

tracts, and they have given uniformly negative responses to such extracts.

One process which has been found to give excellent results, making use of human placenta, is as follows:

The placental tissue is finely pulped and treated with either one and one-quarter volumes of acetone or two volumes of 95 per cent ethyl alcohol. When one is using alcohol as the extracting vehicle one may with safety add several volumes of this reagent to the freshly ground glands, as the active principle which one wishes to obtain appears to be soluble in all strengths of grain alcohol. The mixture is kept agitated for some time and is then allowed to stand at room temperature for twenty-four hours. The fluid content is next separated from the mixture by the use of a suitable press. It is filtered, and to the filtrate one adds one-half c.c. of glacial acetic acid per litre. The reagent, acetone or alcohol, as the case may be, is removed by distillation at low temperature and reduced pressure. Concentration of the aqueous phase is continued until one has obtained a volume equal to one-half that of the placental tissue originally extracted. A filtering agent is now added to facilitate rapid filtration and to remove by adsorption a certain amount of undesirable material. An acid-washed hydrated aluminum silicate, such as Lloyd's reagent, has given excellent results in our hands. The filtrate is returned to the still and very cautiously concentrated under reduced pressure to the consistency of a thin syrup. Ten volumes of alcohol, either absolute or 95 per cent strength, are added. It is important that the alcohol be added very slowly and that the mixture be kept violently agitated during the process. The purpose of adding such a quantity of high grade alcohol at this stage is to remove by precipitation undesirable material and to retain in alcoholic solution the bulk of the active principle. It may be noted that herein our process differs materially from other processes which have been suggested for the purification of maturity-producing factors.

The mixture of concentrate and ten volumes of alcohol may be placed in a freezer at a temperature of -10°C . for several hours. This, however, is not an essential step. The mixture is next filtered and the residue may be again extracted with hot alcohol and the extract thus

obtained filtered and added to the first extract. The combined filtrates are then concentrated under reduced pressure to a thin syrup and again treated with alcohol, preferably absolute, in the manner above described. The mixture is filtered and again concentrated to remove all traces of alcohol. The aqueous mixture remaining in the still is diluted with sufficient distilled water to allow of the extraction of lipoids by ether. This is done in a separatory funnel in the usual manner. The process of ether extraction should be repeated at least five times. The ether is removed from the aqueous solution by distillation at reduced pressure and the concentration process is then continued until the material in the distilling flask is almost dry. (An aqueous or dilute alcoholic solution of the product at this or an earlier stage—as, for example, after one treatment with ten volumes of alcohol followed by the removal of œstrin by ether—has been found to be satisfactory for most clinical needs). The residue is then extracted several times with small amounts of absolute ethyl alcohol.

The combined alcoholic extracts are filtered and the filtrate is made strongly ammoniacal by the addition of one-third volume of saturated aqueous ammonia. The mixture is placed in a crystallizing dish and allowed to concentrate by slow evaporation at room temperature. Several days may be allowed for this stage and small amounts of ammonia and alcohol may be added from time to time as indicated. The semi-crystalline solid material which separates out may be separated from the mother liquor and washed three times with dilute cold aqueous ammonia. The washed solid material is then extracted with hot absolute alcohol faintly acidified with glacial acetic acid. The alcoholic extract is treated with one-third volume of saturated aqueous ammonia and set aside. The solid material which separates is again treated as outlined above. One then obtains the final product by chilling the absolute alcohol extract of the above substance.

The precipitate from alcohol may be further purified by repeated solution in hot alcohol and separation by chilling of the filtered alcoholic solution. The purified product is relatively insoluble in water, and indeed its