7253 C

Metallic Glutamates in Nutritional Anemia.*†

ERWIN BRAND AND CHARLES J. STUCKY.

From the Department of Chemistry, New York State Psychiatric Institute and Hospital, New York.

Our knowledge of some of the many aspects of the problem of anemia has been enlarged recently by studies on the milk anemia of the rat. Thus from the work of the Wisconsin investigators the importance of Cu in Hb regeneration is now generally appreciated. Cu therapy, however, does not present any particular difficulty, while Fe deficiencies and Fe therapy still remain important problems in the clinic. For such investigations milk anemia is a convenient experimental approach. Although much work has been done there is still some uncertainty regarding the response of anemic rats to small doses of Fe.[1, 2] Certain investigators[3, 4] have reported that glutamic acid supplements Fe in nutritional anemia, but this has not been confirmed by others.[5]

The following experiments were carried out with about 300 rats in order to clarify some of the discrepancies in regard to the effect of Fe in anemia and to determine whether glutamic acid could be employed as an effective vehicle for the administration of minerals in secondary anemias.

Experimental. Most of the rats employed in the experiments were bred according to a modification of Elvehjem's technique.[6] The anemia producing diet was powdered whole milk (Klim, containing 1.8-2.0 p.p.m. of Cu and 14-17 p.p.m. of Fe) which was fed in the solid form. Hb estimations (Newcomer) were made weekly and red blood counts at frequent intervals. The inorganic Fe and Cu salts employed for supplements were of the highest purity; the

* In cooperation with the Research Department of the Calco Chemical Co., Bound Brook, N. J.

† Part of this paper was presented before the Division of Biological Chemistry at the Spring Meeting of the American Chemical Society, Washington, D. C., 1933.

[1] Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

[2] Mitchell, H. S., and Miller, L., *J. Biol. Chem.*, 1931, **92**, 421.

[3] Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **93**, 39.

[4] Rider, T. H., *J. Biol. Chem.*, 1933, **100**, 243.

[5] Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197. Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1932, **97**, 115.

[6] Elvehjem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

ferrous glutamate was Cu-free and the glutamic acid contained less than 0.2 mg. Cu per 100 gm.

Some of the experiments with $FeCl_3$, ferrous glutamate and glutamic acid are summarized in Table I. It appears from this table that Hb regeneration is just as effective with ferrous glutamate (Group 1) as with ferric chloride (Group 2) and that addition of glutamic acid produced similar results (compare groups 2 and 3). When glutamic acid (Group 5) was added to supplements of Fe+Cu (Group 4) the hematopoietic response appeared to be slightly greater.

TABLE I.
Response of Anemic Rats to $FeCl_3$, Ferrous Glutamate and Glutamic Acid.

Group	Supplement	No. of Rats		Aver. Hb Initial	2nd wk.	4th wk.	6th wk.	8th wk.
1	.25 mg. Fe as Ferrous Glutamate	5	a[1]	3.3	4.7	4.9	5.4	7.7
			b[2]	.21	.32	.37	.46	.74
2	.25 mg. Fe as $FeCl_3$	10	a	3.3	5.3	6.0	7.1	8.1
			b	.17	.33	.46	.63	.79
3	.25 mg. Fe ($FeCl_3$) + 70 mg. Glutamic Acid	14	a	3.2	4.7	5.6	6.5	8.0
			b	.20	.37	.50	.67	.88
4	.25 mg. Fe ($FeCl_3$) + .01 mg. Cu ($CuSO_4$)	7	a	3.9	6.7	9.5	11.6	13.7
			b	.33	.64	1.04	1.33	1.73
5	.25 mg. Fe ($FeCl_3$) .01 mg. Cu ($CuSO_4$) + 70 mg. Glutamic Acid	5	a	3.6	7.9	11.2	14.2	15.3
			b	.28	.72	1.13	1.48	1.75

[1] a = Hb in gm. per 100 cc. of blood.
[2] b = "Total" Hb in gm., *cf.* Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, XIII.

In view of this possible supplementary effect of glutamic acid when fed in conjunction with Fe and Cu, additional experiments were conducted. Groups of rats were fed the following combinations of supplements: 1. Mixtures of ferrous glutamate and copper glutamate at several levels of Fe and Cu. 2. Mixtures of ferrous glutamate and inorganic copper at several levels of Fe. 3. Mixtures of ferrous glutamate and inorganic Cu at several levels of Cu. 4. Mixtures of inorganic Fe and copper glutamate at several levels of Cu. 5. Copper glutamate at several levels of Cu. 6. Iron magnesium glutamate at several levels of Fe and Mg. 7. Iron magnesium glutamate plus glutamic acid. 8. Mixtures of Fe, Cu, Mg and Mn

as (a) inorganic salts, (b) glutamates, (c) inorganic salts plus glutamic acid, (d) glutamates plus glutamic acid. Because of the similarity of the results, only the data from group 8 (a-d) will be presented in Table II.

TABLE II.
Response of Anemic Rats to Metallic Glutamates.

Gr.	Supplement	No. of Rats		Average Hb Values Weeks 0	1	2	3	4	5	6
A	.25 mg. Fe as $FeCl_3$ .01 " Cu " $CuSO_4$ .30 " Mg " $MgCl_2$ .20 " Mn " $MnSO_4$	9	a[1]	2.8	5.6	7.0	8.4	9.6	12.3	12.7
			b[2]	.15	.37	.55	.72	.90	1.23	1.33
B	.25 mg. Fe .01 " Cu .30 " Mg .20 " Mn glutamates (Bivalent)	10	a	3.0	5.1	6.8	8.3	9.8	11.6	12.0
			b	.16	.34	.51	.68	.86	1.08	1.19
C	.25 mg. Fe as $FeCl_3$ .01 " Cu " $CuSO_4$ .30 " Mg " $MgCl_2$ .20 " Mn " $MnSO_4$ + 67 " Glutamic Acid	4	a	2.9	5.0	6.7	8.1	10.0	10.7	12.6
			b	.19	.36	.58	.79	1.05	1.22	1.49
D	.25 mg. Fe .01 " Cu .30 " Mg .20 " Mn glutamates (Bivalent) 60 " Glutamic Acid	4	a	3.8	4.6	6.4	7.8	9.1	10.0	12.0
			b	.25	.36	.57	.80	1.03	1.21	1.51

[1] Hb in gm. per 100 cc.
[2] "Total" Hb in gm., *cf.* Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, XIII.

It may be seen (Table II) that the glutamate compounds of Fe, Cu, Mg and Mn produced almost identical Hb regeneration and growth as equivalent amounts of these metals in inorganic form (See groups A and B). The addition of large amounts of glutamic acid (67 mg.) did not accelerate blood regeneration and growth (See groups C and D).

It should be noted that in all cases where Fe supplements only (Table I) are fed, there is a slow regeneration of Hb, which result

is in accord with the findings of Mitchel and Miller.[2] On the other hand in practically every group reported in Table I some of the rats failed to survive the experimental period of 8 weeks, which is in agreement with the observations of the Wisconsin investigators.

Summary. The influence of glutamic acid in the remission of milk anemia in rats was investigated and the efficiency of this substance as a vehicle for the administration of metals as hematopoietic agents tested. The effect of inorganic salts and of metallic glutamates was essentially the same and the further addition of glutamic acid did not alter this result.

7254 C

Pigmentation and Growth Rate in Gray Breast Feathers of Adult Mallard Drakes.

R. GEORGE JAAP. (Introduced by L. J. Cole.)

*From the Department of Genetics (No. 171), University of Wisconsin.**

Before the physiology of sexual dimorphism in birds can be completely understood the normal change from the male winter plumage to the female-like summer plumage in the Mallard drake must be explained. This report embodies certain facts regarding feather pigmentation and growth rate in Mallard drakes which must be considered in future studies of the physiological basis for this "eclipse" or "summer plumage" change of the adult male duck.

The males used in these experiments are semi-domesticated Mallards, the majority of which are genetically "dark-phase mallard" *(M Li)*.[1] These drakes are very uniform in breeding, and in adult body weight, between 980 and 1160 gm. The males are left in outside yards until they are to be used in an experiment, when they are brought indoors and kept in artificially heated and lighted pens, the hours of illumination being approximately the same in all trials. At the beginning of the experiment, 50 to 60 feathers are plucked from the middle of the ventral surface on either side of the keel, the plucked area being in the same location on each drake. All trials

*Published with the approval of the Director of the Agricultural Experiment Station. This investigation has received support from the Wisconsin Alumni Research Foundation and the Committee for Research in Problems of Sex of the National Research Council.

[1] Jaap, R. G., *J. Her.*, 1933, **24**, 467.