TABLE V.
Effect of Sulfonamides on Varying Concentrations of Hemolytic Streptococci (Strain C203MV) at 37°C.

Hr. of incubation	No. of organisms (× 1000) per cc Sulfadiazine 99 μg/cc	Sulfapyridine 99 μg/cc	Broth
0	292,000	292,000	292,000
24	190,000	240,000	260,000
0	2,920	2,920	2,920
24	208,000	252,000	285,000
0	29	29	29
24	5,400	8,500	122,500

rapid multiplication occurred in the presence of either sulfadiazine or sulfapyridine.

In the presence of a small initial number of organisms per cc rapid multiplication occurred in plain broth whereas much slower multiplication occurred in the presence of sulfadiazine or sulfapyridine.

It is apparent that the sulfonamides were effective only after a lag period during which the organisms were in contact with the drug. On the addition of sulfadiazine or sulfapyridine after the organisms had passed into the phase of growth acceleration, no bacteriostasis took place. Neither sulfadiazine nor sulfapyridine acted on initially large numbers of organisms in spite of active multiplication. This is in contrast to the action of penicillin.

Summary. Under the experimental conditions used there was no evidence of a synergistic action between sulfadiazine or sulfapyridine and penicillin. Para-aminobenzoic acid did not increase the titer of penicillin although it does increase its rate of action.

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Inhibitory Effect of Certain Amino Acids on Growth of Young Male Rats.

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Previous attempts to supplement gelatin with known amino acids so as to provide optimum growth have been unsuccessful (Jackson *et al.*[1] Kruse[2]). Threonine was not recognized at the time and we attempted to determine whether this was the necessary factor.

Arginine, histidine, lysine, valine, leucine, isoleucine, methionine, tryptophane, phenylalanine, and threonine were fed at levels in excess of Rose's minimum levels[3] with 20% gelatin (Group 2). A second group was fed the amino acids alone (Group 3). A third group was fed gelatin alone (Group 1).

The animals fed the supplemented gelatin made slight gains whereas loss of weight occurred in the same period on gelatin alone. The animals fed the amino acids alone grew better than those on the gelatin-amino acid diet, despite the fact that gelatin supplied "non-essential" amino acids. However, the rate of growth on the amino acids alone was not optimal.

It seemed to us that gelatin might be in-

[1] Jackson, R. W., Sommer, B. E., and Rose, W. C., *J. Biol. Chem.*, 1928, **80**, 167.

[2] Kruse, H. D., Day, H. G., and McCollum, E. V., *Am. J. Hygiene*, 1934, **19**, 260.

[3] Rose, W. C., *Physiol. Rev.*, 1938, **18**, 109.

TABLE I.
Nitrogen Supplement to Experimental Diets.

Group No.	Supplement g/100 g diet						
	Gelatin*	Amino acid mixture	Casein	Glycine (Merck)	*l*-Proline (Merck)	*dl*-Phenyl-alanine (Merck)	Fibrin
1	20						
2	20	†					
3		†					
4			20				
5			20	6			
6			20		5		
7			20			5	
8			50				
9			15				
10			15	6			
11							15
12				6			15

* Wilson's Pure-Food Gelatin.
† See Table II.
Diet 13—Stock diet.
Diet 14—94 g stock diet plus 6 g Glycine.

TABLE II.
Amino Acid Supplements to Diets 2 and 3.

Amino acid	Amt fed, g	Amt active, g	Rose essential amino acid requirements, g
dl-Methionine (Merck)	0.80	0.80*	0.6
l(+)Arginine HCl (Mearl)	0.40	0.33†	0.2
l(—)Histidine HCl (Paul Lewis)	0.60	0.49‡	0.4
l(+)Lysine HCl (Merck)	1.50	1.2 §	1.0
l(—)Tryptophane (Merck)	0.25	0.25	0.2
dl-Phenylalanine (Merck)	1.00	1.0 *	0.7
dl-Threonine (Merck)	1.50	0.75‖	0.6
dl-Valine (Merck)	1.80	0.90‖	0.7
dl-Leucine (Merck)	2.10	1.05‖	0.9
dl-Isoleucine (Merck)	1.20	0.60‖	0.5
Total	11.15	7.37	5.8
$NaHCO_3$	1.13 g ≅ 0.49 g HCl		

* Both isomers active.
† Contains 0.44 × .173 = 0.076 g HCl.
‡ ” 0.60 × .190 = 0.114 g ”
§ ” 1.50 × .20 = 0.300 g ”
‖ Only 50% active.

hibiting growth due to its marked amino acid imbalance, particularly its high content of glycine and proline. We therefore performed feeding experiments comparing growth on good basal diets with and without the additions of single amino acids.

Experimental Procedure. Male albino rats, 21 days old, were divided into groups of 2 of equal average weight, and fed the diets *ad libitum* assigned to the group. The diets, except for 13 and 14 contained the following basal ingredients.

Corn Oil	4 g
Salt Mixture	4 ”
Celluration	4 ”
Liver Conc. 1-20	1.5 ”
Choline	0.3 ”
Thiamine	0.3 mg
Pyridoxine	0.3 ”
Riboflavin	0.3 ”
Niacin	2.5 ”
d-Ca Pantothenate	1.0 ”

In addition, each rat received 2 drops of haliver oil per week.

The nitrogen supplements shown in Table I and the above basal ingredients were mixed with sufficient sucrose to make 100 g.

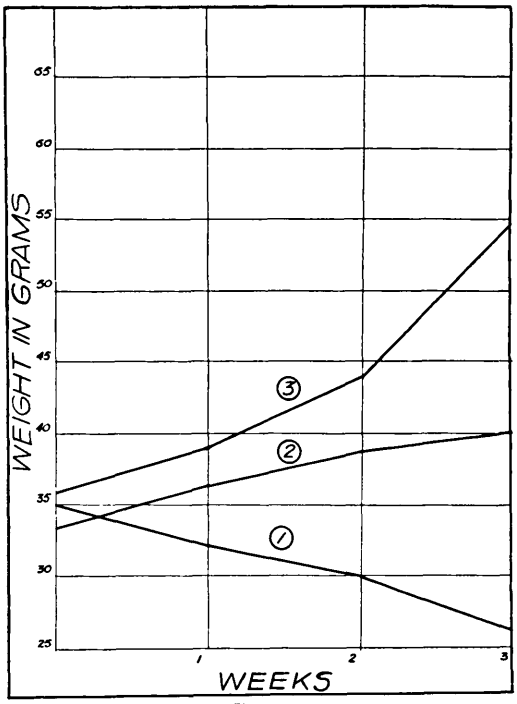

FIG. 1.

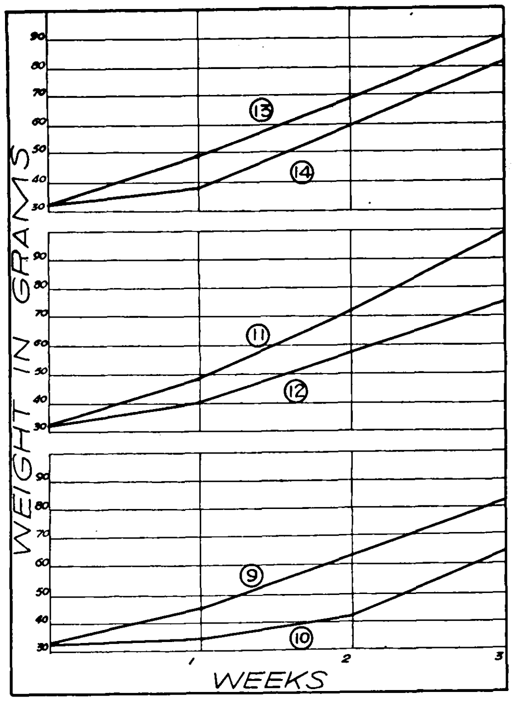

FIG. 3.

Results. Fig. 1 shows the superior growth obtained on amino acids (3) as source of nitrogen compared to growth on gelatin plus amino acids (2), or gelatin alone (1).

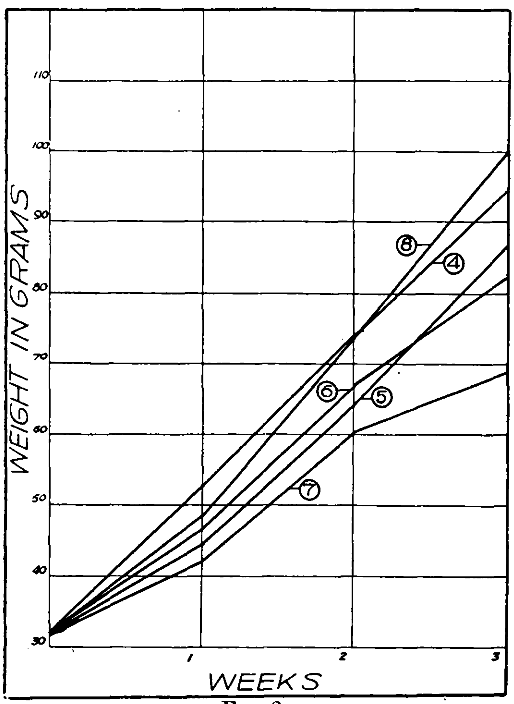

FIG. 2.

Fig. 2 shows the inhibitory effect of glycine on 20% casein diet (5) compared to 20% casein (4), and the inhibiting effect of l-proline (6) and dl-phenylalanine (7) and non-inhibiting effect of 50% casein (8).

Fig. 3 shows the inhibitory effect of glycine on a 15% casein diet (10) compared to 15% casein alone (9), the inhibiting effect of glycine on 15% fibrin diet (12) compared to 15% fibrin alone (11) and the inhibiting effect of glycine on stock diet (14) compared to stock diet alone (13).

Discussion. The data indicate that glycine at 6% or l-proline at 5% of the diets used have an inhibitory effect on growth and may explain the difficulty in supplementing gelatin with amino acids so as to make it adequate for growth.

dl-Phenylalanine at 5% has an inhibitory effect on growth even more severe than glycine and l-proline.

These effects are not due to high nitrogen intake alone since 50% casein has no deleterious effect although it does supply considerably more nitrogen.

Sullivan *et al.*[4] reported growth inhibition and toxicity of glycine on a diet containing 4% casein at levels of amino acid from 2.5 to 20%. Jackson *et al.*[1] reported growth inhibition by 9% glycine as well as 35% gelatin when added to a normal casein diet. Their effects with glycine were not as marked as those reported in this paper. This may be due to their use of older animals (60-90 g) while our animals were started from weaning (35 g).

These data indicate the danger of imbalancing amino acid proportions by introducing high levels of a single amino acid even though it may be an essential amino acid. This is of particular importance in preparing experimental diets as some investigators have used high levels of glycine to balance nitrogen intake in protein and amino acid studies. Studies on other amino acids, to be reported, indicate that not all amino acids show this inhibitory effect.

Conclusion. 1. Gelatin will not allow optimum growth even when supplemented with all reported essential amino acids. In fact, less growth is obtained with gelatin plus amino acids than with the amino acids alone at the same level in the diet. 2. The presence of 6% glycine or 5% dl-phenylalanine or 5% l-proline in certain diets has an inhibitory effect on growth. 3. The inhibitory effect of glycine and proline on growth may explain the difficulty in supplementing gelatin for optimum growth.

4 Sullivan, M. X., Hess, W. C., and Sebrell, W. H., *Pub. Health Rep.*, 1932, **47**, 75.

We wish to acknowledge and thank Miss Joan Staats for technical assistance.

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Failure to Demonstrate an Interrelationship Between Inositol and p-Aminobenzoic Acid in the Rat.

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Martin[1] has pointed out that although purified rations containing 6 B complex factors (thiamine hydrochloride, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid and choline chloride) maintained apparently normal nutrition in the Rockland strain rat, addition of p-aminobenzoic acid or inositol to the above ration at levels of 0.01 and 0.02% respectively precipitated syndromes that failed to develop if all 8 factors were present. In the present experiment rats of another strain were placed at weaning on a purified ration containing the 6 B complex factors listed above to which p-aminobenzoic acid and inositol were added in doses ranging from 0.05 to 1.0% of the ration, and the effect of feeding these diets determined.

Procedure and Results. The basal ration consisted of sucrose 73.5, Vitamin Test Casein* 22, and Sure's Salt Mixture No. 1[2] 4.5. To each kg of the above were added 20 mg thiamine hydrochloride, 20 mg riboflavin, 20 mg pyridoxine hydrochloride, 100 mg calcium pantothenate, 100 mg nicotinic acid, 1200 mg choline chloride and 5 mg 2-methyl-naphthaquinone. Daily supplements of 800 mg corn oil (Mazola), 0.5 mg alpha-tocopherol, and an A-D concentrate† equivalent to 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D were also administered.‡ Inositol and p-aminobenzoic acid

1 Martin, Gustav J., *Am. J. Physiol.*, 1942, **136**, 124.

2 Sure, Barnett, *J. Nutrition*, 1941, **22**, 499.

* S.M.A. Corporation, Chagrin Falls, Ohio.

† Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units vitamin D per gram.

‡ The vitamin content of the above ration is considerably in excess of minimum levels compatible with growth and reproduction.