

ity both eventually result in sap hyperacidity. The untreated loam produced more vigorous plants than the untreated, low-mineral humus though both soils were strongly acid. The higher mineral content of the acid loam may in part account for the better growth of wheat on this soil.

Fluctuations in light intensity and soil acidity produced smaller changes in sap hydrogen concentration of plants grown on the loam soil. Under certain conditions, such as lack of balance among nutrients of mineral insufficiency, it seems that the effect of lime in altering the free acidity of the sap may outweigh its other functions as a nutrient.

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Blood Findings in Albino Rats Suffering From Lack of Vitamin A.

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The literature regarding blood changes in vitamin A deficiency disease deals chiefly with blood counts, platelets and hemoglobin findings. Cramer, Drew and Mottram¹ in a study of blood platelets and corpuscle counts stated that there were no constant differences in the number of white or red corpuscles, although in advanced stages of deficiency there may be a distinct anemia. Koessler, Mauer and Loughlin² report that they have produced conditions similar to human pernicious anemia in experimental animals deprived of vitamin A. Hopp³ studied the occurrence of anemia in rats on deficient diets and concluded that diets deficient in vitamins A and B do not produce anemia in the rat.

Damianovich and collaborators⁴ did not observe an anemia in rats suffering from lack of A or B. Falconer⁵ reports a slight drop in platelets and a small rise in red and white corpuscles, but con-

¹ Cramer, W., Drew, A. H., and Mottram, J. C., *Proc. Royal Soc., London*, Series B, 1922, xciii, 499.

² Koessler, Karl K., Maurer, S., and Loughlin, R., *J. Am. Med. Assn.*, 1926, xxvii, 476.

³ Hopp, W. H., *Johns Hopkins Bull.*, 1922, xxxiii, 163.

⁴ Damianovich, H., Bianchi, A., and Savazzini, Lilia A., *Compt. Rend. Soc. de Biol.*, 1923, xxviii, 377.

⁵ Falconer, E. H., and Peachy, G., *Am. J. Physiol.*, 1926, lxxvi, 145.

cludes the changes are not sufficient to constitute specific lesions of vitamin A deficiency. Recently Anderson and his co-workers⁶ published values on the composition of normal rat blood. They find that in most respects it is quite similar in composition to human blood. The most marked exception is a higher value for non-protein nitrogen.

This study was to determine whether there is any decided change in the relative number of red blood corpuscles or the amount of hemoglobin, uric acid, non-protein nitrogen, creatinine, urea nitrogen, chlorides and sugar in the blood of xerophthalmic animals as compared to that of normal stock rats and control animals. The latter received cod liver oil in addition to the vitamin A-free diet.⁷ The animals were placed on diet at varying ages. They were not all obtained from the same stock colony which accounts for the variation in the length of time required for development of xerophthalmia. To determine the amounts of these blood constituents in the rat it was necessary to take the combined blood of 4 animals. The blood for chemical examination was obtained as follows: The animal was first placed in a state of complete surgical anesthesia. The hair was removed from the under side of the neck, and the carotid artery exposed and severed. By holding the animal in the hand the blood was poured into an oxylated test tube which was held at the opening in the neck. The findings of the determinations in each group of the combined bloods tested were averaged and taken for the standard of that group. The hemoglobin and red cell counts given were taken for each individual rat making a total of 28 stock, 58 xerophthalmic, and 13 control animals.

The combined bloods of each 4 rats were deproteinized by the method of Folin and Wu.⁸ Uric acid was determined by the method of Benedict,⁹ non-protein nitrogen and creatinine by the method of Folin and Wu.⁸ Urea nitrogen was estimated by Folin's method, using the aeration process.¹⁰ Chlorides were determined by Whitehorn's method.¹¹ Sugar values were obtained by the method of Folin and Wu.¹² The acid hematin method of Newcomer¹³ was

⁶ Anderson, A. K., Honeywell, H. E., Santy, A. C., and Pedersen, S., *J. Biol. Chem.*, 1930, lxxxvi, 157.

⁷ Turner, R. G., *Proc. Soc. Exp. Biol. and Med.*, 1928, xxvi, 23.

⁸ Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, xxxviii, 81.

⁹ Benedict, S. R., *J. Biol. Chem.*, 1922, liv, 233.

¹⁰ Hawk, P. B., and Bergeim, O., "Practical Physiological Chemistry," 1927, P. Blakiston's Son & Co., 371.

¹¹ Whitehorn, J. C., *J. Biol. Chem.*, 1920-21, xlv, 449.

¹² Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, xli, 367.

¹³ Newcomer, H. S., *J. Biol. Chem.*, 1919, xxxvii, 465; 1923, lv, 569.

used for estimating hemoglobin. Red cell counts were made by the Levy-Burker-Neubauer modification of the Thoma method.¹⁴

The blood obtained for hemoglobin and red blood cell counts was taken from the tail of the rat. The end of the tail was clipped and the blood allowed to flow freely before taken for the tests.

All the tests on animals suffering from lack of vitamin A were made at varying times after symptoms of definite xerophthalmia appeared.

The results are given in the following tables. Table I gives the average blood chemistry findings for each group, Table II, hemoglobin and red blood cell counts as found in the blood of normal stock, xerophthalmic, and control animals.

As seen in Table I for all 3 groups, uric acid, creatinine, sugar, and chloride values are similar to those given by Anderson for normal rat blood. They all fall, except the latter, within the range for normal human blood. Chloride values are slightly below normal. Non-protein nitrogen averages below the range for normal rat blood in stock and control animals. The xerophthalmic animals show an average within the range as compared with his findings. Two of the 7 determinations in this group gave values strikingly above the normal

TABLE I.
Average Blood Findings in Normal, Xerophthalmic and Control Animals.
Results in mgm. per 100 cc. of blood.

Normal Stock Animals.						
No. of Animals	Uric Acid	Non-Protein N.	Creatinine	Urea N.	Chloride as NaCl	Sugar
12						
Range	2.6-3.1	30.9-31.9	1.5-1.6	15.0-16.0	330-410	100-129
Ave.	2.9	31.5	1.5	15.4	400	115
Xerophthalmic Animals.						
36						
Range	1.6-3.0	35.4-58.8	1.4-1.9	15.2-28.6	320-440	97-130
Ave.	2.5	40.1	1.5	19.8	390	112
Control Animals.						
16						
Range	2.5-3.8	36.0-37.2	1.5-1.6	16.0-18.2	270-630	95-129
Ave.	2.5	36.5	1.55	16.7	440	111
Normal Rat Blood (6)						
Range	1.35-2.4	38.6-49.1	1.2-1.4	9.9-20.6	472-572	110-132
Ave.	1.86	45.2	1.28	15.6	515.2	122.2

¹⁴ Morris, R., "Chem. Laboratory Diagnosis," Appleton & Co., 1923.

TABLE II.
Hemoglobin and Red Blood Cell Count in Normal, Xerophthalmic, and Control Animals.

Normal Stock Animals.							
No. of Animals	Age in Days	Body Weight in gm. Aver.	Sex	Hb gm. per 100 cc. Aver.	Devia-tion	R.B.C. Million per cmm. Aver.	No of Days on Diet
8	80	114	♂	16.64	±3	8.72	59
4	78±2	90	♀	15.65	±1	8.20	57
16	65±4	76	♀	12.94	±2	8.18	44
Aver. Unweigt.	74	93		15.07		8.37	53
Xerophthalmic Animals.							
29	98±2	119	♀	13.58	±3	9.16	54
8	80	94	♀	14.99	±1	8.30	38
2	70±4	52	♂	15.49	—	10.06	45
19	200±5	110	♀	13.51	±2	8.24	130
Aver. Unweigt.	112	93		14.39		8.94	66
Control Animals.							
13	223±5	140	♀	13.31	±2	8.39	153

(48.7 and 58.8). Figures corresponding to these high values were found by Anderson in rats with bad lungs. His animals ranged from 7 to 12 months in age. Our animals at necropsy showed no macroscopical lung lesions. They ranged from 2 to 7 months in age.

The urea nitrogen findings show an average of 15.4 mg. per 100 cc. blood for normal stock animals, 19.8 for xerophthalmic, and 16.7 for control animals. The basal ration for xerophthalmic and control animals was lower in nitrogen content than the stock ration. The greater increase as found for the diseased animals may be due to the emaciated condition of these animals because of excess nitrogen formed through destruction of protein tissue.

The hemoglobin determinations given in Table II do not show an increase in xerophthalmic animals.

The red blood cell counts show an average increase of 560,000 cells in the xerophthalmic animals as compared with stock and control animals.

Summary. From the results given it is concluded that the relative amounts of uric acid, non-protein nitrogen, creatinine, urea nitrogen, chlorides and sugar in the blood of xerophthalmic animals are not striking enough or constant enough to constitute a decided

change. Further, the hemoglobin and red cell count are not sufficiently altered to show specific lesions in vitamin A deficient animals.

It is believed that anemia does not result from lack of vitamin A.

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A Test to Differentiate Irradiated Cholesterol From Non-Irradiated Cholesterol and Irradiated Ergosterol From Non-Irradiated Ergosterol.

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Cholesterol dissolved in chloroform gives with a mixture of sulphuric and selenious acids (125 mg. of sodium selenite in 25 cc. of concentrated sulphuric acid) a purple color in the upper chloroform layer and a red-brown color in the lower acid layer, which displays no green fluorescence.¹ Solid cholesterol, irradiated with the mercury arc lamp for 30 minutes at a distance of 16 inches, when dissolved in chloroform gives with the sulphuric and selenious acid mixture a dark wine-red color in the chloroform layer, and a still darker wine-red color in the acid layer with the absence of green fluorescence. This reaction takes place in the 1.0% and in the 0.5% solution. In the 0.25%, 0.10%, 0.025% and 0.01% solutions the color reaction is negative. The chloroform layer is more or less purple, while the acid layer is brown and free from fluorescence.

Ergosterol, non-irradiated and dissolved in chloroform, gives with sulphuric acid a colorless chloroform layer and a brownish red, cherry red or orange color in the acid layer in direct light and a green fluorescence in transmitted light.² With the sulphuric acid-selenious acid mixture the chloroform layer also remains uncolored, while the acid layer gives a brown color in direct light and no green fluorescence in transmitted light.

Solid ergosterol, irradiated with the mercury lamp for 30 minutes at a distance of 16 inches, dissolved in chloroform and treated with

¹ Levine, V. E., and Richman, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, xxvii, 832.

² Levine, V. E., and Richman, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, xxvii, 833.