graphs similar to all the stages of disintegration of hemolyzed erythrocytes reported by Furchgott from light microscopy are represented and observed.

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Action of Vitamin B_{12} in Counteracting Glycine Toxicity in the Chick.* (18125)

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Cary *et al.*(1) and Rubin and Bird(2), working with rats and chicks, respectively, have observed growth-inhibitory effects from high levels of protein in diets now known to be low in vitamin B_{12}. McGinnis *et al.*(3) reported that the nonprotein nitrogen content of the blood in chicks fed diets high in protein and deficient in the "animal protein factor" was higher than in chicks fed the same diets supplemented with this factor. Zucker and Zucker(4) observed a similar condition in rats. Since that time, vitamin B_{12} has been shown by Ott *et al.*(5), Lillie *et al.*(6), and others, to be an important part of the animal protein factor. Recently, Charkey *et al.*(7) have demonstrated that the levels of nonprotein nitrogen and amino acids in the blood were higher in vitamin B_{12}-deficient chicks than in chicks fed vitamin B_{12} (Merck & Co., APF Supplement No. 3). These workers concluded that vitamin B_{12} appears to function in metabolism by enhancing the utilization of circulating amino acids for building fixed tissues. These observations are in agreement with those noted in this laboratory (unpublished data).

In view of these findings, the present study was conducted to determine the effect of feeding different levels of glycine to chicks receiving various amounts of vitamin B_{12}. Evidence is presented to indicate that vitamin B_{12} is concerned in the metabolism of glycine in the chick.

Experimental. New Hampshire chicks of mixed sexes, obtained from dams kept on raised wire platforms and fed a ration low in vitamin B_{12}, were used in these experiments. The chicks were maintained in electrically heated batteries, and feed and water were supplied *ad libitum*. All glycine supplements were made at the expense of cerelose (glucose). Merck & Co. APF Supplement No. 3 was used as the source of vitamin B_{12}.

In Exp. 1, day-old chicks first were fed basal diet 122 (developed in this laboratory, Table I) during an 18-day preliminary period to further deplete them of vitamin B_{12}. This diet was deficient in vitamin B_{12} and contained 35% protein. At the end of the depletion period, six comparable groups of 15 chicks each were selected on the basis of body weight. Groups 1, 3, and 5 were fed the basal diet plus 0, 3, and 30 μg of vitamin B_{12} per kilo, respectively. The other 3 groups were fed these same diets except that 1%

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1. Cary, C. A., Hartman, A. M., Dryden, L. P., and Likely, G. D., *Fed. Proc.*, 1946, v5, 128.

2. Rubin, M., and Bird, H. R., *J. Nutrition*, 1947, v34, 233.

3. McGinnis, J., Hsu, P. T., and Graham, W. D., *Poultry Sci.*, 1948, v27, 674.

4. Zucker, L. M., and Zucker, T. F., *Arch. Biochem.*, 1948, v16, 115.

5. Ott, W. H., Rickes, E. L., and Wool, T. R., *J. Biol. Chem.*, 1948, v174, 1047.

6. Lillie, R. J., Denton, C. A., and Bird, H. R., *J. Biol. Chem.*, 1948, v176, 1477.

7. Charkey, L. W., Wilgus, H. S., Patton, A. R., and Gassner, F. X., PROC. SOC. EXP. BIOL AND MED., 1950, v73, 21.

TABLE I. Composition of Basal Diet 122.

	%
Cerelose	51.67
Alpha soybean protein	38.00
Soybean oil	3.00
Cod liver oil (3000 A; 400 D)	.50
DL-methionine	.60
L-leucine	.20
Mineral mixture 1 M*	6.00
Iodinated casein (Protamone)	.03
	mg/100 g
Thiamin HCl	1.00
Riboflavin	1.00
Calcium pantothenate	2.00
Pyridoxine HCl	.60
Niacin	5.00
Folic acid	.30
p-Aminobenzoic acid	.20
Menadione	.50
Biotin	.02
Choline chloride	200.00
i-Inositol	100.00
alpha-tocopherol acetate	.50

1200 I.U. vit. A, and 0.5 mg of alpha-tocopherol acetate were administered by dropper weekly.
* Described by Briggs(8).

glycine was also added. A 3-week experimental period was employed.

In Exp. 2, the protein level of the basal diet was lowered to 21% by reducing the level of alpha soybean protein from 38 to 23%. Additional cerelose was used in compensation. A preliminary vitamin B_{12}-depletion period was omitted in this trial since the parent stock had been maintained on the vitamin B_{12}-deficient ration for a longer period of time. Six groups, each containing twelve day-old chicks, were fed the modified basal ration plus different supplements. Combinations of 0, 3, and 30 μg of vitamin B_{12} per kilogram of diet, with and without 4% added glycine, were tested. This experiment was discontinued at the end of two weeks, since the addition of 4% glycine to the vitamin B_{12}-low basal diet resulted in 75% mortality. Nevertheless, the inhibitory action of this level of glycine on vitamin B_{12}-deficient chicks appears clearly evident even in this brief test period.

Results and discussion. The result obtained in Exp. 1 are given in Table II. It is evident that the growth of vitamin B_{12}-deficient chicks was depressed by the addition of 1% glycine to the diet when no vitamin B_{12} was supplied. This growth-inhibitory action of glycine was counteracted by the addition of either 3 or 30 micrograms of vitamin B_{12} per kilogram of diet. The slight difference in the average gains of the two groups of chicks (Groups 5 and 6) fed diets containing 30 micrograms of vitamin B_{12}, with and without added glycine, is not statistically significant. A previous study (unpublished) supports this conclusion. In that test, chicks fed basal diet 122, supplemented with 1% added glycine and 30 micrograms of vitamin B_{12} per kilo, grew somewhat more

TABLE II. Effect of 1% Added Glycine in Combination with Various Amounts of Vitamin B_{12} on Chick Growth. (Experiment I).

Group No.	Supplement to basal diet 122	No. of surviving chicks†	Avg gain during 3 wk exp. period (g)
1	None	14	146
2	1% glycine	13	99*
3	3 γ B_{12}/kg	14	227
4	3 γ " + 1% glycine	14	209
5	30 γ B_{12}/kg	15	336
6	30 γ " + 1% glycine	14	309

* The difference between this value and that of group 1 is statistically significant to the 5% level.
† Each group contained 15 chicks at the start.

TABLE III. Effect on Chick Growth and Mortality Obtained from the Addition of 4% Glycine in Combination with Different Amounts of Vit. B_{12}. (Experiment II).

Group No.	Supplement to modified basal diet	No. of surviving chicks*	Avg gain of surviving chicks during 2 wk exp. period (g)
1	None	11	42
2	4% glycine	3	36
3	3 γ B_{12}/kg	12	65
4	3 γ " + 4% glycine	11	75
5	30 γ B_{12}/kg	12	73
6	30 γ " + 4% glycine	12	71

* Each group contained 12 chicks at the start.

8. Briggs, G. M., *J. Nutrition,* 1946, v31, 79.

rapidly than those fed the same diet without additional glycine.

The results of Exp. 2 are presented in Table III. The effect of 4% added glycine in the vitamin B_{12}-deficient diet was striking. Only 3 of the 12 original chicks fed this diet (Group 2) survived the 2-week period. However, the addition of as little as 3 μg of vitamin B_{12} per kilo of diet (Group 4) prevented the excessive mortality and completely counteracted the growth-inhibitory action of the added glycine, confirming the results obtained in Exp. 1. Since Merck's APF supplement No. 3 was used as the source of vitamin B_{12}, the possibility exists that the effects observed may have been the result of something other than vitamin B_{12}. This possibility, however, is not considered to be very great.

The inhibitory action of the 4% added glycine in the vitamin B_{12}-deficient diet containing 21% protein (Exp. 2) cannot be attributed to its effect on the protein level of the diet, since the addition of 1% glycine to the 35% protein diet (Exp. 1) did not cause excessive mortality. Therefore, the retarded growth and extreme mortality which was observed in vitamin B_{12}-deficient chicks fed the high level of glycine may be assumed to have resulted from a specific glycine imbalance rather than from a change in the protein level of the diet. Since the addition of vitamin B_{12} to all diets containing 1% or 4% added glycine counteracted the inhibitory action of this amino acid on chick growth, it appears that vitamin B_{12} functions in some manner in the metabolism of glycine.†

This finding is comparable with those of Groschke and Briggs(9) and Anderson *et al.* (10) of this laboratory, who found that niacin and pyridoxine, respectively, are also concerned in the metabolism of glycine. Groschke and Briggs(9) demonstrated that glycine was highly "pellagragenic" when fed to chicks receiving a niacin-low diet. However, they found that as much as 6% glycine could be included in the chick diet with no adverse effects when an adequate amount of nicotinic acid was supplied. Anderson *et al.* (10) similarly showed that the addition of 4% glycine to a pyridoxine-low diet exerted a growth-depressing action in the chick. This was overcome by the addition of pyridoxine.

The growth-depressing effect of large amounts of glycine fed to rats and its counteraction by certain vitamins has been reported by other workers. Dinning *et al.*(11) and Martel *et al.*(12) observed that rats fed diets containing 10% glycine grew at a subnormal rate. A marked improvement in growth rate was noted upon addition of folic acid. Pagé and Gingras(13) demonstrated that large intakes of glycine inhibited growth of rats fed a pyridoxine-deficient diet, but that the addition of pyridoxine to the diet overcame this inhibition. Furthermore, Martin(14) reported that glycine was more toxic when fed at high levels to riboflavin-deficient rats than when fed to rats maintained on a diet adequate in riboflavin. The results of the present investigation together with those of the workers referred to above indicate that vitamin B_{12}, nicotinic acid, pyridoxine, folic acid, and riboflavin are required in the metabolism of glycine. This appears to be particularly true when unusually large amounts of glycine are fed.

Summary. Vitamin B_{12}-deficient chicks were fed vitamin B_{12}-deficient basal diets supplemented with 0, 1 and 4% glycine in combination with 0, 3, and 30 μg of vitamin B_{12} (supplied by Merck's APF Supplement No. 3) per kilogram. The growth of the chicks that received either 1 or 4% added glycine in the vitamin B_{12}-deficient basal diets

† At the conclusion of this investigation, it was learned that Machlin *et al.* at the Poultry Section, B. A. I. Beltsvile, Md., had obtained similar results.

9. Groschke, A. C., and Briggs, G. M., *J. Biol. Chem.*, 1946, v165, 739.

10. Anderson, J. O., Combs, G. F., and Briggs, G. M., *Poultry Sci.*, 1949, v28, 755.

11. Dinning, J. S., Keith, C. K., Day, P. L., and Totter, J. R., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 262.

12. Martel, F., Pagé, E., and Gingras, R., *Rev. Canadienne de Biologie,* 1947, v6, 802.

13. Pagé, E., and Gingras, R., *Trans. Roy. Soc. Can.*, 1946, v40, 119.

14. Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 528.

was depressed. Those chicks which received 4% added glycine without vitamin B_{12} suffered excessive mortality. The addition of as little as 3 micrograms of vitamin B_{12} per kilo of diet overcame the inhibitory action of both levels of glycine. These results indicate that vitamin B_{12} functions in the metabolism of glycine.

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Inhibition of Calcification *in vitro* by Surface Active Compounds.* (18126)

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The observation that some types of pathological lipid deposition are often followed by mineralization suggests that lipid substances may enhance mineral salt deposition. An *in vitro* study of the role of lipids in the calcification of hypertrophic epiphyseal cartilage of the rachitic rat may further our understanding not only of normal calcification, but may also provide a clue to the nature of abnormal mineralization. This method, which enables one to observe the selective formation of new bone salt in rachitic epiphyseal cartilage, depends on the functioning of the complete system essential for calcification(1-3, 6,11,12). This technic has provided, in previous experiments, evidence that lipids are related to calcification. For example, bone formation is inhibited in the rachitic tibia if it has been previously extracted with alcohol, acetone, or chloroform(2). Moreover, the calcifying mechanism is injured by phloridzin and iodoacetic acid; two compounds which are inhibitors not only of phosphorylative glycogenolysis, but also of fat absorption(3,4). That these inhibitions can be overcome with excess inorganic or organic phosphates does not preclude the possibility that a system handling lipids is directly involved in the local deposition of bone salts(5,6). Levine and Follis(7,8) have demonstrated the presence of a lecithinase in cartilage. They postulated that it is part of an independent system for elevating the local concentration of phosphate ions. Another line of investi-

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1. Shipley, P. G., *Bull. Johns Hopkins Hosp.*, 1924, v35, 304.

2. Shipley, P. G., Kramer, B., and Howland, J., *Biochem. J.*, 1926, v20, 379.

3. Gutman, A. B., Warrick, F. B., and Gutman, E. B., *Science*, 1942, v95, 461.

4. Verzar, F., *Int. Physiol. Congress, Moscow Abstr.*, 1935, v15, 419.

5. McLean, F. C., Lipton, M. A., Bloom, W., and Barron, E. S. G., 14th Conference of metabolic aspects of convalescence, Symposium on bone metabolism, 1946, pp. 17, Josiah Macy, Jr. Foundation, New York.

6. Robison, R., Significance of phosphoric esters in metabolism, 1932, pp. 85, New York University Press.

7. Levine, M. D., and Follis, R. H., *Fed. Proc.*, 1949, v8, 458.

8. Follis, R. H., and Berthrong, M., *Bull. Johns Hopkins Hosp.*, 1949, v85, 281.

9. Levander, G., *J. Surg. Gynec. Obstet.*, 1938, v67, 705.

10. Pfeifer, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 388.

11. Sobel, A. E., Cohen, J., Kramer, B., *Biochem. J.*, 1935, v29, 2640.

12. Sobel, A. E., Nobel, S., and Hanok, A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 68.