THE EFFECT OF COPPER OF EVAPORATED MILK ON HEMOGLOBIN REGENERATION IN NUTRITIONAL ANEMIA

by

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INTRODUCTION

The question of blood regeneration has commanded the attention of investigators since the discussion was opened before the beginning of the present century. Numerous experiments gave varied results and conclusions, and the subject was really not well understood. Since more has been learned of the vitamins, interest in hemoglobin formation and regeneration has been again aroused. Recently, certain mineral elements have been found to play an important part in nutrition, particularly in blood regeneration.

Within the last few years, authorities have shown that copper plays a leading role in hemoglobin building. As a study of the process of evaporation of milk seems to indicate that larger amounts of copper may be present in the finished product than are normally found in corresponding amounts of ordinary milk, it seemed of interest to determine whether or not the amount of copper in evaporated milk is sufficient to aid in blood regeneration in nutritional anemia.

HISTORICAL

Evidence concerning the utilization of inorganic and organic iron in the animal body has been vigorously sought and attacked from various angles. Inorganic iron has been variously designated as absolutely ineffective or as actually participating in hemoglobin building. Certain types of food iron have been reported as especially efficient blood builders. Those early experiments many of which were very comprehensive and apparently carefully performed, now require reinterpretation in the light of more recently developed knowledge. One point which seems to need special consideration is whether or not the experimental diet was deficient in vitamins. Also, the superiority of a diet in building hemoglobin might have been due to the presence of iron in a more available form or to some accompanying substance needed with iron for hemoglobin synthesis. Strong support for this view was given in the work of Hart and co-workers (1) in 1925. They found that the addition of inorganic iron to a basal ration of milk plus sodium citrate did not restore to normal the blood of anemic rabbits. But inorganic iron in the presence of fresh cabbage, an iron-free alcoholic extract of dessicated cabbage or an iron-free extract of chlorophyll, or yellow cornmeal, prevented or cured such an anemia. Williamson and Ets (2) in 1925 found inorganic iron, given to dogs made anemic by bleeding, was absorbed but not converted into hemoglobin. The iron, therefore, had no therapeutic value in anemia. In 1927, (3) Hart and Elvehjem, Waddell, and Herrin, found the ash of lettuce or cabbage corrected nutritional anemia induced by a diet of whole milk plus ferric oxide. Hart here pointed out the necessity of working with purified iron salts since he obtained prevention or cure of anemia when using ferrous sulfate which was labeled C.P., but was really impure. This material was much less effective when purified. Robscheit-Robbins and Whipple in 1927 (4) obtained contradictory results when anemic dogs were given iron salts. Here again the purity of the compounds used was questioned.

Many of the inconsistent results of previous work were certainly due to deficiency diseases rather than to lack of iron only. Recently, Whipple and his associates have shown the diet to be of the greatest importance in this type of work. Tests have been made with various foods and some found superior to others. In 1920 (5) these workers reported liver feeding to have a favorable influence in short anemia periods in dogs; since that time they (6) have shown a liver diet or an extract of liver, to have an extraordinary effect. In 1928 (7) the inorganic ash of apricots was found as potent for blood regeneration as the fresh apricot feeding.

Since the findings of Hart, Elvehjem, and Waddell, (3) were that ferric oxide alone cannot correct or prevent anemia but that it is effective when accompanied by iron-free organic compounds as chlorophyll, the attention of

Mitchell and Schmidt (8) in 1926 was turned to the comparisons of iron from various sources, organic and inorganic. Molasses, meat, ovoferrin, egg yolk, and spinach were found good as organic sources; ferric chloride and ferric ammonium citrate were found very good as inorganic sources while ferric oxide and ferrous carbonate were found poor. These observations suggested that a new line of differentiation be drawn as regards availability of iron in the animal organism, namely, soluble versus insoluble. The last four compounds named showed such interesting results that the attention of Mitchell and Vaughn (9) was directed to further studies of the availability of inorganic iron. Evidence shows that inorganic iron salts (chloride, sulfate, acetate, citrate, and phosphate) fed at a level of 0.5 mg. iron daily, failed to increase the hemoglobin level materially in anemic rats. Ashed residues of dried beef liver, dried lettuce, and yellow corn, and acid extracts of the same, fed at a level of 0.5 mg. iron intake, were very effective in anemia. The assumption is made that ashes and ash extracts contain in addition to iron some other inorganic substance vitally concerned in hemoglobin building.

McHargue, (10) in a publication on the significance of manganese, copper, zinc in forage crops and food, pointed out marked results he had obtained in plant experiments using some of the so-called non-essentials as copper, zinc, and manganese which appear to be factors in animal nutrition. In 1928, (23) McHargue, Healy, and Hill presented data to show that copper added to the diet of rats increased hemoglobin. Hart and coworkers found in 1928 (17) in analysis of lettuce ash, that copper was an active agent. Too, (18) these same workers in 1929 found the supplementing of a basal diet of whole milk and iron with several liver preparations, with hydrogen sulphide fractions of the acid extracts (of ashes of two of them) and with copper as a solution of copper sulphate, all on same level of copper intake, served equally well to cure nutritional anemia. This discovery is additional proof that the deficiency of this basal diet is inorganic in nature and is probably copper. It is not now the question of inorganic versus organic, or soluble versus insoluble but the heretofore unappreciated constituent copper which plays a role in hemoglobin building.

Titus and Cave (12), of this college, have presented data showing that manganese is effective in hemoglobin building in nutritional anemia, using the rabbit as the experimental animal. Titus, Cave, and Hughes (13) also found a combination of copper, manganese and inorganic iron to be more efficient in hemoglobin building in anemic rats than either iron and copper, or iron and manganese. Evidently manganese, as well as copper, plays some part in nutrition.

Working (14) in experiments conducted in the same laboratories, found that the combination of copper, manganese, and inorganic iron gave better hemoglobin regeneration when fed to chicks made anemic on milk plus wheat middlings diet than either iron and copper or iron and manganese.

Titus and Hughes (16) found that the copper of powdered whole milk plus 0.5 mg. iron caused excellent growth and hemoglobin production in anemic rats.

Since copper has been found an essential factor in hemoglobin building, Elvehjem, Peterson, and Lindow in 1929 (15), analyzed 160 common foods for copper content. Lindow and Peterson in 1927 (19), analyzed 84 common foods for manganese content. It is of interest to note that those foods found by different workers to be excellent blood regenerators, contain large amounts of copper. For example, calf liver contains 164.4 mg. copper per kilo of dry material, and 12 mg. manganese per kilo. Spinach rich in iron but classed as only moderately favorable in hemoglobin regeneration, contained 86.5 mg. manganese per kilo, dry basis, but only 6.9 mg. per kilo of copper. Pineapple like spinach, moderately favorable in hemoglobin building, contained 133.9 mg. manganese per kilo dry material, and 8.3 mg. copper per kilo. It appears that pineapple and spinach lay their potency to manganese content, but that this must be in conjunction with copper to bring about outstanding results. Because of the limited data available it will not be possible to know the average manganese and copper content of many plant foodstuffs until an extensive series of samples grown on different soils and under climatic conditions has been analyzed.

Since the role which copper plays in nutrition is still comparatively little known, it seemed of interest to conduct the following experiments to further study the role of copper in hemoglobin regeneration in four common brands of evaporated milk.

PROCEDURE

Normal albino rats four weeks of age, taken from the Sherman, Iowa, and Wisconsin rations, were earmarked, placed in five groups of ten each and fed the following kinds of milk:

Lot I	Brand P	Evaporated Unsweetened
Lot II	Brand V	Evaporated Unsweetened
Lot III	Brand C	Evaporated Unsweetened
Lot IV	Raw Whole Milk	
Lot V	Brand B	Evaporated Unsweetened

The evaporated milks were diluted one to one with distilled water and fed ad libitum in porcelain dishes sterilized daily. Raw milk was obtained fresh daily very soon after milking to eliminate contamination by copper and iron from any source. For the same reason the evaporated milks were placed in glass bottles after cans were opened. Since milk contains the necessary vitamins, except D, a few drops of cod liver oil were given daily in the morning feeding.

The animals were weighed and hemoglobin determinations made weekly throughout the experiment. In obtaining blood samples, the caudal vein was pricked with a sharp scalpel, a few centimeters from the end of the tail, giving a sufficient drop of blood. The samples were taken in 1:300 dilutions, diluted with a one-tenth of one per cent solution of sodium carbonate. After each blood sample was taken, the pipette was carefully washed with distilled water, alcohol and ether, and dried by suction. Determinations of grams hemoglobin in 100 cc. of blood were made by means of the Fleischl-Miescher Hemoglobinometer.

Diluted evaporated milk and raw whole milk comprised the diet of the respective groups until hemoglobin was sufficiently reduced, to five per cent, indicating anemia. The time required to produce this condition varied from two to eight weeks. Animals were then placed in individual wire cages. The corrective supplement, 0.5 mg. iron, was given in about two cc. of the morning feeding. Further food was withheld until supplement was consumed, when a liberal amount of milk was given each animal. Four animals of the raw milk group received 0.05 mg. copper in addition to iron in the basal ration. For a short time after rats became anemic, the diluted form of evaporated milk was given. It appeared that there animals were not receiving sufficient copper, hence through the remainder of the experiment the undiluted form was given.

The iron used as supplement was in the form of iron chloride. This was prepared from Mallinchrodt's iron wire such as is used for standardizing, dissolved in a calculated amount of Mallinchrodt's quality reagent hydrochloric acid. This solution was treated with hydrogen sulphide under pressure twelve hours to precipitate any copper present, filtered, and excess hydrogen sulphide removed by boiling. The filtered solution was then diluted to volume so that one-half cc. contained the 0.5 mg. iron to be given daily.

The copper used as a supplement for the raw milk group was prepared from Baker's reagent quality copper sulphate, C. P. A calculated amount was dissolved in distilled water, so that one-half cc. contained 0.05 mg. copper. The iron supplement which this group received was prepared from Baker's quality ferric chloride. A calculated amount was dissolved in distilled water so that one-half cc. contained 0.5 mg. iron.

The four brands of evaporated milks tested were analyzed in triplicate for copper content by the colorimetric method by Biazzo (20). Analysis showed copper content as follows:

Brand P	0.51 mg. per liter
Brand V	0.84 mg. per liter
Brand C	0.00 mg. per liter
Brand B	0.59 mg. per liter

The average consumption was 20 cc. each for all groups for a 60 to 75 gram rat, then each rat would receive daily approximately the following amounts of copper:

Brand P	0.0102 mg. copper for 20 cc. of milk
Brand V	0.0168 mg. copper for 20 cc. of milk
Brand C	0.0000 mg. copper for 20 cc. of milk
Brand B	0.0118 mg. copper for 20 cc. of milk

While the experiment was being conducted two of the brands of evaporated milk used in diluted form as basal diet showed ability to sustain normal hemoglobin. The question was raised whether all brands tested would give the same results used in the undiluted form with 0.5 mg. iron as supplement. Therefore, an experiment was carried out in which 20 normal albino rats, from the Iowa and Wisconsin rations, four weeks of age, were placed in individual cages and given the following rations:

Lot A 5 animals, Brand P undiluted plus 0.5 mg. iron

Lot B 5 animals, Brand V undiluted plus 0.5 mg. iron

Lot C 5 animals, Brand C undiluted plus 0.5 mg. iron

Lot D 5 animals, Brand B undiluted plus 0.5 mg. iron

Initial weights and hemoglobins were taken and biweekly thereafter. At the end of four weeks, one Iowa and one Wisconsin animal of each of the four groups was given 0.05 mg. copper in addition to the basal ration of milk and iron.

Composite tables and curves were prepared, showing relative weights and hemoglobin readings for the animals throughout the experiments. Complete data is given, including hemoglobin determinations expressed in grams of hemoglobin per 100 cc. of blood and weights of the animals in grams. For each lot of animals the data is given in two sets, namely, for the fore or preparatory period and also for the anemic or final period.

TABLE I

BRAND P, LOT I, FOREPERIOD

Hemoglobin

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.
1	8.16	6.54	5.70		
2	6.73	6.54			
3	6.10	6.93	5.91		
4	8.79	7.14	7.35	6.93	
5	6.73	8.47	7.14		
6	8.98	7.54	6.33	5.91	
7	8.97	6.73	5.91	6.33	
8	10.22	6.33	6.93		
9	7.74	6.93	5.31		
10	7.18	4.69			
Av.	7.96	6.78	6.32	6.39	

Weights in Grams

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.
1	50	60	62		
2	45	65			
3	47	63	65		
4	47	66	71	75	
5	50	63	77		
6	43	54	60	61	
7	48	60	64	66	
8	49	58	65		
9	40	52	57		
10	43	53			
Av.Wt.	46.2	59.4	65.1	67.3	
Av.Gain		13.2	18.9	21.1	

TABLE II

BRAND P PLUS IRON, LOT I, FINAL PERIOD

Hemoglobin

No.	Initial: Wt.	1wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.
1	4.08	Dead						
2	4.50	3.87	5.10	6.54	4.89	Dead		
3	4.69	3.66	5.31	6.73	6.54	7.35	7.74	
4	5.91	7.14	8.16	6.12	5.10	Dead		
5	4.50	3.27	4.89	8.16	8.97	9.39	11.01	
6	3.80	6.73	9.58	10.52	8.97	7.74	7.54	
7	3.27	Dead						
8	5.70	4.69	5.10	5.50	3.87	2.46	2.65	
9	4.08	3.31	4.08	Dead				
10	4.50	3.33	2.65	2.85	Dead			
Av.	4.50	4.50	5.60	6.66	6.39	6.73	7.23	

Weights in Grams

No.	Initial: Wt.	1wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.
1	54	Dead						
2	73	77	80	82	61	Dead		
3	67	70	82	88	81	76	64	
4	74	73	68	66	80	Dead		
5	79	78	76	78	86	96	100	Dead
6	56	66	77	76	75	66	59	Dead
7	67	Dead						
8	65	64	61	62	55	55	64	
9	56	55	47	Dead				
10	58	56	46	Dead				
Av.Wt.	64.9	67.3	67.1	75.3	69.6	73.2	71.7	
Av.Gain		2.4	2.2	10.4	4.7	8.3	6.8	

TABLE III BRAND V, LOT II, FOREPERIOD

Hemoglobin

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.
11	7.89	4.29							
12	11.44	8.97	8.97	8.77	9.78	11.01	9.18	7.74	
13	9.52	9.18	7.95	6.26	6.33	6.93	Dead		
14	11.22	8.47	6.12						
15	8.16	7.95	6.93						
16	9.78	5.50	5.50						
17	9.58	6.26	5.91						
18	8.16	5.10							
19	4.89	6.54							
20	8.26	7.95	6.54	7.54	8.58	8.58	7.54	Dead	
67	8.16	8.16	8.77	9.99	10.62	10.22	10.41	9.58	Disc.
68	7.74	8.97	8.77	8.97	8.58	8.16	8.58	10.20	Disc.
Av.	8.73	7.27	7.38	8.30	8.78	8.98	8.92	9.17	

Weights in Grams

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.
11	49	59							
12	60	78	91	88	89	87	83		
13	48	62	74	76	77	65	Dead		
14	57	66	81						
15	53	61	75						
16	54	63	70						
17	50	64	75						
18	57	71							
19	66	78							
20	58	68	80	90	92	90	88	Dead	
67	84	95	98	87	81	79	82	102	Disc.
68	65	75	79	83	90	88	94	101	Disc.
Av.Wt.	58.4	69.8	80.3	84.8	85.8	81.8	89.2	101.5	
Av.Gain		11.4	21.9	26.4	27.4	23.4	30.8	43.1	

TABLE IV BRAND V PLUS IRON, LOT II, FINAL PERIOD

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.	9 wk.
					Hemoglobin					
11	4.08	4.89	5.70	9.18	9.58	10.41	12.24	12.43	8.97	Dead
12	6.54	6.52	Dead							
14	5.50	4.08	7.14	11.62	11.22	11.22	Dead			
15	5.50	5.50	5.31	Dead						
16	4.89	Dead								
17	5.10	6.54	6.73	7.14	7.14	9.78	12.63	12.63	14.67	Disc.
18	4.89	4.08	5.91	8.58	7.95	6.33	8.97	10.23	11.01	Dead
19	4.89	5.91	6.93	Dead						
69	6.50	8.97	9.58	10.20	12.03	13.26	13.47	13.05	Disc.	
70	6.50	10.20	12.24	12.24	13.26	12.43	13.47	13.05	Disc.	
Av.	5.43	6.28	7.44	9.82	10.19	10.57	12.15	12.27	11.55	
					Weights in Grams					
11	72	74	82	82	86	100	106	104	100	Dead
12	68	65	Dead							
14	86	81	75	89	103	108	Dead			
15	75	71	64	Dead						
16	76	Dead								
17	80	87	87	92	103	112	121	129	135	
18	86	91	101	109	113	125	118	99	84	
19	82	84	79	Dead						
69	74	88	100	105	116	124	131	Disc.		
70	71	83	95	102	115	124	124	Disc.		
Av.Wt.	77.0	80.4	85.3	96.5	106.0	115.5	120.0	110.6	106.3	
Av.Gain		3.4	8.3	19.5	29.0	38.5	43.0	33.6	29.3	

TABLE V

BRAND C, LOT III, FOREPERIOD

No.	Initial Wt.	1 wk.	2 wk.	3 wk.
Hemoglobin				
21	12.43	6.80	6.12	Dead
22	7.14	7.35		
23	9.39	5.70		
24	10.62	7.35		
25	6.80	6.54		
26	10.41	5.31		
27	11.43	7.95	6.12	
28	7.45	4.89		
29	7.45	6.12		
30	7.14	5.70		
72	8.47	6.54	7.14	7.14
81	7.54	7.95	8.16	6.12
Av.	8.85	6.51	6.88	6.63
Weights in Grams				
21	62	71	72	
22	50	67		
23	53	68		
24	44	57		
25	45	59		
26	40	62		
27	51	68	68	
28	42	55		
29	47	60		
30	59	71		
72	90	102	102	95
81	52	65	71	73
Av.Wt.	52.9	67.0	78.2	84
Av.Gain		14.1	25.3	31.1

TABLE VI

BRAND C PLUS IRON, LOT III, FINAL PERIOD

Hemoglobin

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.
22	4.89	4.89	3.87	Dead	
23	4.89	4.29	4.29	4.69	Dead
24	5.70	4.08	3.06	Dead	
25	3.87	Dead			
26	4.89	3.87	Dead		
27	3.31	Dead			
28	3.87	Dead			
29	5.50	4.89	5.50	5.91	Dead
30	5.70	5.31	5.31	Dead	
72	6.54	6.12	Dead		
81	6.12	4.29	Dead		
Av.	5.02	4.71	4.40	5.30	

Weights in Grams

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.
22	65	68	66	Dead	
23	68	73	71	66	
24	58	65	60	Dead	
25	62	Dead			
26	67	69	Dead		
27	69	Dead			
28	57	57	Dead		
29	68	70	70	64	Dead
30	81	84	86	Dead	
72	88	89	Dead		
81	73	67	Dead		
Av.Wt.	68.7	71.3	70.6	65.0	
Av.Gain		2.6	1.9		

TABLE VII

RAW MILK, LOT IV, FOREPERIOD

Hemoglobin

No.	Initial Wt.	1 wk.	2 wk.	3 wk.
31	13.04	6.54	6.73	
32	9.78	6.12		
33	13.05	3.87		
34	9.78	6.33	5.70	
35	8.97			
36	8.16	6.73	7.35	
37	12.63	6.12		
38	9.99	6.33		
39	8.08	6.33		
40	7.14	5.70		
Av.	10.62	6.00	6.59	

Weight in Grams

No.	Initial Wt.	1 wk.	2 wk.	3 wk.
31	59	71	90	
32	58	70		
33	46	59		
34	48	54	67	
35	59	76		
36	59	74	87	
37	51	61		
38	50	64		
39	51	64		
40	53	70		
Av.Wt.	53.4	66.3	81.3	
Av.Gain		12.9	27.9	

TABLE VIII

RAW MILK PLUS IRON, LOT IV, FINAL PERIOD

Hemoglobin

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.	9 wk.	10 wk.	11 wk.
32	4.29	4.08	4.08	3.31	2.46	2.46						
34	4.08	3.31	3.66	Dead								
35	4.50	5.50	4.89	4.89	2.46	Dead						
37	4.69	3.66	Dead									
39	4.55	4.50	5.91	8.47	7.35	6.12	7.74	7.60	5.10	4.69		
40	4.69	3.66	3.31	4.08	2.46	Dead						
Av.	4.46	4.11	4.37	5.18	3.68	4.29						

Weights in Grams

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.	9 wk.	10 wk.	11 wk.
32	86	87	81	83	86	85	Dead					
34	70	61	70	Dead								
35	92	96	92	99	88	Dead						
37	65	66	Dead									
39	76	80	77	85	105	115	118	101	99		105	Dead
40	83	90	88	91	85	82	Dead					
Av.Wt.	78.6	80.0	81.6	89.5	91	94						
Av.Gain		1.4	3.0	10.9	12.4	15.4						

TABLE IX

RAW MILK PLUS COPPER AND IRON, LOT IV, FINAL PERIOD

Hemoglobin

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.	9 wk.
31	4.50	5.91	13.86	15.30	12.84	12.03	15.90	14.07		
33	3.06	2.65	5.31	9.58	11.22	11.62	11.22	13.26	12.24	
36	5.10	7.17	12.84	9.80	10.62	11.82	14.28	14.28		
38	4.08	3.66	6.73	13.86	11.82	12.43	13.66	11.43	13.86	
Av.	4.18	4.84	9.68	12.13	11.62	11.97	13.76	13.26	13.05	

Weights in Grams

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.	9 wk.
31	90	92	93	90	107	133	161	185	195	205
33	65	63	54	68	89	118	136	160	177	186
36	93	101	97	112	142	155	170	175	181	
38	70	67	61	76	91	120	131	141	149	157
Av.Wt.	79.5	80.7	76.2	86.5	107.2	131.5	149.5	165.2	175.5	182.6
Av.Gain		1.2		7.0	27.7	52.0	70.0	85.7	96.0	103.1

TABLE X BRAND B, LOT V, FOREPERIOD

Hemoglobin

No.	Initial Wt.	1wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.
41	9.24	8.77	8.44	8.77	9.91	7.14	Dead		
42	8.98	9.39	11.22	9.39	11.01	10.81	8.98	9.24	Dead
43	13.86	10.41	9.78	Dead					
44	12.03	7.95	7.95	9.18	8.97	8.16	8.16	8.16	Dead
46	9.80	9.78	8.97	9.18	7.95	8.58			
47	7.74	7.34	9.18	6.54					
48	8.77	8.58	8.77						
49	7.95	9.18	7.35						
50	10.20	7.14	7.14	8.77	6.73	7.35	Dead		
95	8.77	7.74	8.77	8.58	7.54	8.58	9.78	Disc.	
99	7.74	8.16	8.58	Dead					
Av.	9.55	8.58	8.74	8.63	8.68	8.43	8.97	8.70	

Weights in Grams

No.	Initial Wt.	1wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.
41	42	51	54	54	51	48	Dead		
42	45	62	63	66	66	65	60	55	Dead
43	45	52	54	44	Dead				
44	41	49	50	57	57	59	59	55	Dead
46	48	51	60	73	75	79			
47	55	55	62	74					
48	54	54	60						
49	55	55	62						
50	56	56	65	76	81	85	82	Dead	
95	58	67	66	71	78	80	85	Disc.	
99	66	78	72	Dead					
Av.Wt.	51.3	57.2	60.7	64.3	68.0	69.3	71.5	55.0	
Av.Gain		5.9	9.4	13.0	16.7	18.0	20.2	3.7	

TABLE XI BRAND B PLUS IRON, LOT V, FINAL PERIOD

Hemoglobin

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.
46	6.33	6.12	Dead						
47	5.31	5.10	Dead						
48	5.31	7.54	7.54	Dead					
49	5.70	4.89	Dead						
93	6.73	7.14	8.16	11.62	11.62	10.62	9.58	Disc.	
94	6.73	7.14	6.93	Dead					
98	5.70	6.73	Dead						
100	6.93	9.78	9.99	10.20	12.24	Dead			
Av.	6.09	6.80	8.01	10.91	11.93				

Weights in Grams

No.	Initial Wt.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.
46	76	64	Dead						
47	80	71	Dead						
48	77	84	80	Dead					
49	77	81	Dead						
93	66	74	93	100	110	113	117	Disc.	
94	54	63	67	Dead					
98	51	60	58						
100	62	74	81	82	78	Dead			
Av.Wt.	67.8	71.3	75.8	91.0	94.0				
Av.Gain		3.5	8.0	23.2	26.2				

TABLE XII

NORMAL RATS - BASAL DIET OF UNDILUTED EVAPORATED MILK PLUS

0.5 MG. IRON, BRAND P, LOT A

Hemoglobin

No.	Initial Hb.	2 wks.	4 wks.	6 wks.	
1	8.72	10.81	12.63	7.54	
2	6.54	10.81	13.26	14.47	+ Cu at 4th wk.
3	10.20	11.43	12.03	11.43	+ Cu at 4th wk.
4	12.43	12.63	12.84	10.82	
5	11.43	12.84	13.47	10.81	
Av.Hb.	9.86	11.70	12.84	9.72 + Fe	
				12.95 + Cu	

Weights in Grams

No.	Initial	2 wks.	4 wks.	6 wks.	
1	51	72	92	109	
2	62	104	134	174	+ 0.05 mg. Cu at 4th wk.
3	83	125	158	185	+ 0.05 mg. Cu at 4th wk.
4	86	112	130	132	
5	73	110	117	125	
Av.Wt.	71	104.6	126.2	122 + Fe	
Av.Gain		33.6	55.2	179 + Cu	
				51.+ Fe	
				108 + Cu	

TABLE XIII

NORMAL RATS - BASAL DIET OF UNDILUTED EVAPORATED MILK

PLUS 0.5 MG. IRON, BRAND V, LOT B

Hemoglobin

No.	Initial: Hb.	2 wks.	4 wks.	6 wks.	
6	7.54	10.20	10.20	12.84	
7	7.14	9.58	9.18	15.18	+ Cu at 4 wks.
8	12.03	10.62	11.43	13.47	+ Cu at 4 wks.
9	11.01	13.26	13.47	11.22	
10	11.01	11.01	13.05	10.20	
Av.Hb.	9.74	10.73	11.46	11.42	+ Fe
				14.32	+ Cu

Weights in Grams

6	51	84	105	112	
7	58	77	105	83	+ 0.05 mg. Cu at 4 wk.
8	94	130	157	185	+ 0.05 mg. Cu at 4 wk.
9	70	110	146	162	
10	79	106	123	137	
Av.Wt.	70.4	101.4	127.2	137	+ Fe
Av.Gain		31.0	56.8	134	+ Cu
				66.6	+ Fe
				63.6	+ Cu

TABLE XIV

NORMAL RATS - BASAL DIET OF UNDILUTED EVAPORATED MILK
PLUS 0.5 MG. IRON, BRAND C, LOT C

Hemoglobin

No.	Initial Hb.	2 wks.	4 wks.	6 wks.	
11	8.47	8.97	7.95	Dead	
12	8.47	9.78	8.58	9.18	+ Cu at 4th wk. on Expt.
13	12.03	11.43	8.58	12.03	+ Cu at 4th wk. on Expt.
14	11.43	11.43	9.18	6.73	
15	11.01	11.62	8.16	8.16	
Av.Hb.	10.28	10.64	8.49	7.44	+ Fe
				10.60	+ Cu

Weights in Grams

11	51	76	79	Dead	
12	65	97	110	133	+ 0.05 mg. Cu at 4th wk.
13	76	112	135	162	+ 0.05 mg. Cu at 4th wk.
14	81	110	135	133	
15	75	107	132	136	
Av.Wt.	69.6	100.4	118.2	134.5	+ Fe
Av.Gain		30.8	48.6	147.5	+ Cu
				64.9	+ Fe
				77.9	+ Cu

TABLE XV

NORMAL RATS - BASAL DIET OF UNDILUTED EVAPORATED MILK PLUS 0.5 MG. IRON, BRAND B, LOT D

Hemoglobin

No.	Initial: Hb.	2 wks.	4 wks.	6 wks.	
16	10.41	10.62	10.20	10.20	
17	8.58	11.01	11.01	13.26	+ Cu at 4th wk. on Expt.
18	8.16	8.16	10.41	13.47	+ Cu at 4th wk. on Expt.
19	12.64	11.43	9.18	9.18	
20	10.20	13.26	11.82	11.43	
Av.Hb.	9.99	10.89	10.52	10.27	+ Fe
				13.36	+ Cu

Weights in Grams

16	52	85	100	118	
17	51	90	122	161	+ 0.05 mg. Cu at 4th wk.
18	65	97	128	161	+ 0.05 mg. Cu at 4th wk.
19	80	110	140	160	
20	71	106	119	128	
Av.Wt.	63.8	97.6	121.8	135.3	+ Fe
Av.Gain		33.8	58.0	161.0	+ Cu
				71.5	+ Fe Av. Gain
				97.2	+ Cu Av. Gain

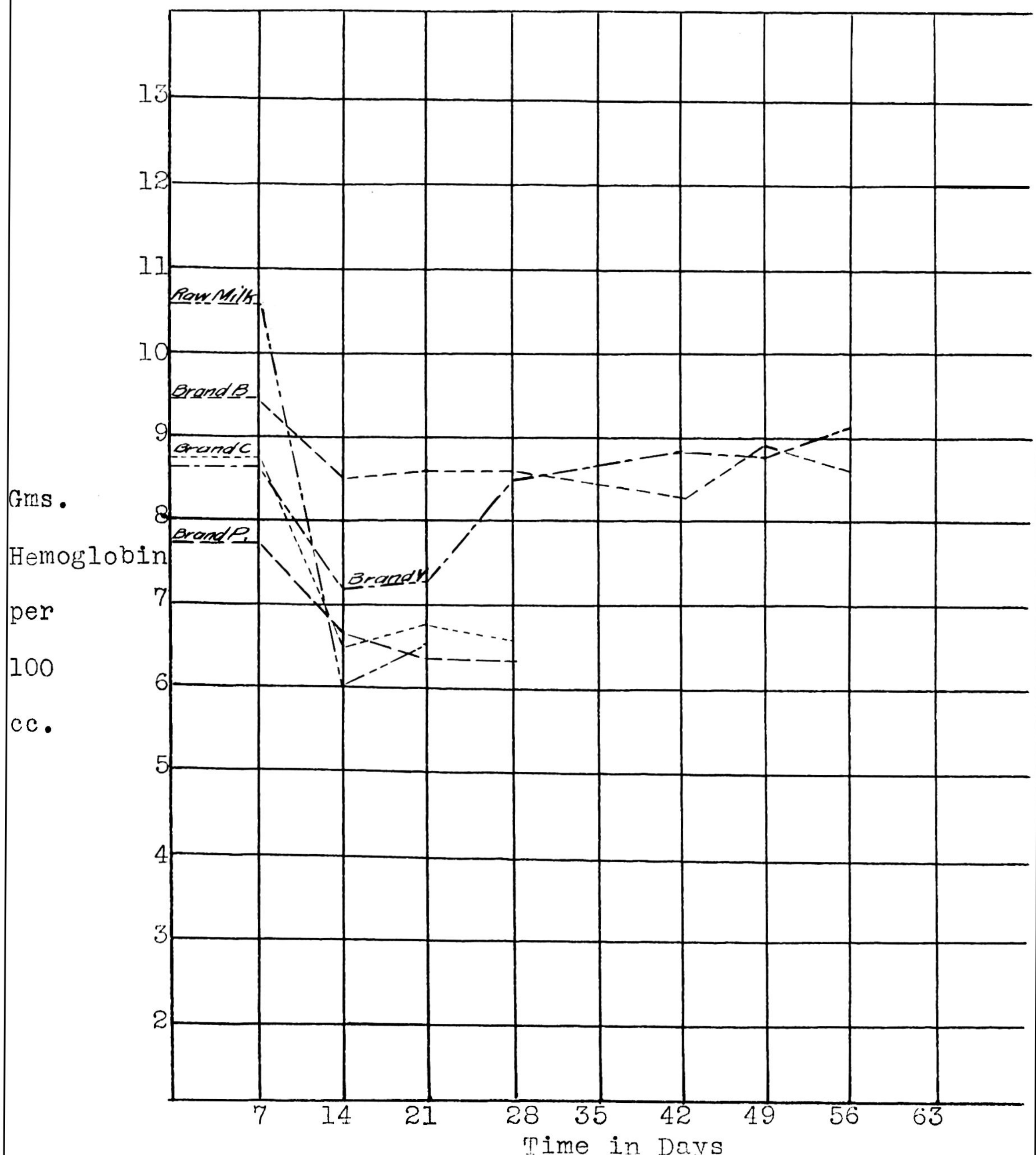

Fig. 1. Hemoglobin Foreperiod.

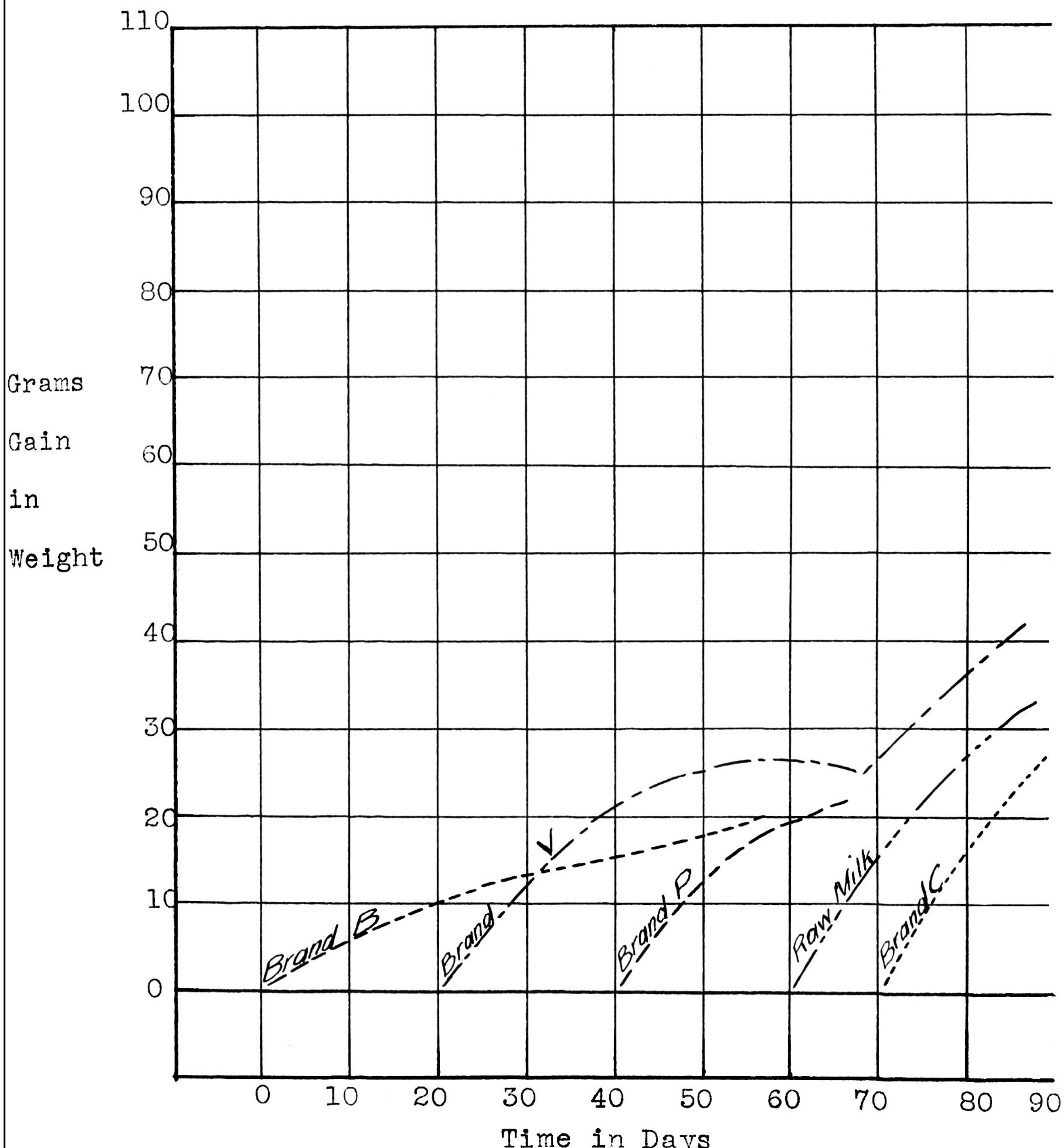

Fig. 2. Weights foreperiod.

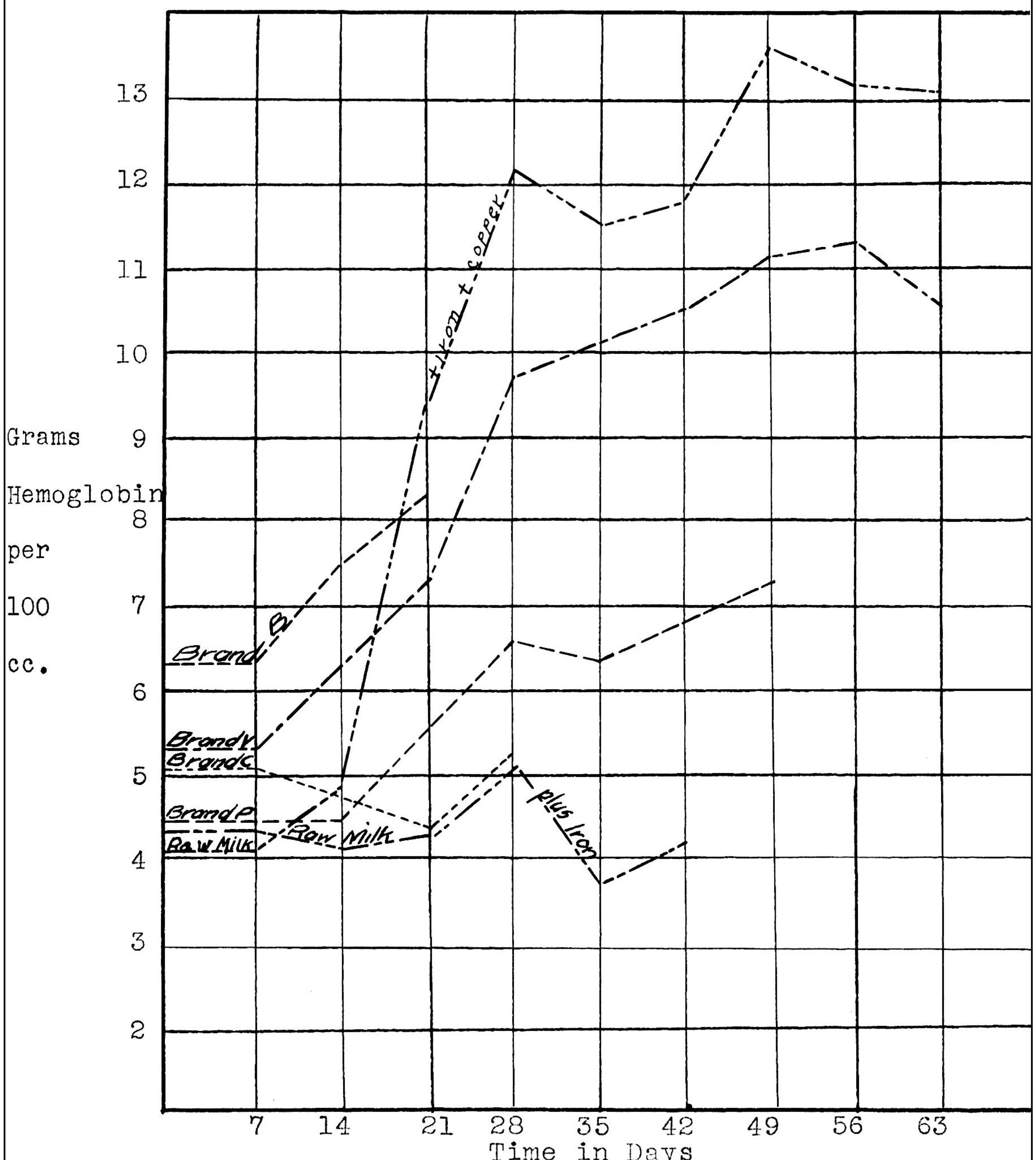

Fig. 3. Hemoglobin anemia plus iron.

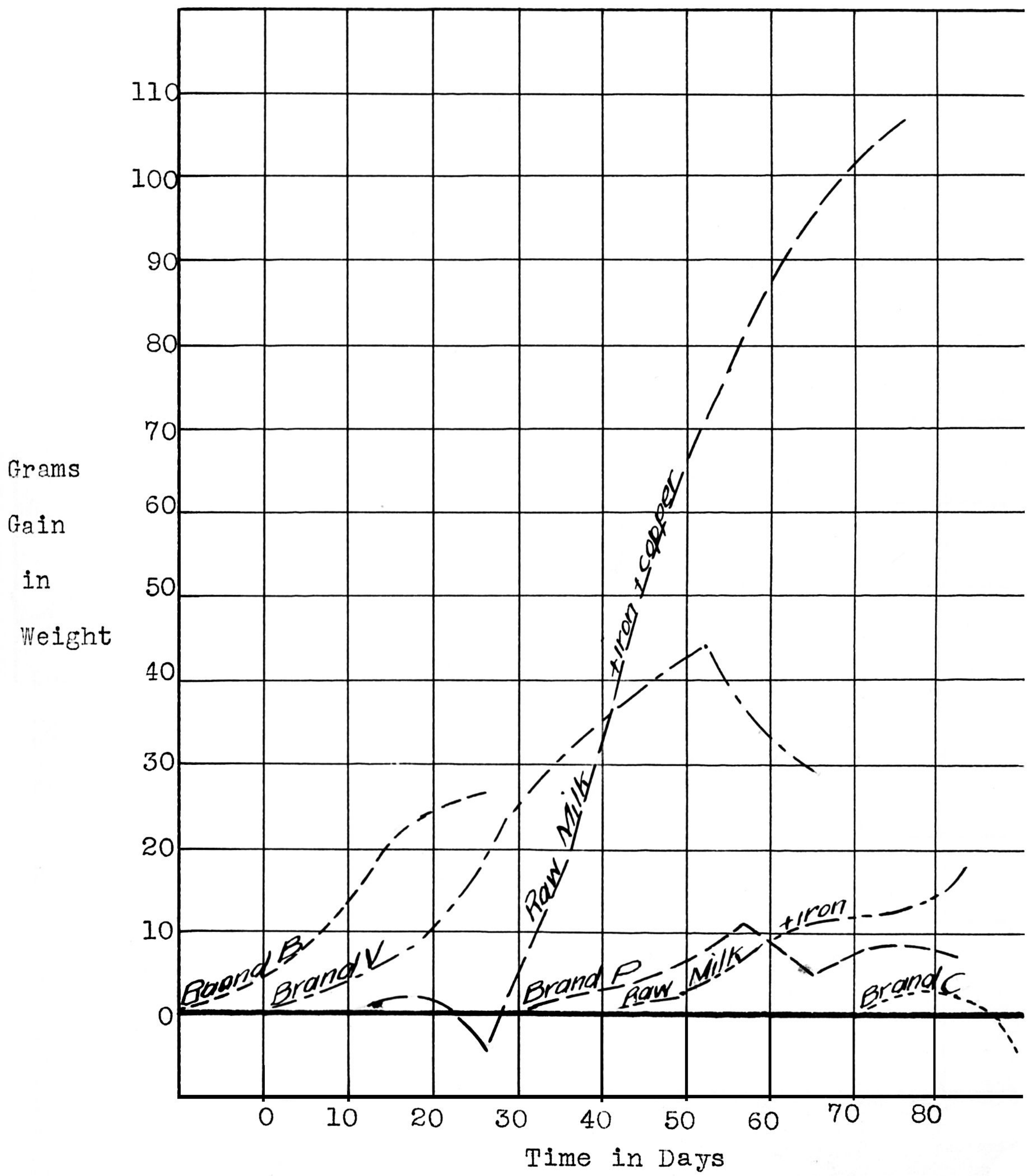

Fig. 4. Weights anemia plus iron.

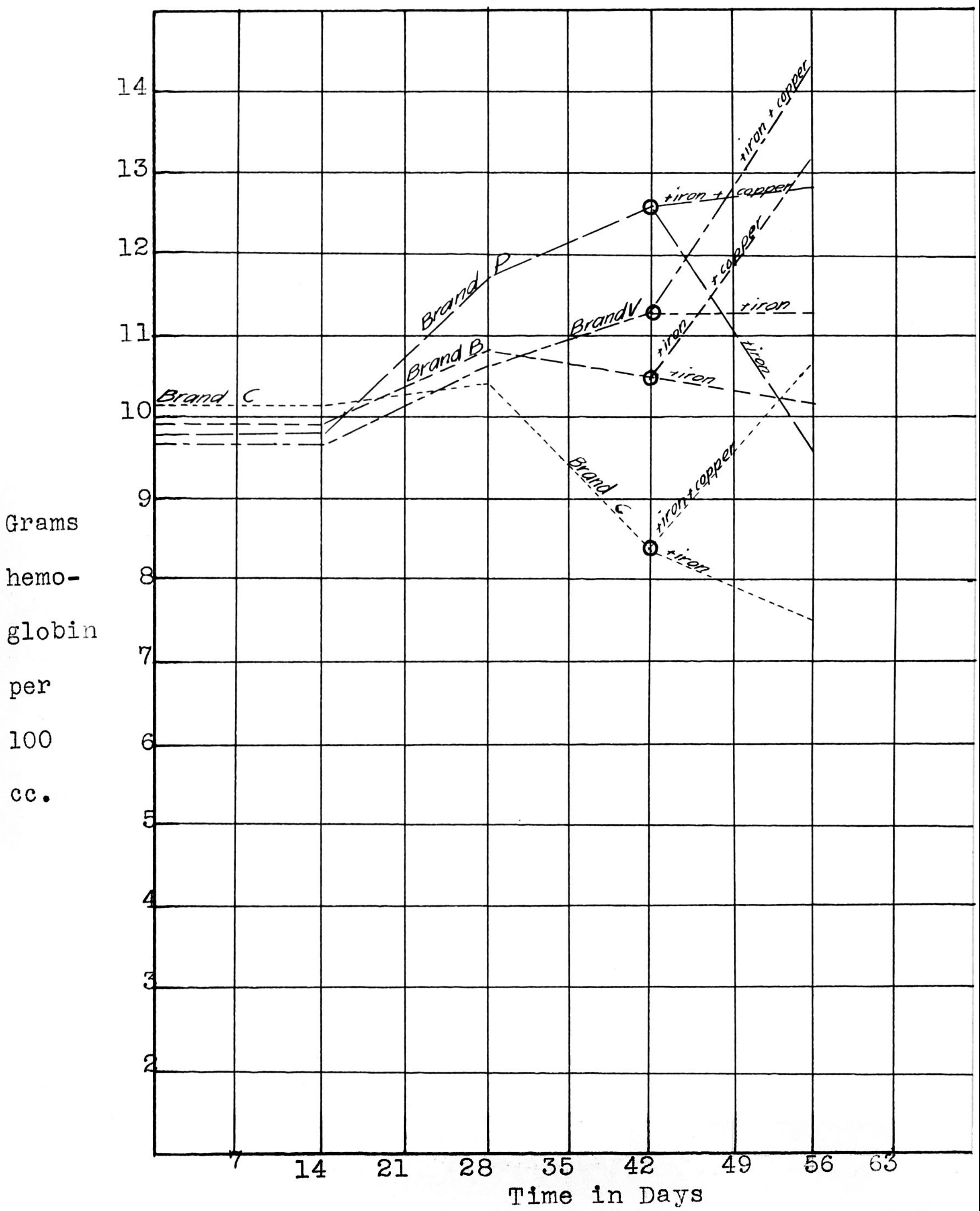

Fig. 5. Normal hemoglobin plus 0.5 mg. Fe.

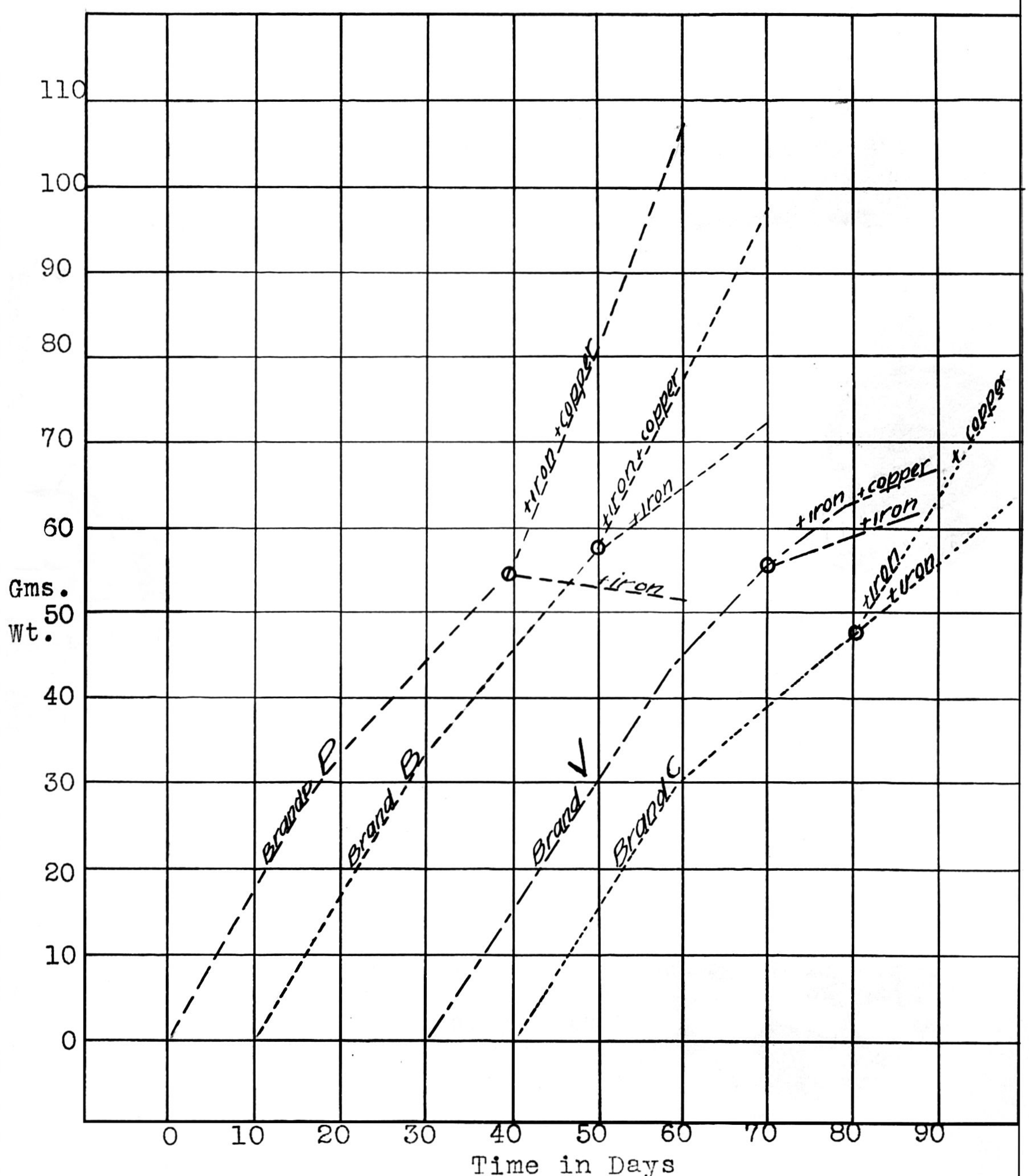

Fig. 6. Weights. Normal Hemoglobin ÷ 0.5 mg. Iron.

Fig. 7.

Lot I Brand P

Age: four months, one week

Weight: 65 grams. Hemoglobin: 6.33%

On Experimental diet plus iron for 10 weeks.

Fig. 8.

Lot II Brand V

Age: 4 months, one week.

Weight: 135 grams. Hemoglobin: 14.67%

On experimental diet plus iron for nine weeks.

Fig. 9.

Lot V Brand B

Age: Three months

Weight: 100 grams. Hemoglobin: 9.18%

On experimental diet for six weeks plus iron.

Fig. 10.

Lot IV Raw Milk

Left of picture:

Age: four months

Weight: 180 grams. Hemoglobin: 14.28%

On experimental diet for nine weeks plus copper and iron.

Right of picture:

Age: four months

Weight: 101 grams. Hemoglobin: 4.69%

On experimental diet for ten weeks plus iron.

DISCUSSION

The experiments carried out here serve again to emphasize the importance of certain nutritional elements in the animal organism, especially the importance of copper for hemoglobin formation.

Analyses of the different brands of milk tested show Brand V to have the highest copper content with the remaining brands in order named: Brand B second, Brand P third, Brand C fourth. This is in accordance with experimental findings.

Since the copper content of raw milk has been previously determined by other authorities, an analysis was not carried out here. However, there is a variance in results of analyses. Supplee and Bellis (20) found the copper content of cow's milk to be 0.52 mg. per liter. Quam and Hellwig (21) placed the figure at about 0.4 mg. per liter. On the assumption that 0.52 mg. is correct, a rat consuming 40 cc. daily would receive 0.024 mg. copper. This amount would account for regeneration of hemoglobin, when fed in connection with an iron salt. Since regeneration of hemoglobinglobin has not been secured on raw milk and iron, Hart and co-workers (22) were interested in following the copper content of milk to determine the actual amount present and to detect any variation which might occur under various conditions. They found milk produced by cows on a normal ration, contained about 0.15 mg. per liter. This substantiated their biological findings. They found a definite regeneration of hemoglobin produced by an addition of 0.005 mg. copper and 0.5 mg. iron to a basal ration of milk fed to anemic rats, each rat receiving 0.01 mg. copper daily. In a previous experiment (17) these workers found those animals receiving 0.05 mg. and 0.1 mg. levels of copper daily had a much more rapid recovery to normal; while those receiving 0.01 mg. copper slowly showed marked increases with a final return to normal. These authors did not determine conclusively how much copper a rat needed in order to maintain its synthesis of hemoglobin at a normal rate.

The analyses of milks tested in this experiment were subject to the many difficulties encountered in the determination of minute amounts of copper. However, much care was exercised in using copper and iron-free solutions and avoiding contamination from any source. If all analyses were correct, 0.01 mg. copper seemed insufficient for hemoglobin regeneration as indicated in Figures 3 and 4 in which composite curves show relative comparison of all milks tested. Those animals receiving Brand P consuming 20 cc. each, received 0.01 mg. copper daily as a maximum average. Only those animals consuming at least 20 cc. or more showed a rise in hemoglobin. It is probable that scarcely 0.01 mg.

copper was received by the majority of animals, which would account for lack of regeneration to normal. One remaining animal of this group showed interesting and questioning results. At the end of seven weeks of anemia, hemoglobin was 3.54 per cent. Feeding was then begun from a new case of Brand P milk. An immediate rise in hemoglobin was noted; in four weeks had reached 10.20 per cent. A second group of animals placed on this milk at this time was much more difficult to make anemic than previous groups. The question was raised whether this new lot of milk might not contaon a greater amount of copper, since the results obtained could be attributed to no other variable. It appeared that different lots of the same brand of evaporated milk might contain different amounts of copper.

Brand C brought no beneficial results whatever. Animals became anemic in two weeks on the basal ration, lost steadily and all were dead at the end of four weeks as shown in Table VI. Analyses and experimental evidence indicated this brand to be low in copper.

Those animals receiving Brand B presented interesting data. Rats were difficult to make anemic on this milk. Fig. 1 and Table X showing hemoglobin of the foreperiod indicates this milk maintained hemoglobin above eight per cent for a period of eight weeks. At this point a number of the animals were discontinued. But those animals falling below six per cent hemoglobin of this group did not seem to be able to regenerate, however, this point was not proved. Apparently this milk was able to maintain hemoglobin over long periods but was not able to promote regeneration. Throughout the experiment, this milk did not seem to agree with the rats as well as the other brands, difficulty being encountered in preventing infection.

Brand V was found best of the evaporated milks tested, comparing closely to the group receiving raw milk with added copper in regard to hemoglobin regeneration. This evaporated milk was also better in promoting growth but did not give the good growth found in the raw milk group with added copper, as shown in Fig. 4. Brand V also brought about a long period of sustained hemoglobin level on the basal ration of diluted milk. A number of these animals were discontinued after eight weeks when anemia was not secured. This group showed regeneration when hemoglobin was reduced to four per cent which was not found true of Brand B. Animals of this group were thrifty, vigorous, showed excellent coats, and had good color. At the end of 30 days hemoglobin was normal.

Authorities have found that a basal diet of raw milk plus inorganic iron does not bring about regeneration of blood. This fact was again shown in this experiment. As Tables VIII and IX indicate, those animals receiving iron showed no regeneration, while those receiving 0.05 mg. copper in addition to 0.5 mg. iron, regenerated hemoglobin to normal in 21 days. Growth was very rapid, and an excellent state of nutrition resulted in the animals receiving the two minerals.

It was apparent that some factor was missing in all of the evaporated milks since the growth curve of the group receiving raw milk with added copper was much greater than any of those of the evaporated milk group.

Figures 7, 8, 9 and 10 show comparisons of the appearance and size of typical animals of all the groups. In Fig. 10 the superiority of the animal of the raw milk group, receiving copper in addition to iron, over the one receiving iron only can readily be seen. Fig. 8 shows that Brand V of the evaporated milks produced superior animals, although not equal to those animals receiving whole milk and copper in addition to iron. The animals of Brand C did not survive until the animals of the other lots were in a suitable condition for showing differences by photograph.

A second experiment conducted to determine the effect of a basal diet of undiluted evaporated milk plus 0.5 mg. iron daily on normal hemoglobin, again showed the necessity of a sufficient amount of copper in the utilization of inorganic iron. As Fig. 5 indicates, Brand P sustained normal hemoglobin for a period of four weeks then dropped, but a rapid rise is noted when 0.05 mg. copper is added to the daily ration. Those animals of this group receiving iron only and no copper, continued to drop slowly.

Brands B and V sustained normal hemoglobin throughout but those animals of both groups receiving 0.05 mg. copper in addition to the 0.5 mg. iron showed a greater increase in hemoglobin than those animals receiving only iron. Apparently the amount of copper received in the milk was not optimal. Again Brand V was best of all evaporated milks tested.

Those animals receiving Brand C again demonstrated the importance of copper in utilization of inorganic iron. A steady decline in hemoglobin resulted when these animals received only 0.5 mg. iron in addition to the daily ration, but a rapid rise was noted on addition of 0.05 mg. copper.

As Fig. 6 indicates, throughout the experiment the growth curve rises as does hemoglobin with added copper.

SUMMARY AND CONCLUSIONS

Experiments were conducted to determine the effect of copper of four common brands of evaporated milk on hemoglobin regeneration in nutritional anemia in rats made anemic on a basal milk diet.

The following points were outstanding:

1. Brand P evaporated undiluted milk contained in-

sufficient copper to be of value in nutritional anemia.

2. Biological analysis and experimental feedings indicated Brand C to be almost devoid of copper.

3. Brands V and B (diluted) sustained hemoglobin above seven and one-half per cent for a period of eight weeks.

4. Brand V regenerated hemoglobin to normal and promoted the greatest growth of any of the four evaporated milks tested.

5. Raw milk plus iron and added copper promoted growth and hemoglobin production over and above that of any of the four evaporated milks.

6. 0.01 mg. copper appeared to be an insufficient amount for promotion of growth and hemoglobin production. It is probable that some other necessary factor was missing.

7. 0.05 mg. copper appeared to be the optimum amount for hemoglobin production and promotion of growth.

8. Inorganic iron was utilized in the presence of sufficient copper, in rats made anemic on a basal milk diet.

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