is equivalent to approximately half of the cysteine dose used for comparison (950 mg/kg I.V.) in terms of sulfhydryl content.

Sodium sulfide and colloidal sulfur were included to test further the possible importance of sulfur in the protection afforded by cysteine against x-rays. The former was ineffectual, while the latter gave equivocal results. Ascorbic acid selected because of its reducing properties and its presumed importance in biologic oxidation-reductions failed to alter survival of the irradiated rat. A sulfhydryl group is the most evident distinguishing feature between the substances which definitely protect and those which do not. It should be borne in mind, however, that all sulfhydryl-containing materials may not necessarily protect and that other reducing substances which are properly distributed, temporally and spatially, may.

Since cysteine, but not cystine, is effective in altering sensitivity to radiation, one may speculate that the cysteine acts by protecting certain cellular components from oxidation by the products of irradiated water. This could be accomplished indirectly by the reduction of one or more of the critical links in the metabolic chain or directly by neutralization of the oxidizing agents presumably produced by radiation. Barron and co-workers(3) have reported that small doses of x-rays inactivate sulfhydryl enzymes *in vitro* by oxidation of their -SH groups (reversible inhibition) and large doses by denaturation of the protein moiety (irreversible inhibition). Non-sulfhydryl enzymes may be irreversibly inhibited by still larger amounts of x-rays, primarily by protein denaturation. If enzyme inactivation of the types reported by Barron is an important factor leading to death of the irradiated animal, it would appear from the above considerations and our data on the time course of the cysteine protection that the inactivation is of the irreversible non-specific type resulting from protein denaturation.

Summary. Cysteine greatly reduces the sensitivity of rats and mice to lethal amounts of x-rays delivered at either high or low dose rates provided that the amino acid is given before the exposure. Rather comparable protection is obtained when cysteine is injected intravenously in rats immediately or 1 hour before irradiation with 800 r (90% reduction in the 28-day mortality). Injection immediately after exposure or 6 or 24 hours before is without influence. Cysteine also improves survival significantly when it is given orally 30 to 60 minutes prior to the irradiation. Glutathione (I.V. but not oral) can also diminish radiation toxicity. Cystine, methionine, ascorbic acid, and sodium sulfide do not alter survival of the irradiated animal. Results with colloidal sulfur are equivocal. The possible significance of these observations is discussed.

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Vitamin B-12 in Amino Acid Metabolism.* (17562)

L. W. CHARKEY, H. S. WILGUS, A. R. PATTON AND F. X. GASSNER

From the Chemistry and Poultry Husbandry Sections, Colorado Agricultural Experiment Station, Colorado A. and M. College, Fort Collins, Colo.

McGinnis *et al.*(1) reported that blood non-protein nitrogen content was higher in chicks deprived of animal protein factor (APF) than in normal controls, and suggested that this may have been due to a function of APF in enhancing amino acid utilization. Zucker and Zucker(2) found that non-protein nitrogen, and urea values in rat blood were in-

* Scientific Journal Series No. 311, Colo. Agr. Exp. Station.

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

creased in zoopherin deficiency, and have elsewhere related zoopherin to the animal protein factor. Norris(3) stated that evidence found by his group (not reported in detail) pointed to such a function on the part of APF. The observation of Bird *et al.*(4) that normal chicks have no appreciable requirement for APF after 8 weeks of age may further corroborate the same theory inasmuch as the period during which APF *is* required is the time of most rapid growth, and utilization of amino acids.

It cannot yet be stated with certainty that the effects cited, or those observed in the present work were due entirely to the vitamin B-12 contained in the APF or zoopherin preparations used. There are many indirect indications, however, that the principal activity involved probably was that of vit. B-12. Perhaps the most direct evidence is that obtained by Ott *et al.*,(5) who found that crystalline vit. B-12 exerted APF activity in chicks deprived of animal protein. They concluded that since the crystalline vitamin elicited growth responses comparable to those obtained with crude sources of APF, it is possible that vit. B-12 is identical with or closely related to this factor.

Evidence is presented herewith in more direct support of the theory that vit. B-12 functions in amino acid utilization in chicks. The term 'vitamin B-12' refers hereinafter to an activity provided in the form of the Merck and Co. APF Supplement No. 3.

Experimental. In Exp. 1 mixed single comb White Leghorn chicks from APF-depleted dams were given corn meal for the first 2 days of life, then placed on the experimental diets. Twenty-five chicks were placed in each group, according to a uniform weight distribution pattern. Feed and water were supplied *ad libitum*. The basal diet contained sodium proteinate† 25, corn meal 70, dicalcium phosphate 3, pulverized limestone 1, DL-methionine 0.3, and choline chloride 0.17%. Vitamins and minerals were added, in terms of milligrams per kilogram of diet, as follows: niacinamide 17.5; calcium pantothenate, riboflavin and 2-methyl-1,4-naphthoquinone, each 10; thiamin hydrochloride, pyridoxine hydrochloride, pteroylglutamic acid, p-aminobenzoic acid and alpha tocopherol, each 5; manganese, 27.5; iron, 10; copper, 1; cobalt, 0.1; and sodium chloride, 2500. Vitamin A and D oils were incorporated to provide 6600 I.U. of vit. A and 750 A.O.A.C. units of vit. D per kg of diet. Because certain treatments in this experiment called for the use of iodinated casein, all lots were supplemented to contain 7 mg iodine per kg diet.

In Exp. 2 single comb White Leghorn cockerels from a commercial hatchery were kept on a vit. B-12 depletion diet for 2 weeks; then placed on experimental diets. Twenty-five chicks were placed in each group, according to a uniform weight distribution pattern. The experimental basal diet was as described for Exp. 1 except that iodine supplementation was reduced from 7 to 2.2 mg per kg of diet. The latter iodine level has been found in previous work to be protective against goitre, and experimental treatments did not necessitate balancing at the higher level. The depletion diet was the same as the Exp. 2 experimental basal except that solvent process soybean meal was used at 52.5% and corn meal at 42.5% in place of sodium proteinate at 25% plus corn meal at 70%. To facilitate depletion with respect to vit. B-12,(6) this diet was supplemented with 0.05% iodinated casein (Protamone‡).

Experimental measurements were made of growth, feed consumption, thyroid weight, thyroid histology, and blood contents of: non-protein nitrogen, alpha amino nitrogen, eryth-

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

3. Norris, L. C., Verbal report at 1948 annual meeting of Poultry Science Assn., Fort Collins, Colo.

4. Bird, H. R., Marsden, S. J., Groschke, A. C., and Lillie, R. J., *Poultry Sci.*, 1948, v27, 654.

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

† Derived from soybeans and kindly provided by Dr. J. W. Hayward of the Archer-Daniels-Midland Company, Minneapolis.

6. Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 400.

‡ Through the courtesy of Dr. W. R. Graham, Cerophyl Laboratories, Kansas City, Mo.

rocytes, hemoglobin, total amino acids,(7) and 7§ individual amino acids, namely arginine, lysine, methionine, tryptophan, histidine, threonine and valine.

Blood for analysis was collected by ventricular puncture in Exp. 1, and from the carotids after decapitation in Exp. 2. In both cases potassium oxalate was used as the anticoagulant. Individual samples were retained separately until the effectiveness of anticoagulant measures could be verified. Clotted samples and those containing disproportionately large amounts of oxalate were discarded. The remaining samples were pooled according to experimental treatments, and in Exp. 1 according to sex. The pooled samples were stirred gently and subdivided for preparation of both laked(8) and unlaked(9) protein-free filtrates. The filtrates were analyzed for total non-protein nitrogen by the procedure of Koch and McMeekin,(10) and for each of the 7 amino acids by microbiological methods employing *Streptococcus faecalis*, A.T.C.C. 9790.

Of all the various measures taken only growth, feed consumption, non-protein nitrogen and the specific amino acids showed meaningful variations. These are summarized in Table I. The blood contents shown are those obtained by the assay of unlaked protein-free filtrates. Values were obtained by the use of laked filtrates also; but are not reported in detail since this technic provided relatively poor sensitivity to the experimental treatments. The variations encountered in these values show in most instances the same trends as those shown in Table I; thereby lending a degree of further corroboration.

Discussion. It is apparent from Table I that the circulating blood of the birds receiving vit. B-12 contained less non-protein nitrogen, and less of each of the amino acids measured than did the blood from birds deprived of B-12. It also is apparent that the

TABLE I.
Effect of Dietary Vitamin B-12 on Growth, Feed Utilization and Certain Blood Contents.

Exp. No.		Avg init. wt, g	Avg wt gain, g	Avg gain per g feed, g	Blood components—Avg mg % in blood							
					Total NPN	Arginine	Lysine	Methionine	Tryptophan	Histidine	Threonine	Valine
1*	Basal	41	250	0.56	20.7	6.6	5.8	0.77	1.56			
					(5)‡	(4)	(2)	(3)	(4)			
	Basal B-12§	41	266	0.61	18.6	6.6	5.0	0.69	1.43			
					(4)	(6)	(2)	(3)	(6)			
2†	Basal	97	153	0.53	19.0	6.7	7.4	0.57	1.53	1.46	4.7	5.4
					(2)	(3)	(2)	(1)	(3)	(2)	(1)	(1)
	Basal B-12§	96	164	0.60	16.1	5.8	5.3	0.40	1.36	1.11	3.2	4.0
					(1)	(3)	(2)	(1)	(4)	(2)	(1)	(1)

* Duration, 1st 4 weeks of life. Exp. 1 values are averages of both sexes. No sex differences were found in blood contents.
† Duration, 3rd and 4th weeks of life. Exp. 2 was performed with cockerels.
‡ Number of replicate determinations for each value shown in parentheses. Values are averages except those determined only once. Limitations were imposed by exhaustion of materials (protein-free filtrate) for analysis.
§ Merck and Co. APF Supplement No. 3, kindly provided by Dr. D. F. Green. Diet contained the equivalent of 50 μg of vit. B-12 per kg of feed.

7. Danielson, *J. Biol. Chem.*, 1933, v101, 505.
§ Only the first 4 in the case of Exp. 1.
8. Folin and Wu, *J. Biol. Chem.*, 1919, v38, 81.
9. Folin, *J. Biol. Chem.*, 1930, v86, 173.
10. Koch and McMeekin, *J. Am. Chem. Soc.*, 1924, v46, 2066.

birds given B-12 grew more rapidly. Since better growth was obtained at lower blood levels of amino acids, it appears likely that one function of vit. B-12 is to enhance anabolic processes, which remove amino acids from the blood to form fixed tissues. More efficient utilization of blood components leading to lower blood levels could well result in reduction of renal wastage. This in turn should be reflected in greater weight gain per unit of feed; which was found to be the case in both experiments.

The high efficiency of feed utilization observed in these experiments is of interest. Mishler, Carrick and Hauge(11) reported that when a similar ration containing presumably adequate B-12 (as fish solubles) was further supplemented with 0.3% DL-methionine, the methionine gave improved growth and feed utilization. In the present studies the diets were supplemented with 0.3% of DL-methionine, and feed utilization efficiency was very high even in the absence of B-12; although still further enhanced by its presence. A practical type, high energy starter containing animal products (2.5% fish meal, 7.5% meat and bone scrap, 2.5% dried whey) has given at best about 0.45 g gain per g feed. This ration presumably contained ample B-12. Methionine supplementation may have been a requisite condition for the high feed utilization efficiency observed in the present experiments.

The blood methionine levels were unexpectedly low compared to those of the other amino acids, since examination of the amino acid composition of both chicken muscle and of the diets used showed much higher relative contents of methionine than that found in blood. Similar low methionine values (0.106 to 0.317 mg%) were reported relative to other amino acid blood levels in rats.(12) These several findings may reflect some unique relationship of methionine in metabolism, particularly as to the role of vit. B-12 in amino acid utilization.

Summary. Vit. B-12 has been shown to reduce blood levels of non-protein nitrogen and of 7 individual amino acids from the levels found in vit. B-12 deficient chicks.

Chicks given vit. B-12 grew more rapidly and utilized feed more efficiently than B-12 deficient controls, although the latter had higher blood levels of amino acids.

Vit. B-12 appears to function in metabolism by enhancing utilization of circulating amino acids for building fixed tissues.

11. Mishler, D. H., Carrick, C. W., and Hauge, S. M., *Poultry Sci.*, 1948, v27, 263.

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Action of Aureomycin and Chloromycetin on the Virus of Primary Atypical Pneumonia.* (17563)

MONROE D. EATON (With technical assistance of Charlotte G. Levenson and Charlotte Hanks)

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston.

Recently published reports of clinical studies on the treatment of primary atypical pneumonia with aureomycin(1-4) have indicated effectiveness of this antibiotic in shortening the course of the disease. Additional evidence for the effectiveness of aureomycin against the virus of atypical pneumonia will be found in the experimental studies with cotton rats and chick embryos to be reported in this paper.

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4. Meiklejohn, J., and Shragg, R. I., *J. Am. Med. Assn.*, 1949, v140, 391.