Summary. The Syrian hamster is highly susceptible to intracerebral and intranasal inoculation with the virus of St. Louis encephalitis.

12078

Anemia in Vitamin C Deficiency and Its Response to Iron.

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Experimental scurvy in the guinea pig is usually associated with anemia,¹ and antiscorbutic food² or vitamin C³ improves the anemia together with the scurvy. In human nutrition, hypochromic anemia frequently accompanies scurvy in infants,⁴ and it is also commonly found in adults with vitamin C deficiency. Mettier, Minot and Townsend⁵ postulate that vitamin C exerts a specific effect on erythropoiesis. Dunlop and Scarborough⁶ reported 2 cases of scurvy with anemia which disappeared on supplementing a deficient diet with 60 mg of ascorbic acid daily. On the other hand, Keefer and Yang⁷ state that in man scurvy may exist without anemia, although, if the disease is of considerable duration and associated with hemorrhage, infection or general undernutrition, anemia may be present. Likewise, Abt and Farmer⁸ feel that when anemia accompanies lack of vitamin C, it is probably due to a generally deficient diet in which substances other than vitamin C, especially iron, are lacking. The recent experimental production of scurvy in a human subject without appearance of anemia⁹ also supports the latter viewpoint.

In view of the existing controversy in regard to the rôle of vitamin C in anemia, the following data obtained in the Spring of

² Mettier, S. R., and Chew, W. B., J. Exp. Med., 1932, 55, 971.

³ Aron, H. C. S., J. Nutrition, 1939, 18, 375.

⁴Hess, A. F., Scurvy, Past and Present, Philadelphia, Lippincott Co., 1920; Parsons, L. G., and Hawksley, J. C., Arch. Dis. Child., 1933, **8**, 117.

⁶ Dunlop, D. M., and Scarborough, H., Edinburgh Med. J., 1935, 42, 476.

7 Keefer, C. S., and Yang, C. S., Nat. Med. J. China, 1929, 15, 419.

⁸ Abt, A. F., and Farmer, C. J., J. Am. Med. Assn., 1938, 111, 1555.

⁹ Crandon, J. H., Lund, C. C., and Dill, D. B., New England J. Med., 1940, 223, 354.

¹ Meyer, A. W., and McCormick, L. W., Stanford Univ. Pub. Med. Sc., 1928, 2, 96, 199.

⁵ Mettier, S. R., Minot, G. R., and Townsend, W. C., J. Am. Med. Assn., 1930, 95, 1089.

1939 in the course of a nutritional survey of 238 boys in a municipal relief institution may be of interest. These boys, ranging from 11 to 20 years of age, lived on a monotonous diet of millet and corn with small amounts of vegetables in season. Most of them appeared to be underdeveloped and sparely nourished. Sixty of them had pallor, 112 had hypertrophied and bleeding gums and 87 had positive Chvostek's sign. However, none showed bleeding tendency elsewhere, manifest tetany, ocular or cutaneous manifestations of avitaminosis A or neurological evidence of vitamin B_1 deficiency.

Of this series, 44 inmates who had hypertrophied gums were selected for more detailed study. Their Pelidisi indices varied between 87 and 99, averaging 92. Twenty-six had underdeveloped external genitalia. Eighteen showed latent tetany, as evidenced by Chvostek's or Trousseau's sign or both. Three were suspected to have pulmonary tuberculosis, 3 had enlarged liver and 4 enlarged spleen. The plasma vitamin C values were all low : 28 cases ranging between 0.10 and 0.19, 9 cases between 0.20 and 0.29, and 7 cases between 0.30 and 0.40 mg per 100 cc. However, only 21 cases showed increased capillary fragility by the Gothlin's test. Anemia was present in more than half of the cases, the hematocrit reading being from 27.2 to 30% in 3 cases, from 30 to 40% in 30 cases and from 40 to 45.6% in 16 cases. The anemia observed was mostly of the hypochromic type, with considerable achromia and anisocytosis. No nucleated erythrocytes were noted in the peripheral blood. Platelet counts varied between 210,000 and 333,000 per cu mm, and reticulocyte counts between 0.7 and 3.2%. Bleeding time, coagulation time and prothrombin time (Quick) were all normal.

In order to determine the cause of the anemia, we selected for therapy 16 boys of the above series who had varying degrees of anemia without apparent explanation. They were evenly divided into 2 groups. The subjects in the first group each received 50 mg of vitamin C (Cebion, Merck) daily for 4 weeks, and those of the second group 1.5 g of ferrous carbonate daily for the same length of time. During the period of treatment, the boys remained in the relief institution without any change in their diet or routine. Hematological studies and plasma vitamin C determinations made before and after the medication are set forth in Table I. The observations before and after therapy have been paired and tested for significance by means of the *t*-test,¹⁰ and probability values of 0.05 or less are considered significant.

¹⁰ Fisher, R. A., Statistical Methods for Research Workers, 7th ed., 1938, Oliver and Boyd, London.

						Mean	Mean		
		Hemato	· Ervthro	Hemo-	Mean	corpus-	corpus-		Plasma
		crit	cvte	globin	corpus-	cular	cular	Reti-	vita-
Case		% cell	million	g per	cular	hgb.	hgb.	culo-	$\min C$
No.		volume	per mm ³	100 cc	vol., μ ³	10-12 g	conc., %	cyte, %	$\mathrm{mg}\%$
Vitamin C. Treated Series									
т	Doforo	90.0	0 02	51	20.7	99 Q	95 5	18	0.38
T	Aftor	18.3	2.20	5.9	82.0	02 2 02 2	20.0	2.0	1 40
9	Riter	10.0	2.23	7.0	04.0	40.0 02.0	20.1	1.0	0.12
4	After	20.0	2 / 2	70	82.0	20.2 93 A	21.0	17	0.98
2	Bofore	20.1	0.40 9.02	0.0	116.6	23.0	28.9	2.8	0.20
9	After	34.3	2.00	10 4	106.5	22.2	30.4	24	0.97
4	Before	354	3.22	10.4	02.0	27.8	30.0	14	0.15
Ŧ	After	32.0	3.08	10.0	106 7	32.5	30.4	31	0.73
5	Bofore	35.3	3 76	10.0	94.8	28.9	30.9	10	0.13
0	After	35.5	2.98	11.8	119.0	39.6	33.3	2.5	0.49
6	Refore	35.9	3.22	11.0	11115	34.2	30.7	3.2	0.25
Ū	After	35.0	3.36	10.1	104.0	30.0	28.9	2.3	1.27
7	Before	36 1	3.89	118	92.8	30.3	32.7	1.0	0.35
•	After	34.8	3 16	11.6	110.0	36.7	33.3	1.8	0.92
8	Before	38.0	3 14	121	121.0	38.5	31.8	1.8	0.15
0	After	38.0	2.70	12.9	140.0	47.5	33.9	2.1	0.50
Mean	Before	32.9	3 30	99	100.3	30.0	29.8	1.8	0.23
	After	32.1	3.02	10.0	106.2	33 1	30.8	20	0.91
	t	0.22	1.68	0.44	1 60	1.70	1.93	1.07	7 09
Probał	nility	0.8	0.1	0.7	0.2	0.1	0.1	0.3	0.001
2 1 0 0 a 0 1 a 0 j		Iron-Treated Series							
1	Before	27.2	3.71	6.9	73.2	18.6	25.4	2.6	0.38
	After	38.0	4.22	13.1	90.0	31.0	34.5	2.8	0.22
2	Before	27.8	2.96	6.8	94.0	22.9	24.4	1.2	0.14
	After	33.2	3.68	10.1	90.4	27.4	30.4	1.7	0.23
3	Before	34.1	2.56	9.1	133.1	35.5	26.7	2.0	0.18
	After	40.2	3.99	12.2	100.7	30.6	30.3	2.1	0.27
4	Before	33.8	2.99	9.7	113.0	32.4	28.7	2.0	0.12
	After	37.0	3.93	12.6	94. 0	32.1	34.1	1.9	0.22
5	Before	31.8	3.27	9.0	97.3	27.5	28.3	1.0	0.12
	After	36.2	3.25	11.8	111.2	36.3	32.6	2.7	0.22
6	Before	38.3	3.19	11.8	120.0	37.0	30.7	1.8	0.21
	After	42.3	4.06	14.5	104.0	35.7	34.3	2.1	0.21
7	Before	35.2	3.84	9.7	91.7	25.2	27.5	1.4	0.18
	After	36.5	4.20	12.3	86.8	29.3	33.7	2.4	0.17
8	Before	34.2	3.11	11.0	110.0	35.4	32.2	2.5	0.17
	After	34.7	2.62	11.2	132.5	4 2.7	32.2		0.19
Mean	Before	32.8	3.20	9.2	103.0	29.3	28.0	1.8	0.19
	After	37.3	3.74	12.1	101.2	33.1	32.8	2.2	0.22
t		3.77	2.51	5.26	0.38	1.87	5.16	0.21	0.94
Probability		0.01	0.05	-0.001	0.7	0.1	0.001	0.8	0.4

TABLE I.

It will be noted that vitamin C administration, while capable of raising the plasma vitamin C concentration in every instance with an average increase from 0.23 to 0.91 mg %, failed to influence the hematocrit or hemoglobin values. The slight reduction in the average erythrocyte count after vitamin C therapy was not significant, likewise the changes in the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were insignificant. There was no significant rise in reticulocyte per-

605

centage following vitamin C medication. On the other hand, iron therapy was followed in most cases by a significant increase in hematocrit, erythrocyte and hemoglobin values, although, as expected, the plasma vitamin C concentration remained subnormal as before in all instances. The average increase of hematocrit was from 32.8 to 37.3%, that of erythrocyte count from 3.20 to 3.74 million per cu mm, and that of hemoglobin from 9.2 to 12.1 g per 100 cc. The mean corpuscular volume and the mean corpuscular hemoglobin showed insignificant variations, while the mean corpuscular hemoglobin concentration evinced a definite increase after the iron administration. No distinct rise in reticulocyte count was noted.

Summary. Anemia appears to be common in association with vitamin C deficiency among the inmates of a municipal relief institution studied. Therapeutic results indicate that the anemia is not due to lack of vitamin C per se, but related in all probability to a concomitant iron deficiency.

12079

Attempts to Grow Leishmania donovani in Tissue Cultures.

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The parasites of kala-azar, *Leishmania donovani*, live as nonmotile forms only in the cells of the mammalian hosts. When they are cultured in the ordinary artificial media at 37° C, no growth occurs, but at room temperature, $22-25^{\circ}$ C, or when brought to the digestive tract of certain insects, they develop into motile flagellates. It seems, therefore, that in order to cultivate the oval non-motile form of the parasites *in vitro*, an opportunity must be given them to grow in the living cells, and the tissue-culture method naturally suggests itself.

Gavrilov and Laurencin¹ have already made use of this method to cultivate these parasites, using the tissue of the infected hamsters. They found that the oval forms survived in the cells for only about 10 days. The following experiments were undertaken to determine whether it is possible to get a more successful growth of these parasites in the tissue cultures.

¹ Gavrilov, W., et Laurencin, S., Ann. Soc. Belge Med. Trop., 1938, 18, 1.