

anoxemia and toxins altering capillary permeability, etc., contribute to the formation of edema. By reducing water intake it was possible to reduce the extent of the edema in some cases with low osmotic pressure of the plasma proteins. We are now attempting to determine experimentally the level of osmotic pressure of the plasma proteins below which edema must form even with good renal function and no salt retention. When the osmotic pressure of the plasma proteins is below the hydrostatic pressure at the venous end of the capillary system, undoubtedly edema must form because water can not stay in the capillaries.

Our figures are in essential agreement with those obtained by Schade,² Govaerts,³ and Krogh⁴ on similar cases.

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A Method for Preparing an Active Adrenal Cortex Extract.

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(Introduced by P. E. Smith.)

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A number of investigators have recently^{1, 2} reported active extracts of the adrenal cortex, but at the time we were ready to use cortical extracts on test animals we were unable to find detailed directions for their preparation. As a result we have developed the following method.

Beef adrenals obtained from the slaughter house immediately after the kill are transported to the laboratory, packed in ice. The connective tissue and fat are dissected off, and the glands ground in a meat chopper equipped with a fine cutting disc. The minced glands are weighed and placed in 95% ethyl alcohol, one liter of alcohol per kilogram of gland, and allowed to stand in the cold over night. The next morning the alcohol is poured off, employing

² Schade, H., *Ergeb. d. Inn. Med. Kinderh.*, 1927, **32**, 425.

³ Govaerts, P., *Bull. d. l'Acad. Roy. d. Med. d. Beligigne.*, 1924, **4**, 161.

⁴ Krogh, A., *Anat. and Physiol. of the Capillaries*, Yale Univ. Press, New Haven, 1922.

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¹ Swingle, W. W., and Piffner, J. J., *Science*, 1930, **71**, 321.

² Hartman, F. A., *Endocrinol.*, 1930, **14**, 229.

a coarse cheese cloth as a strainer. The alcohol is then filtered through a coarse filter paper and the filter washed with fresh alcohol. This alcohol, together with the washings, is saved.

The adrenals are placed in a continuous extraction apparatus and extracted with alcohol for 84 hours under reduced pressure, and at a temperature between 35°C. and 40°C. After extraction the alcohol extract is combined with the alcohol in which the glands were dehydrated and placed in the cold for 12 hours, during which period a white precipitate settles out. The extract is then filtered and concentrated to half its volume under reduced pressure and again filtered. The remaining alcohol is removed under reduced pressure and the aqueous residue placed in the cold for 24 hours. This extract is centrifuged in ice at a speed of 2000 R.P.M. for 10 minutes. A light brown, slimy precipitate settles out and a few drops of orange colored oil appear on the surface of the supernatant fluid; the supernatant fluid is withdrawn with a pipette and its equivalent in grams of gland computed. It may be passed through a Seitz filter for purposes of sterilization.

Further purification is obtained by shaking the supernatant fluid with an equal quantity of benzene in a separatory-funnel. The benzene fraction is freed from the benzene by evaporation under reduced pressure over a small quantity of water. The aqueous residue is extracted with ether. The ether fraction is shaken up with distilled water and the ether then removed by reduced pressure. The ether-free aqueous solution is the potent extract and is diluted with water when needed for injection. It is adrenin-free by the Folin-Denis phenol reagent.

The activities of the various fractions obtained during this procedure were tested by their effect on blood constituents of normal and adrenalectomized cats, as well as their effect on the general health, weight and appetite of the animals. The results are described in a following paper by Zwemer and Sullivan.³

³ Zwemer, R. L., and Sullivan, R. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 723.