

decrease in rate over a period of hours was observed in each instance, the decrease being of similar degree both in normal preparations and those from thyroid-treated animals.

It is obvious that the preparations from hyperthyroid animals beat at a much faster rate than those from normal animals. This was a constant and striking phenomenon which persisted throughout each experiment.

The effects of various drugs and solutions are summarized below: Iodine and iodides in concentrations of 1:100,000 to 1:4,000,000 had no significant effect on the isolated auricles but caused moderate decrease in rate of 5 of 11 and in amplitude of 8 of 11 isolated hearts. There was no difference in the response of normal preparations and those from thyroid-treated animals. No constant changes were observed in coronary flow.

Thyroxine and desiodothyroxine, in acute experiments, had no constant effect on normal preparations or on those from thyroid-treated animals.

Preparations from thyroid-treated animals responded to epinephrine with increases in rate and amplitude (*percent*) similar to those of the normals. Acceleration of rate (*beats per minute*) was greater in the former preparations than in the normals. There was no difference in sensitivity to high dilutions (1:100,000,000) of epinephrine.

5394

Effect of Withdrawal of Vitamin-A on Leukocyte and Differential Count in the Albino Rat.

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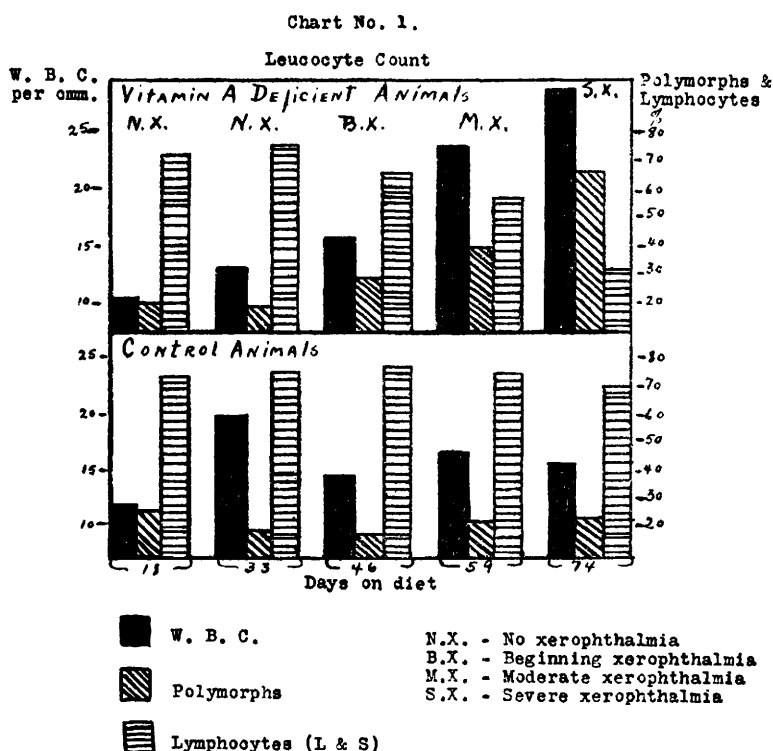
It is generally accepted that animals deprived of Vitamin A develop a characteristic susceptibility toward infection.¹ Suppurative lesions of the upper respiratory tract, sinuses and middle ear occur

¹ McCollum, E. V., *J. Am. Med. Assn.*, 1917, **68**, 1379; Drummond, J. C., *Biochem. J.*, 1919, **13**, 95; Macy, Icie G., Outhouse, J., Long, M. L., and Graham, A., *J. Biol. Chem.*, 1927, **78**, 152; Sherman, H. C., and Burtis, M. P., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25**, 649.

rather frequently in rats deprived of this nutritional element.² The relation of this characteristic as compared to the leukocyte and differential count at various stages of the disease is of interest.

Cramer, Drew and Mottram³ observed a lympho-paenia in the circulating blood of rats placed on diets deficient in water-soluble vitamin B. They found no change in the differential or W. B. C. count in rats placed on vitamin A deficient diet. Their report includes data on only 2 rats fed on diets lacking vitamin A, at 50 to 70 days of age, for a period of 6 to 7 weeks. One of the animals developed xerophthalmia, the other did not. From the growth curves presented for these animals they could not have shown more than a mild form of the disease as they were still gaining weight at the end of the experiment.

Our animals were studied until they showed severe symptoms of vitamin A deficiency with marked loss in weight and gross xeroph-



² Daniels, Amy L., *J. Am. Med. Assn.*, 1923, **81**, 828; Turner, R. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **26**, 233; Shurly, B. R., and Turner, R. G., *J. Am. Med. Assn.*, 1930, **94**, 539; Turner, R. G., Anderson, Dorothy E., and Loew, E. R., *J. Infect. Dis.*, 1930, **46**, 328.

³ Cramer, W., Drew, A. H., and Mottram, J. C., *Lancet*, 1921, **2**, 1202.

thalmia. Total leukocyte count and differential count of the blood of rats during various stages of the disease are reported for 13 xerophthalmic animals and 5 control animals. A graphical diagram is given in Chart 1.

The average white blood cell count for animals placed on vitamin A deficient diet did not show any increase over the average count of the controls on the 46th experimental day. At this time most of the animals showed a mild form of xerophthalmia. At 59 days the average count shows an increase of 4,000 W. B. C. per cmm. over the highest average found in the controls. The animals at this time showed a moderate xerophthalmia, both eyes affected, with loss of weight. The average count for 6 animals remaining on the deficient diet for 74 days continued to show this increase in the W. B. C. These animals were very weak and emaciated and would probably have succumbed within several days from the effect of lack of vitamin A in their diet.

The average differential count taken on the 46th experimental day showed a slight increase in the relative percentage of polymorphs with a corresponding decrease in the lymphocytes for the vitamin deficient animals. This change became more marked as the animals remained on the diet. In the animals showing gross pathological symptoms the increase in polymorphs reached 67% while the lymphocytes dropped to 31%. The relative percent of polymorphs and lymphocytes as found in the control animals remained nearly constant throughout the experimental period. These findings show the normal lymphocyte count to constitute about 75% of the total white blood cells in the rat, the polymorphs averaging about 20%. Cramer, Drew and Mottram³ found the percentage value of small lymphocytes to be about twice that for the polymorphonuclear leukocytes in the rat. The lymphocyte count in the data presented includes both large and small lymphocytes. The stain used for differentiating was Wright's stain. The Wistar Institute⁴ reports contrary findings for the normal rat, *i. e.*, a higher percentage of polymorphs than lymphocytes.

Variations in the leukocyte count of control animals may be interpreted as possible fluctuating differences due to physical and metabolic activities. Müller, Peterson and Holscher⁵ believe that many factors other than disease may produce significant physiologi-

⁴ The Rat: Memoirs of the Wistar Institute of Anatomy and Biology No. 6. H. H. Donaldson, 2nd Edition, 1924.

⁵ Müller, E. F., Peterson, W. F., and Holscher, Rose, *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 544.

cal fluctuations of considerable magnitude. Garrey and Butler⁶ believe that passive or active postural changes raise the count instantly from 60 to 100% in humans. The normal leukocyte count for albino rats on a complete vitamin A synthetic diet (controls) may range from 9,000 to 24,000 W. B. C. per cmm., the highest average being 20,000. The highest counts obtained on the 33rd experimental day, did not remain constant and at no other time in the experiment reached as high a limit. The final counts taken on all animals are summarized as follows:

20% of the control animals showed a count between	9,000 and 10,000 per cmm.
80% " " " " " " " "	10,000 " 20,000 " "
7% of the diseased " " " " " "	9,000 " 10,000 " "
38% " " " " " " " "	10,000 " 20,000 " "
30% " " " " " " " "	20,000 " 30,000 " "
25% " " " " " " " "	30,000 " 50,000 " "

Fifty-five percent of the animals placed on vitamin A deficient diet showed a leukocytosis as compared with the W. B. C. findings in the control animals. The xerophthalmic animals likewise showed a reverse picture in the relative percent of polymorphs and lymphocytes as compared to the controls, and as found in the test animals before the onset of xerophthalmia, an increase in the polymorphs with a decrease in the lymphocytes, becoming more noticeable as the disease becomes more severe. Control animals showed a higher percentage of lymphocytes than polymorphs throughout the experiment. The generally accepted theory is that suppurations which develop in the cavities of these animals deprived of vitamin A are secondary to the metaplasia of cells and xerophthalmia. This theory apparently coincides with the variation found in the leukocyte counts. Metchnikoff's theory of phagocytosis suggests that the polymorphonuclear variety of cells is especially adopted to attack bacteria. Leukocytic defence of each individual rat studied begins after xerophthalmia sets in as noted by the increase in W. B. C. count. At the same time there is an increase in the multicellular leukocytes. This increase in polymorphs continues until the animal succumbs. The majority of bloods from xerophthalmic rats examined bacteriologically were found to be sterile. A very mild form of septicemia, undetected by bacterial culturing, may be the cause of increased phagocytosis. Various investigators⁷ believe that upper respiratory, sinus and middle ear infections may be the direct cause

⁶ Garrey, W. E., and Butler, Virginia, *Am. J. Physiol.*, 1929, **90**, 355.

⁷ Smith, D. L., *South. Med. J.*, 1929, **22**, 918; Dixon, O. J., *J. Am. Med. Assn.*, 1928, **91**, 1280; Cone, A. J., *Laryngoscope*, 1927, **37**, 19.

of numerous other systemic conditions brought about probably by a long continued mild form of septicemia. Evidence has accumulated that lymphocytes and monocytes play a rôle in promoting tissue repair. The experimental animals are all young growing rats and their need for tissue repair and rebuilding may be the cause of the high percentage of lymphocytes as found in the control animals.

The leucocytosis observed in the xerophthalmic animals together with an increase in the relative percent of polymorphonuclear cells and a decrease in the lymphocytes, logically, must be considered as a leucocytic defence of the individual animal to combat the invasion of virulent organisms. Is there then a lowered resistance of the specific membrane on which the organism gains control? Lowered resistance of the body is generally noted by an increase in the number of leucocytes, the phagocytes being less in number.

Conclusion. Leukocytosis with an increase in the percent of polymorphonuclear leukocytes and a decrease in the lymphocytes of the circulating blood is evident in the later stages of vitamin A deficiency in the rat.

5395

On the Cortical Hormone of the Adrenal Gland.*

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The crude lipid extract of beef adrenal cortex¹ and the aqueous extract² obtained from the active lipid fraction contain, with the cortical hormone, small quantities of adrenalin. The adrenalin content of the aqueous extract is sufficiently low to permit the demonstration of its efficacy in maintaining indefinitely the lives of adrenalectomized cats.^{2, 3}

A simple method for the separation of adrenalin from the corti-

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¹ Pfiffner, J. J., and Swingle, W. W., *Anat. Rec.*, 1929, **44**, 225; *Am. J. Physiol.*, 1931, **96**, 153.

² Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 321; *Am. J. Physiol.*, 1931, **96**, 164.

³ Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 489.