not harmful when administered mixed in the diet. Some hypotheses on the mechanism of the production of the toxic action of serine are discussed.

[2] Artom, C., and Fishman, W. H., *J. Biol. Chem.*, 1943, **148**, 423.

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Some Dietary Factors Which Reduce the Toxicity of *dl*-Serine in Rats.*

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Previous data[1] point to a relationship between the diet and the severity of the injury, caused by administration of *dl*-serine. Rats on a stock diet, receiving the amino acid by stomach tube, exhibited a transient cessation of growth, and only slight ill-effects; no fatalities were observed. This contrasted with the high mortality observed under the same conditions in animals maintained on both experimental diets 1 and 2. One of these diets (Diet 2, with 30% casein) provided a very satisfactory rate of growth, almost identical with that of rats on the stock diet. It seems, therefore, that the protective action of the stock diet against serine toxicity was not due merely to the maintenance of a state of good nutrition, but rather to some specific factor, not present in sufficient amount in the experimental diets.

In this connection, casein diets are known to be relatively poor in choline, cystine, and glycine. On the other hand, the role of a deficiency of some members of the vitamin B complex could also be suspected, as the amounts of these vitamins present in our experimental diets were probably inadequate.† Accordingly, in the present study, the effect of the administration of a choline, cystine, glycine mixture, pure B vitamins, or both simultaneously, has been investigated. Special attention has been directed to the action of pyridoxine.‡

Experimental. Two experimental diets were used, diet 4[3] and diet 4 supplemented with choline HCl, glycine, and *l*-cystine (50 mg of each, daily). The daily intake of vitamins in animals on the stock diet and in animals receiving pure B vitamins is indicated in Table I. *dl*-Serine was administered by stom-

* This work was aided by a grant from the John and Mary R. Markle Foundation. Preliminary report in *Federation Proceedings*, March, 1944, **3**, 10.

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

† 10 g of diets 1 and 2[1] contained 100 γ nicotinic acid, 10 γ riboflavin, 10 γ thiamin HCl, 1 γ pyridoxine, 1 γ Ca pantothenate.

‡ We are indebted to Dr. E. E. Snell for having pointed out to us the possibility of pyridoxine having an action antagonistic to serine, on the basis of his then unpublished results.[2]

[2] Snell, E. E., and Guirard, B. M., *Proc. Nat. Acad. Science*, 1943, **29**, 66.

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