separation from an alcoholic solution can be greatly facilitated by the addition of distilled water in amount sufficient to reduce the alcoholic concentration to 50 per cent, or even lower.

Some success has also been had with a modified procedure. The principle involved in this modification of the above method consists in the adsorption of the hormone upon calcium phosphate. In this method, therefore, one can work with very dilute aqueous solutions of the placental hormone, and to a large extent shorten the somewhat tedious process with alcohol. One adds to a known potent aqueous solution of the placental hormone, which has been freed of protein and lipoid substances, sufficient neutral sodium phosphate solution to give a concentration of 0.2 per cent. The solution is then made strongly ammoniacal, and 5 per cent calcium chloride solution is slowly added. The mixture is stirred vigorously during the addition of the calcium chloride, enough of which should be added to precipitate all of the phosphate. The precipitate is recovered, washed with dilute ammonia and then extracted at least three times with hot 95 per cent or absolute alcohol which has been faintly acidulated with glacial acetic acid. The alcoholic extracts are combined, filtered, and concentrated to a small volume. The active principle may then be purified from this solution as indicated in the previous paragraphs.

Properties of the Purified Substance

As we have as yet been unable to obtain more than a few milligrams of the purified hormone at any one time, it is impossible in this communication to define with accuracy very much of the chemical properties of the active principle. The yield of the final purified product which has been obtained has been of the order of 1 mgm. per kilo of original placenta. The potency of an active extract has not been appreciably affected by boiling for five minutes in dilute acetic acid solution. We have, however, evidence of deterioration of the saline solution of the purified product. There has been such an urgent demand for the active extract for both laboratory and clinical experiments that the preparation of a sufficiently large sample of the purified product for chemical study has had to be postponed. This investigation is, however, about to be continued, and it is hoped that one will be able to publish a detailed report of such a study very shortly.

Physiological Effects of the Active Principle

The only physiological effect of the hormone which we have been able to study in a quantitative manner has been the production of premature maturity in immature rats or mice. Rats have been used almost entirely for the detailed studies. Also it has been found best to use rats three weeks of age and under 35 grams in weight. In the early stages of the investigation the experimental animals were sacrificed five to seven days after the initial injection, and the vagina, uterus, and ovaries were sectioned and studied microscopically. The positive animals manifested the changes in the uterus and vagina usually associated with œstrus, but in addition the ovaries were enlarged, and on section corpora lutea were usually found as well as normal follicles in varying degrees of development. Later it was found satisfactory to use the vaginal smear method in routine testing of extracts. It has been our practice to make injections twice and at twenty-four hour intervals, the same amount of extract being administered on each occasion. The injections were made subcutaneously and the puncture sealed immediately with collodion. Vaginal smears were taken daily, starting on the third day (72 hours). An epithelial or squamous cell flush occurring up to the sixth day has been arbitrarily read as a positive. One may have some evidence of a positive reaction as early as the third day.

It is also of interest to note that corpora lutea have been seldom found in the ovaries of immature rats which have been treated with either a fraction soluble in 85 per cent alcohol or the recrystallized final product. This observation lends a considerable measure of support to the hypothesis of Wiesner that there is a separate principle which stimulates luteinization. The suggestion which was made in a preliminary paper[24] that the finding of corpora lutea in the treated animal might be made the basis of a test for active extracts of the hormone which we are studying is therefore untenable.

It having been established that the immature rat was a satisfactory test animal for the deter-