

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.

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

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

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

cal hormone has been found.⁴ The 70% alcohol soluble fraction obtained by our previously described method² is transferred to 95% alcohol and filtered through permutit. An extract with an adrenalin concentration of less than 1:2,000,000 (bio-assay-blood pressure) and a tissue equivalent of 30 gm. of cortex per cubic centimeter can be prepared by filtering through permutit twice using 20 gm. per kilo of tissue on the first filtration and 10 gm. per kilo on the second. The active material remaining in the permutit filter after each filtration is washed out with alcohol. The active fraction is transferred to water and the extract clarified by Seitz filtration. Besides adrenalin the permutit removes most of the contaminating pigment substances along with other inert material.

Intravenous injections of this type of extract have been used successfully in the crises of Addison's disease.⁵

Fractionation with permutit has made possible the preparation of active extracts from whole beef adrenal glands thereby doing away with the expense of dissection. These extracts have been found to be just as active in restoring prostrate adrenalectomized cats to apparently normal health as extracts prepared from dissected adrenal cortex. Whole adrenal gland extract (1 cc. equivalent to 50 gm. of whole gland) has an adrenalin content of approximately 1:2,500,000. This extract has been found suitable for subcutaneous, intraperitoneal and intravenous use. The solid content ranges in different batches from 0.3 to 0.4%.

Another method for separating adrenalin from the cortical hormone consists in distributing an active fraction between aqueous alkali and an immiscible solvent such as benzene or ether. Adrenalin passes into solution in the aqueous alkali whereas the cortical hormone is found in the immiscible solvent phase. By means of this fractionation step, highly active extracts (1 cc. equivalent to 30 gm. cortex) have been prepared containing less than one part of adrenalin in 4,000,000. This type of extract can be used subcutaneously, intraperitoneally or intravenously.

Aqueous cortical extracts are rendered inactive by boiling gently in an open flask for 2 minutes.

The unsaponifiable fraction obtained from the 70% alcohol soluble fraction referred to above, using sodium ethylate saponification, is completely inactive as tested on prostrate adrenalectomized cats.

⁴ Swingle, W. W., and Piffner, J. J., *Science*, 1930, **72**, 75; *Am. J. Physiol.*, 1931, **96**, 180.

⁵ Rowntree, L. G., Greene, C. H., Swingle, W. W., and Piffner, J. J., *Science*, 1930, **72**, 482; *J. Am. Med. Assn.*, 1931, **96**, 231.

The cortical hormone is removed from ether solution of an active fraction by washing with dilute acid.

Extract of beef adrenal cortex prepared with permutit fractionation gives a negative biuret, ninhydrin, Hopkins-Cole, Molisch, Pauly, and Liebermann Burchard reaction. It gives a positive xanthroproteic, Millon's, alkaline copper and alkaline phosphotungstate reaction. These 4 positive reactions can be accounted for by the presence of traces of phenolic decomposition products of adrenalin.

It has been found that aqueous extracts containing the cortical hormone can be preserved by the addition of benzoic acid in 0.1% concentration.

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A Precipitant for Material in Liver Active in Pernicious Anemia.

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The identification of γ hydroxyproline¹ among the products of hydrolysis of an acid active in pernicious anemia indicated the possibility of using Reinecke's² salt as a precipitant for this material from liver. Experiments have shown that Reinecke's salt does precipitate this acid and also precipitates active material from liver extract and promises to be useful as a preparative method. This observation is in harmony with the view that the γ hydroxyproline nucleus is an integral part of the active material in liver. The composition of the precipitate derived from the acid closely approximates that required for one molecule of organic acid and 2 molecules of Reinecke's acid.

¹ Dakin, H. D., West, R., and Howe, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **28**, 2.

² Kapfhammer, J., and Eck, R., *Z. Physiol. Chem.*, 1927, **170**, 294.