boxyl groups of amino acids. Other organic acids, such as acetic, lactic, citric, yield no CO_2. Each of the naturally occurring amino acids including proline yields 1 mole of CO_2, except the dicarboxylic acids, aspartic and glutamic. Aspartic yields at once 2 moles of CO_2, while glutamic yields one mole at once, and a second much more slowly (only about 2% in 3 minutes). Peptides do not react, even with the free carboxyl, which is inactivated by the binding of the alpha NH_2 group in peptide linking. Urea does not react.

Because other primary amines, urea and ammonia do not interfere, the determination of amino acid carboxyl CO_2 may be more specific for amino acids in biological material than is the nitrous acid reaction. Because of the non-reactivity of the free carboxyl group in peptides the method appears to provide a sharp distinction between peptides and free amino acids.

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Extraction of Gonadotropic Factors from the Blood. Improved Technic.

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An improved method for gonadotropic hormone (G.H.) extraction from blood was reported from this laboratory in 1935.[1] By means of this method, both follicle stimulating as well as luteinizing effects were obtained with 40 cc. of blood taken from normal menstruating women, from those past the physiological menopause as well as from surgical castrates.[2] This method, which involved desiccation of the blood with sodium sulphate, was used because it enabled both G.H. and E.S. (estrogenic substance) determinations to be performed on the same specimen.

By the present method the deleterious effect of alcohol upon the G.H. factors is avoided, giving a more complete G.H. extraction without impairing the E.S. yield. Moreover, the extracts obtained

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[1] Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1236.

[2] Frank, R. T., Salmon, U. J., and Friedman, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1666.

by this method are non-toxic, in sharp contrast with extracts by the sodium sulphate method which caused the death of many animals because small amounts of sodium sulphate could not be removed.

Forty cc. of freshly drawn blood is run into 150 cc. of cold acetone. A fine precipitate forms which settles to the bottom. The blood-acetone mixture is shaken in a mechanical shaker for 20 minutes, centrifuged and the supernatant acetone poured off. This is repeated twice more with 125 cc. of fresh cold acetone. The acetone fractions are united, evaporated and utilized for estrogenic hormone determinations.

The acetone precipitate is dried under a fan, powdered and then extracted with 100 cc. of water, the mixture being acidified with dilute HCl to pH 4.8 (Brom-cresolgreen). This mixture is stirred by hand for 10 minutes, centrifuged, and the supernatant aqueous extract decanted. This aqueous extract is filtered through a single layer of gauze to remove suspended particles. To the aqueous extract, 400 cc. of cold acetone are added, a fine buff colored precipitate settling out. The mixture is allowed to stand in the refrigerator over night (45°-50°F.). It is then centrifuged and the acetone discarded. The precipitate is extracted with 5 cc. of water, alkalinized with dilute NaOH to pH 8.5 (thymol-blue). The mixture is centrifuged, the supernatant liquid decanted and its pH adjusted to 7 with dilute hydrochloric acid (Brom-thymolblue). This extract is then injected in 5 divided doses over a period of 60 hours, into an immature female rat weighing 24 to 26 gm. The animal is sacrificed at the end of 96 hours and its ovaries studied in serial section.

With this method we have obtained strong follicle stimulating and luteinizing effects with 40 cc. of blood taken from (a) normal women at the 9th to the 13th day of the cycle; (b) women subjected to surgical castration; (c) women who had passed through the physiological menopause; (d) young women affected with secondary amenorrhea.

For the initial precipitation of the blood we have also investigated the use of 95% ethyl alcohol, pure butyl alcohol, alcohol-acetone and ethyl alcohol-butyl, alcohol-acetone mixtures in place of pure acetone.

While both G.H. and E.S. factors can be determined employing these other precipitants in place of acetone, the most uniform results for both factors were obtained with straight acetone.