Full reports of these investigations which are being continued will be published later.

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THE PREPARATION OF ADRENAL EXTRACT

SINCE we have received many requests for the details of our method of preparing an extract of the adrenal cortex, their publication seems desirable.

The medulla contains toxic substances[1] which must be removed if whole adrenals are to be used. We have tried to remove or destroy these substances by washing the ethereal solution with acid or alkali, or by treating the alcoholic or aqueous solutions with aluminum hydroxide, Lloyd's reagent, permutit, kaolin or charcoal. The toxic substances can be removed, but so much cortin is also removed that at present it seems more satisfactory to start with cortex alone.

Fresh adrenal cortex, or cortex from adrenals frozen immediately after killing, is finely ground in a meat chopper. Peroxide-free ethyl ether (a one per cent. $K_2Cr_2O_7$ solution acidulated with H_2SO_4 turns blue in a few seconds after shaking with peroxide ether) is added to the material in a flask as soon as possible. Ether must always be kept in the dark, because peroxides develop within a few hours upon exposure to light. We, therefore, work in a dark room or cover our flasks with opaque cloth. Peroxides destroy cortin and cause toxic substances to develop. A satisfactory proportion for extraction is 4 liters of ether to 3 kilograms of tissue in a 12 liter flask. The air in the flask is replaced with CO_2 and the flask closed with a rubber stopper wired in place. Extraction is facilitated by agitation on a shaker or rocker for four to eight hours. Care must be taken not to produce an emulsion by too vigorous shaking. After pouring off the ether extract, second and third extractions with ether are made in a like manner. The three ether extracts are combined and concentrated almost to dryness by vacuum distillation.

The residue from the ether distillation is extracted four times with 95–98 per cent. ethyl alcohol heated from 45° to 50° C. and kept warm during the extraction so that the fatty material is kept fluid, otherwise the alcohol does not penetrate. For one kilo of gland material about 50–60 cc of alcohol are used in each extraction. One hour on the shaker is adequate for each extraction. The flask is cooled by surrounding with cracked ice so that the alcohol may be easily separated from the oily matter. All fractions are combined and enough water added to make the alcohol content 80 per cent. The solution is chilled to −10° C. ($CaCl_2$ and ice mixture) or below and filtered in a cold atmosphere (4° C. or less) to remove undesirable material. This is extremely important. If the chilling is not sufficient or the solution becomes warmed during filtering, toxic substances are carried through the paper. The alcohol is removed *in vacuo* and the residue again extracted with a small volume of 60–75 per cent. alcohol, then chilled and filtered as above. The alcohol is again removed and the residue extracted with a small volume of ether. The ether is driven off and its residue taken up with sufficient water to make the desired concentration. NaCl is added to make the extract isotonic with the body fluids. After passing through a Seitz filter, the extract is ready for injection.

If the precautions are carefully observed a potent, non-toxic extract can be made. One patient was injected four times daily for more than seven months without untoward effects.

Recently, Britton and Silvette[2] compared our method with that of Swingle and Pfiffner. One of the investigators from Britton's laboratory spent several weeks working with Swingle and Pfiffner,[3] while their knowledge of our method was based only upon the brief published[4] outline of our process. The results of Britton and Silvette are, therefore, what one might expect. Perla and Marmorston-Gottesman[5] have recently used our method successfully. Dr. Perla spent two days in our laboratory.

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THE LIFE HISTORY OF BABESIA BIGEMINA IN THE NORTH AMERICAN FEVER TICK

Babesia bigemina (Smith and Kilbourne, 1893)[1] is the piroplasm which is the causative agent of Texas cattle fever, a disease that at one time threatened the existence of a cattle industry in the United States. Smith and Kilbourne (1893) demonstrated conclu-

[1] E. B. McKinley and N. F. Fisher, *Am. J. Physiol.*, 76: 268, 1926.

[2] S. W. Britton and H. Silvette, SCIENCE, 73: 322, 1931.

[3] W. W. Swingle and J. J. Pfiffner, *Am. J. Physiol.*, 96: 153, 1931.

[4] F. A. Hartman, K. A. Brownell and W. E. Hartman, *Am. J. Physiol.*, 95: 670, 1930.

[5] D. Perla and J. Marmorston-Gottesman, *Proc. Soc. Exp. Biol. and Med.*, 28: 650, 1931.

[1] T. Smith and F. Kilbourne, *Bull.* Bur. Animal Industries, U. S. Dept. Agr., Washington, 1: 177–304, 1893.