3071

Simplified method of preparation of ovarian hormone, and properties of purified product.

J. O. RALLS, C. N. JORDAN and E. A. DOISY.

[*From the Laboratories of Chemistry and Biological Chemistry, St. Louis University School of Medicine, St. Louis, Mo.*]

A detailed study of our former procedure for the preparation of the ovarian hormone from liquor folliculi has shown that several time-consuming steps may be omitted. Tests have indicated that the acetone precipitation of phospholipins may be dispensed with, since the bicarbonate of the liquor folliculi furnishes sufficient alkali to saponify the lipins during the concentration of the alcoholic extract.

Another decided improvement has been effected by a study of the distribution ratios of the hormone and cholesterol between 70 per cent alcohol and petroleum ether. The cholesterol is twenty-nine times as soluble in petroleum ether as in 70 per cent alcohol. A few extractions of the alcoholic solution of the hormone with petroleum ether almost quantitatively remove the cholesterol but only a relatively insignificant fraction of the hormone.

The procedure now advocated is designed to separate the hormone from the soaps and cholesterol. It is the following: The alcoholic extract of fresh liquor folliculi is distilled to dryness and the residue is dissolved in a small amount of water which is transferred to a separatory funnel. The aqueous solution is extracted five times with an equal volume of ether; (in case emulsions form, the addition of a little alkali will aid the separation materially); the ether solutions are combined and washed with a little water, then with dilute acid and again with water. The ether is distilled off and the residue washed with 70 per cent alcohol and petroleum ether into a separatory funnel. If the volume of alcohol used is 100 cc. and the extract represents one liter of liquor folliculi, five washings with 25 cc. of petroleum ether are sufficient to reduce the quantity of cholesterol in the alcohol to less than .01 mg., while the loss of hormone will amount to less than ten per cent. Using this procedure the final product (the 70 per cent alcoholic solution) appears to be a mixture of products

of variable potency, one rat unit ranging from .035 mg. to .075 mg.

Analyses of a purified preparation (.04 mg. = 1 rat unit) by micro methods gave C, 80.8 per cent; H. 10.4 per cent; N, 0.9 per cent; P, 0.00. From the freezing point depression the mean molecular weight was calculated to be 475; based upon the above analyses the smallest gram molecule containing 1 atom of nitrogen must have a weight of about 1500.

3072

The rôle of the sympathetic nervous system in muscle tonus.

JOSEPH C. HINSEY and S. W. RANSON. (Introduced by Leo Loeb).

[*From the Department of Neuro-anatomy, Washington University Medical School, St. Louis, Mo.*]

The left lumbar sympathetic trunk was removed through a median abdominal incision in cats. Autopsy showed the left trunk had been completely removed from the second lumbar to below the brim of the pelvis and that the right trunk was intact. After postoperative periods varying from 50 to 77 days, five of these cats were decerebrated by ligation of the basilar and both carotid arteries. No difference between the sound and the sympathectomized limb could be detected either as to posture or rigidity. Using a simple device, the pressure required to flex the limb was measured in ounces and the time required in minutes. The measurements were repeated several times on each cat and such differences as exist between the two sides, sometimes in favor of the normal side, sometimes against it, disappear when averages of the whole series of measurements are considered.

Three other sympathectomized cats were given tetanus toxin, injecting equal quantities subcutaneously over the femoral trochanter in each hind leg. The degree of rigidity and the abnormal posture which developed in the two limbs were identical. These experiments show that the sympathetic nervous system is not responsible for exaggerated muscle tonus caused by decerebration or the action of tetanus toxin.

The nerve to the vastus internus muscle is being studied to determine the effect of sympthectomy on its unmyelinated fiber content.