decreasing the total output of electric energy by the root, as measured. (d) The simultaneous addition of pure oxygen, after its absence, to all the regions, p, m, and o, produces a sharp drop in the total potential of the length X-Y, followed by a relatively enormous increase, frequently trebling the initial value. Distribution measurements show that in each root, the region, p, becomes highly electropositive to other regions, and the E.M.F.'s of the specific regions between contacts X and Y are each relatively greater in magnitude than in any previous period.

2. When electrodes are placed at the positions X and W, 13 millimeters above X, then: (a) A *drop* in the total E.M.F. of the length X-W is observed when oxygen is removed from the region indicated by the bracket A. On the other hand, a *rise* in the total E.M.F is observed upon the removal of oxygen around the region designated by C. Variable results are obtained when oxygen is removed around the region B The fact that the removal of oxygen around regions which exhibit oppositely oriented electric polarities produce opposite effects on the polarity of the whole furnishes confirmatory evidence of the principle of algebraic summation of cellular E.M.F.'s.

3. When electrode contacts are made at X and Y, and the region C, outside of the contacts, is exposed to absence and presence of oxygen, no change in the total E.M.F. of the length X-Y appears. Neither is a change in the total potential observed when the contacts are made at Y and W, and the region A is exposed to the same change in oxygen concentration.

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Glutamic Acid in Milk Anemia. Effect on Hemoglobin Regeneration in "Cystine Deficient" Animals.*†

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Certain investigators[1] have reported that glutamic acid supplements Fe in the treatment of milk anemia in rats, but this finding

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† This paper was presented in part before the Society of American Biological Chemists, Philadelphia, Pa., 1932.

[1] Drabkin, D. L., and Miller, H. K., *J. Biol. Chem.*, 1931, **90**, 531.

has not been confirmed by others.[2, 3] In our experiments[4] carried out with normal rats from our stock colony the addition of glutamic acid to various metals did not produce any supplementary effect on Hb regeneration and growth.

Certain observations,[5] however, appeared to indicate that factors other than the composition of the milk ration and the supplements *per se* might play a rôle in Hb regeneration. Accordingly the following experiments were undertaken.

A group of 8 rats which had been on a deficient diet was selected. These animals had received for approximately 3 months a ration of the following composition: corn starch 54.5, lard 24.5, casein 9, salt mixture (O.M.) 4.5, sugar 4.5, and cod liver oil 3%.[6] It should be noted that this diet is low in protein and deficient in cystine. This ration was continued for an additional 2 months, supplemented by fresh green lettuce (approximately 20 gm. per rat daily). At the end of this period the rats were bred (normal males were used). The fertility of the animals was found to be low and the mothers had difficulty in nursing their young properly. During the lactation period the mother rat received about 1 oz. of liquid milk per day in addition to the diet. When the pups were 18 days old, whole milk powder (klim) was substituted for the low protein (cystine-deficient) diet above. The pups were weaned when 22 to 25 days old and weighed 22 to 37 gm. Only 3 litters with a total of 11 animals were weaned successfully by the above procedure.

For the production of anemia the procedure previously described[4] was employed. The blood Hb level was reduced to approximately 4 gm. per 100 cc. in 3 to 7 weeks, except in one case in which it required 94 days to produce anemia.

The details of the experiment are shown in Table I. Five animals from 3 litters were fed 0.25 mg. of Fe. None of these rats recovered from the anemia and all of them died before the expiration of the 8th week. An attempt to save some of the animals by transferring them to other supplements was unsuccessful. Three animals were fed a larger dose of Fe (.5 mg.). Two of these rats

[2] Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1931, **93**, 197.

[3] Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1932, **97**, 115.

[4] Brand, E., and Stucky, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 627.

[5] Stucky, C. J., and Brand, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 932.

[6] Vitamin supplements: 200 mg. of vitavose + 50 mg. of yeast concentrate or 150 mg. of dried yeast.

died within a few weeks. The 3rd (Rat 651 of litter 91Y) regenerated, but it grew very slowly (weight 126 gm. at the end of the 12th week). Since 7 of the 8 animals which received Fe supplements (either at the .25 mg. or .50 mg. levels) failed to regenerate

TABLE I.

The Effect of Fe and of Glutamic Acid on Hb Regeneration in "Cystine Deficient" Milk Anemic Rats.

Litter No.	Rat No.	Supplement*	weeks on supplement		Hemoglobin Initial	Hemoglobin Final
89W	642 ♂	.25 mg. Fe + 70 mg. glutamic acid	12	a[1]	4.0	11.1
				b[2]	.28	1.32
	643 ♀	.25 mg. Fe	a†	a	3.9	3.2
				b	.20	.15
90YW	644 ♂	.25 mg. fe + .01 mg. Cu	12	a	3.5	14.2
				b	.20	1.52
	645 ♂	.25 mg. Fe + 70 mg. glutamic acid	12	a	4.4	12.4
				b	.27	1.40
	646 ♂	.5 mg. Fe	1†	a	3.2	2.1
				b	.15	.10
	647 ♂	.25 " "	5†	a	3.6	3.8
				b	.22	.16
	648 ♀	.25 " "	2†	a	3.7	2.7
				b	.20	.15
	649 ♀	.25 " "	6†	a	4.7	3.0
				b	.28	.16
91Y	650 ♂	.25 " "	3†	a	3.3	2.2
				b	.24	.12
	651 ♀	.5 " "	12	a	3.3	12.3
				b	.16	1.02
	652 ♀	.5 " "	3†	a	2.9	2.0
				b	.15	.10

* The Fe was fed as $FeCl_3$ and the Cu as $CuSO_4$.
† Animal died.
a^1 = Hb in gm. per 100 cc.
b^2 = "Total Hb" in gm., *cf.* Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, XIII.

and died, the result obtained with Rat 651 is perhaps due to individual variation.

On the other hand, the 2 rats (642 and 645) which received .25 mg. of Fe supplemented by 70 mg. of glutamic acid were cured of their anemia and showed good growth, attaining a weight of approximately 200 gm. by the end of the 12th week. A control animal (644) which received Fe+Cu showed more rapid Hb regeneration but the growth was slightly less than that of the rats which received glutamic acid.

In the above experiment we used the same milk (klim), Fe and glutamic acid preparations as in our recent experiments with normally bred animals from our stock colony.[4] The "cystine deficient" animals died on Fe supplements alone but recovered from their anemia when glutamic acid was added to the iron, while the "normal" animals survived on Fe supplements and the addition of glutamic acid was without effect.

The number of "cystine deficient" animals was necessarily small, due to the difficulties in breeding and, therefore, no definite conclusions can be drawn. Still it would appear suggestive that, under conditions, glutamic acid may have an hematopoietic effect.

Differences in the animals themselves may be an important consideration in interpreting the discrepancies in the findings of various laboratories.

Secondary anemias and particularly milk anemia in the rat may frequently appear to be only mineral deficiencies, but, under conditions, this simple picture may be complicated by other maladjustments which in turn will determine aspects of hematopoiesis and of therapy.

Summary. Rats bred from mothers subsisting on a low protein (cystine-deficient) ration recovered from their "milk anemia" and showed good growth when glutamic acid (70 mg.) was added to their iron supplement; whereas, those from which glutamic acid was withheld but the iron added, died. The significance of this finding has been indicated.